PhD SCHOOL OF AGRI-FOOD SCIENCES, TECHNOLOGIES AND BIOTECHNOLOGIES

XXXI cycle

"HYDROXYCINNAMIC ACIDS: *IN VITRO* ANTI-PROLIFERATIVE ACTIVITY AND METABOLISM"

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Tutor: **Prof. Angela Conte**
Co-tutor: **Dr. Davide Tagliazucchi**

PhD Programme Coordinator: **Prof. Alessandro Ulrici**

Polyphenols

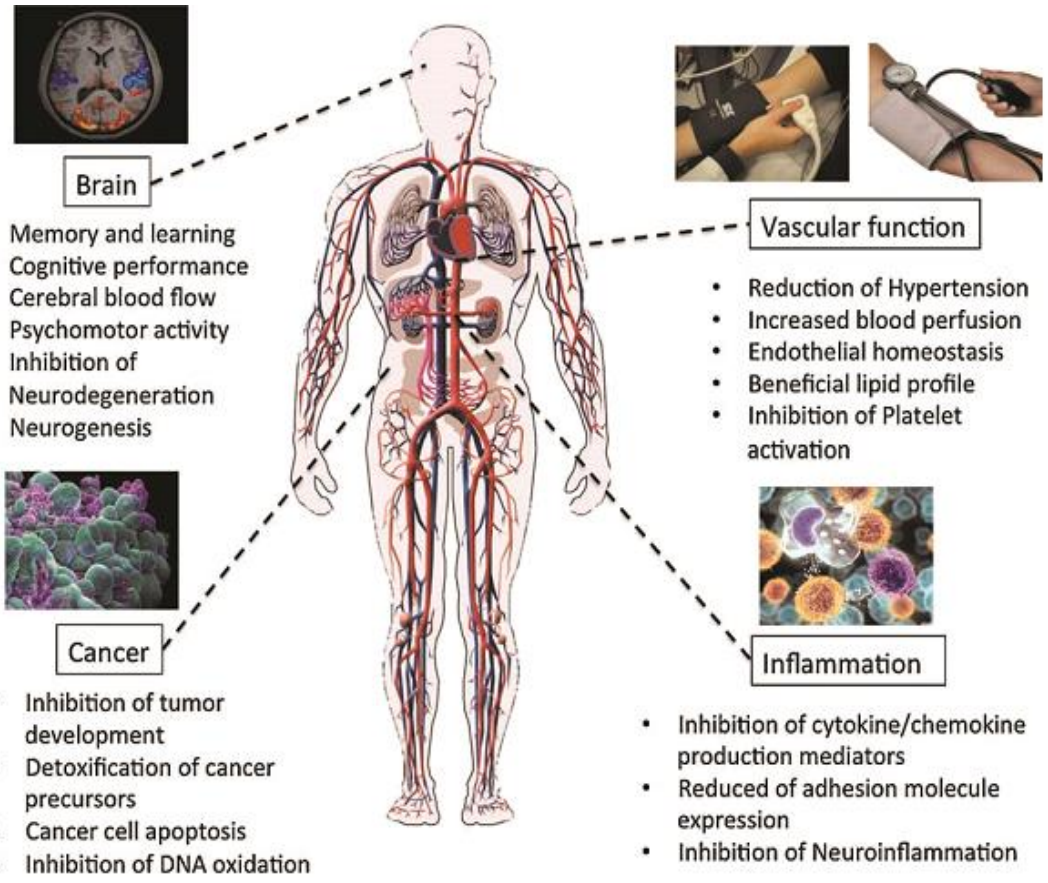


Main sources are fruits, beverages such as tea, coffee, wine and fruit juices, chocolate and, to a lesser extent, vegetables, cereals and legume seeds

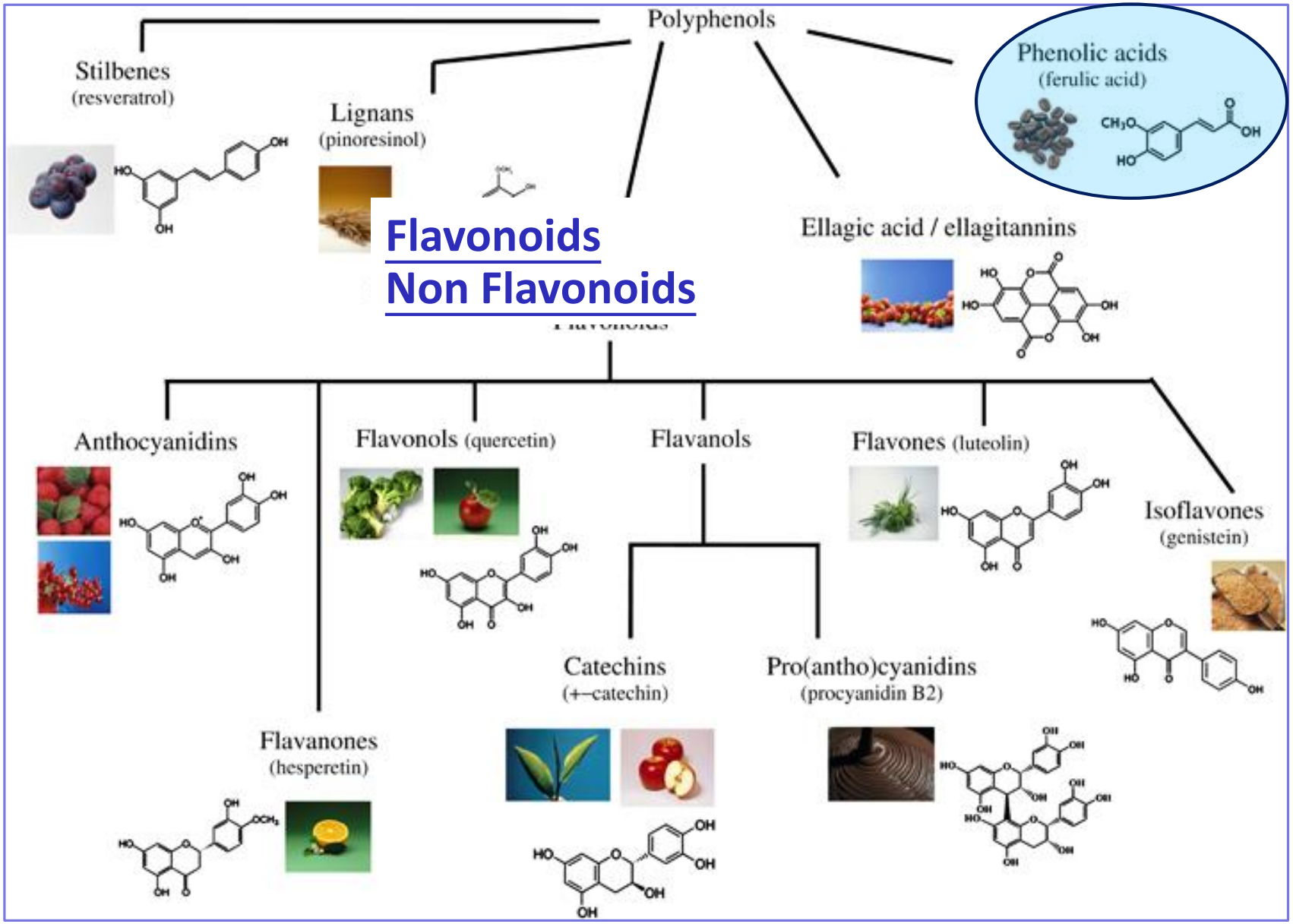


Polyphenols

- More than 8000 chemical structures
- Aromatic benzenoid (phenyl) ring, hydroxyl (-OH) groups
- Two main classes: **Flavonoids**
  Non Flavonoids



Polyphenols are abundant **micronutrients** in our diet, and evidence for their role in the prevention of **degenerative diseases** such as **cancer** and **cardiovascular diseases** is emerging. Their health effects depend on the **amount** consumed and on their **bioavailability**.


Polyphenols
Stilbenes
(resveratrol)
Lignans
(pinoresinol)
Phenolic acids
(ferulic acid)
Flavonoids
Non Flavonoids
Ellagic acid / ellagitannins
Anthocyanidins
Flavonols (quercetin)
Flavanols
Flavones (luteolin)
Isoflavones
(genistein)
Catechins
(+−catechin)
Pro(antho)cyanidins
(procyanidin B2)
Flavanones
(hesperetin)

Cherry cultivars

Light Cherries

Dark cherries

Dark chocolate (70%) bars

Functionalized chocolates

Dark chocolate (70%)

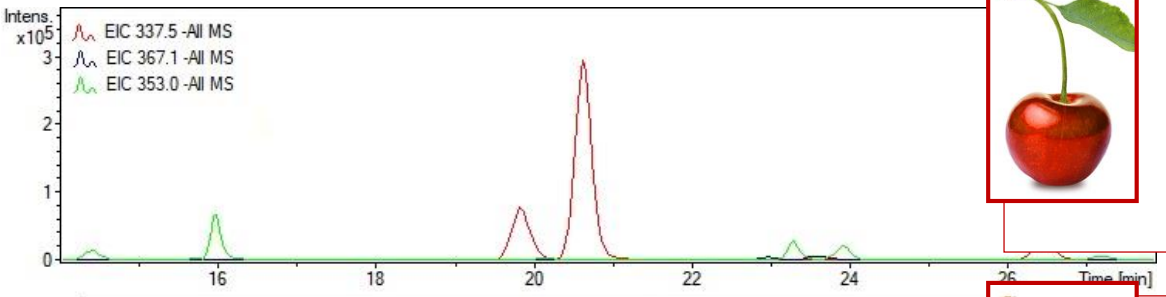
Dark chocolate with Sakura green tea leaves

Dark chocolate with turmeric powder

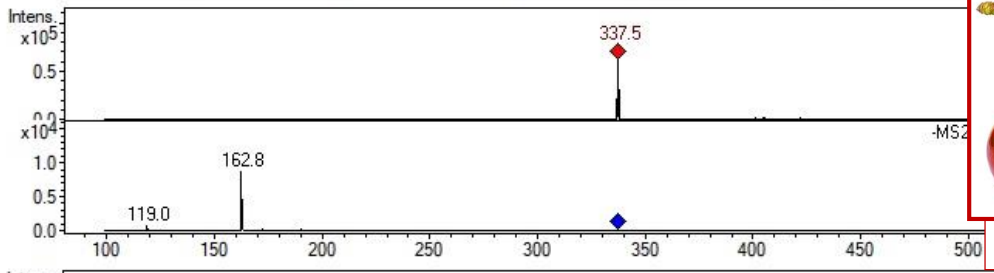
The objectives

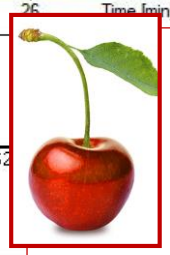
1. • *In vitro* digestion of cherries and chocolate
2. • C18 extraction of phenolic compounds from both digested and undigested samples
3. • Identification and quantification of phenolic compounds by mass spectrometry (LC-ESI-IT-MS/MS)
4. • Evaluation of antioxidant properties and anti-proliferative effect on human colon adenocarcinoma cell lines (Caco-2 and SW 480) and BIOACCESSIBILITY
5. • Anti-proliferative activity of hydroxycinnamic acid standards and Celeste and Dark Chocolate digested hydroxycinnamate-rich fractions
6. • Identification of the major bio-transformations, metabolic fate and enzymatic pathways in Caco-2 and SW 480

LC-ESI-IT-MS/MS identification

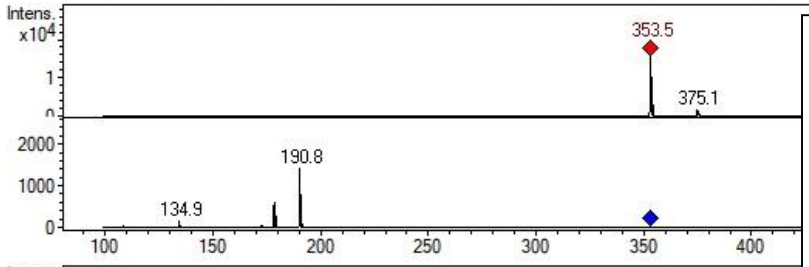


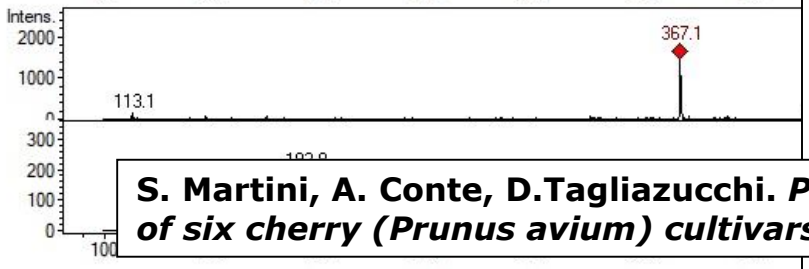
Tentative identification of 86 individual phenolic compounds in cherry cultivars





40 phenolic compounds identified for the first time in cherries.





Phenolic compounds profile and antioxidant properties of six sweet cherry (*Prunus avium*) cultivars

CrossMark

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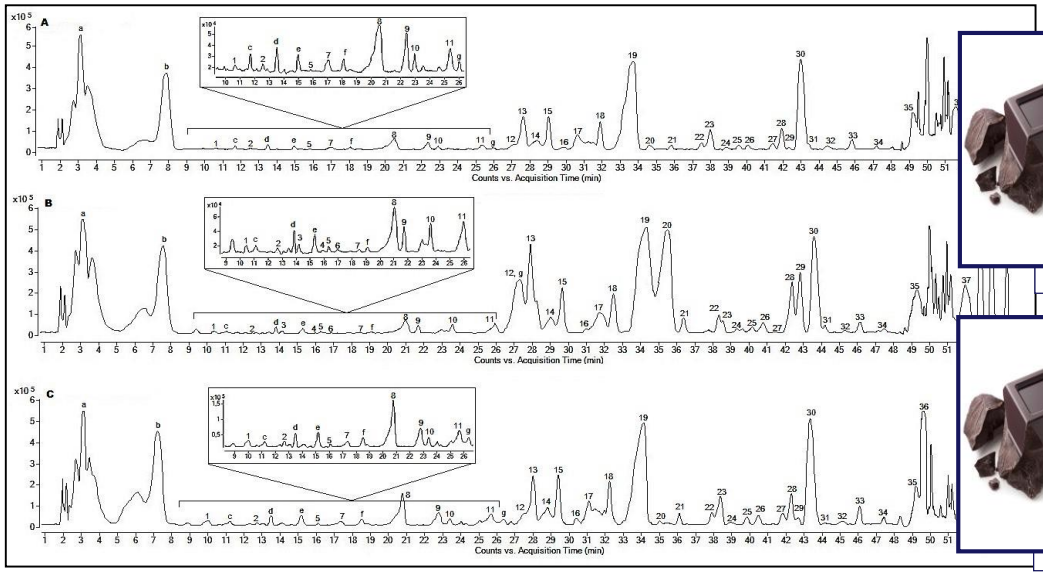
ABSTRACT

Sweet cherry (*Prunus avium*) fruits are a nutritionally important food rich in dietary phenolic compounds. The aim of this study was to investigate the phenolic profile and chemometric discrimination of fruits from six cherry cultivars using a quantitative metabolomics approach, which combine non-targeted mass spectrometry and chemometric analysis. The assessment of the phenolic fingerprint of cherries allowed the tentative identification of 86 compounds. A total of 40 chlorogenic acids were identified in cherry fruit, which pointed out hydroxycinnamic acid derivatives as the main class of phenolics by number of compounds. Among the compounds detected, 40 have been reported for the first time in sweet cherry fruit. Hydroxycinnamic acids are also the quantitatively most represented class of phenolic compounds in the cherry cultivars with the exception of Lapins and Durone della Marca where the most representative class of phenolic compounds were anthocya-

S. Martini, A. Conte, D.Tagliazucchi. *Phenolic compounds profile and antioxidant properties of six cherry (Prunus avium) cultivars.* Food Research International, 2017, 97: 15-26

LC-ESI-IT-MS/MS identification



Tentative identification of 158 individual phenolic compounds in chocolate

67 phenolic compounds identified for the first time in chocolate.

38 phenolic compounds identified for the first time in cocoa-derived products.

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ELSEVIER

Comprehensive evaluation of phenolic profile in dark chocolate and da
chocolate enriched with Sakura green tea leaves or turmeric powder

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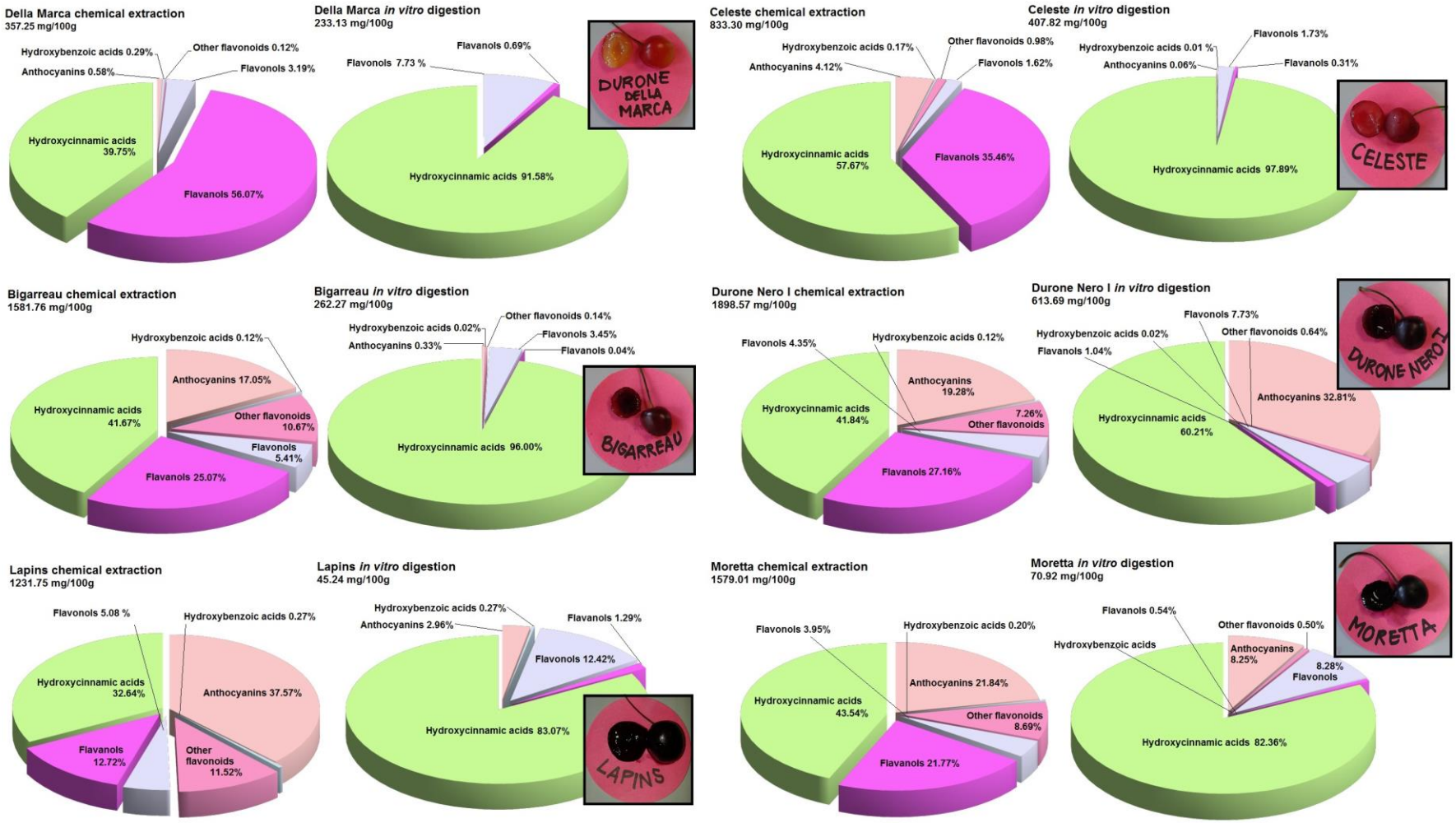
ABSTRACT

Recently, a huge number of studies have confirmed the important role of chocolate polyphenols in human health, underlining its beneficial effects especially in the treatment of cardiovascular diseases. However, a thorough evaluation of chocolate phenolic profile is still lacking. This study aimed at a comprehensive char-
acterization of dark chocolate phenolic profile, using non-targeted mass spectrometry identification. This ap-
proach allowed the identification of 158 phenolic compounds, 67 of which detected in dark
Ellagitannins which have never been reported in cocoa products. The phenolic
influenced and modified the phenolic profile, resulting in a phenolic concentration increase. In this way,
combining the positive
and to limit those reducing the amount of sugars and
calories introduced with chocolate.

S. Martini, A. Conte, D. Tagliazucchi. *Comprehensive evaluation of phenolic profile in dark chocolate and dark chocolate enriched with Sakura green tea leaves or turmeric powder*. Food Research International, 2018, 112:1-16

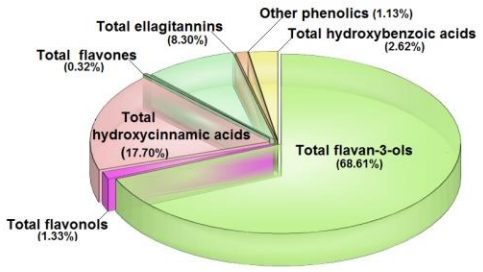
Chemical quantification

Hydroxycinnamic and hydroxybenzoic acids, flavan-3-ols, flavonols, anthocyanins and other flavonoids obtained through chemical extraction or after *in vitro* digestion in six different cherry cultivars

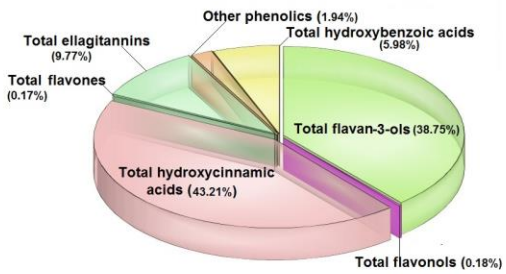


Chemical quantification

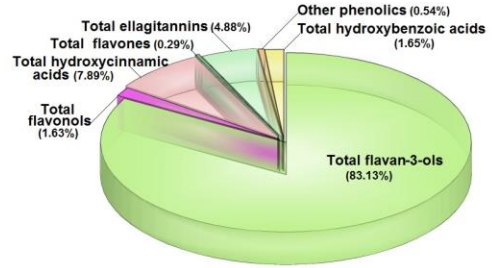
DARK CHOCOLATE 70%
(962 mg/100g)



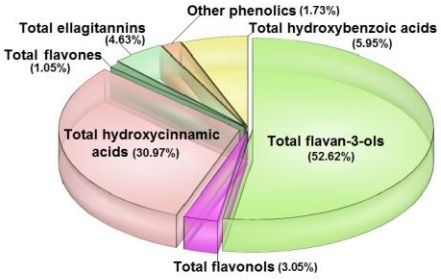
***IN VITRO* DIGESTED DARK CHOCOLATE 70%**
(180 mg/100g)



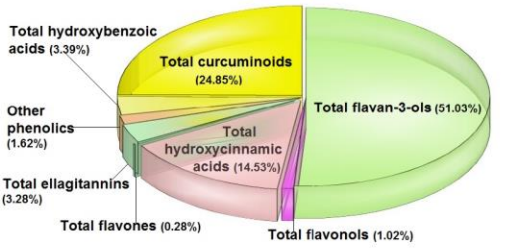
GREEN TEA DARK CHOCOLATE 62%
(1787.5 mg/100g)



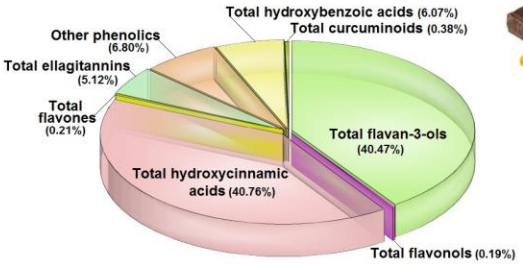
***IN VITRO* DIGESTED GREEN TEA DARK CHOCOLATE 62%**
(215 mg/100g)



TURMERIC DARK CHOCOLATE 70%
(1098 mg/100g)



***IN VITRO* DIGESTED TURMERIC DARK CHOCOLATE 70%**
(173 mg/100g)



- **Hydroxycinnamic acids**
- **Hydroxybenzoic acids**
- **Flavan-3-ols**
- **Flavonols**
- **Flavones**
- **Ellagitannins**
- **Curcuminoids**
- **Other flavonoids**

Obtained through chemical extraction and after *in vitro* digestion of the three types of chocolates

Bioavailability

Proportion of a nutrient that is absorbed from the diet and used for normal body functions

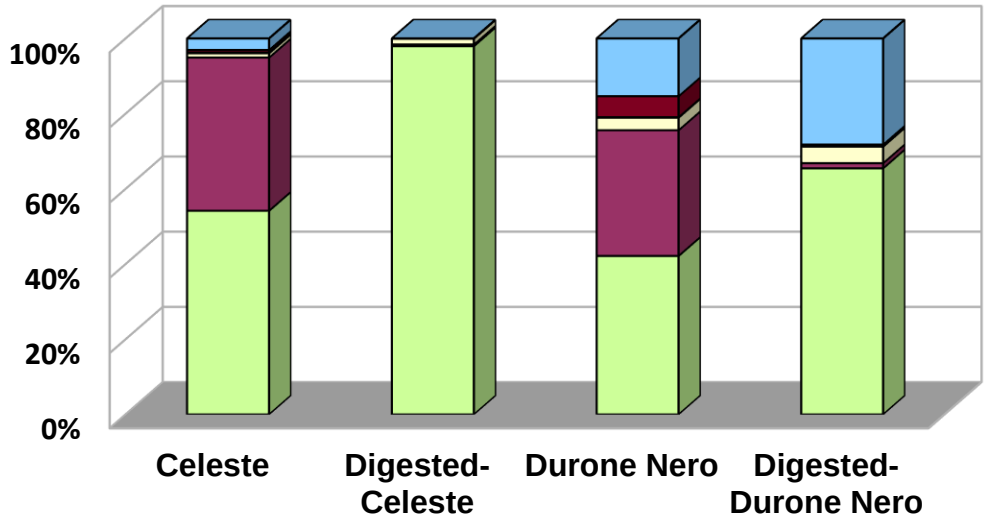
Bioaccessibility	Absorption	Bioactivity
Release of the nutrient from the physicochemical dietary matrix	Transfer across the gut wall (passing through the cells, in-between them or both) to the blood or lymphatic circulation	• systemic distribution • systemic deposition (storage) • metabolic and functional use • excretion (via urine or faeces)

→ **Food matrix**

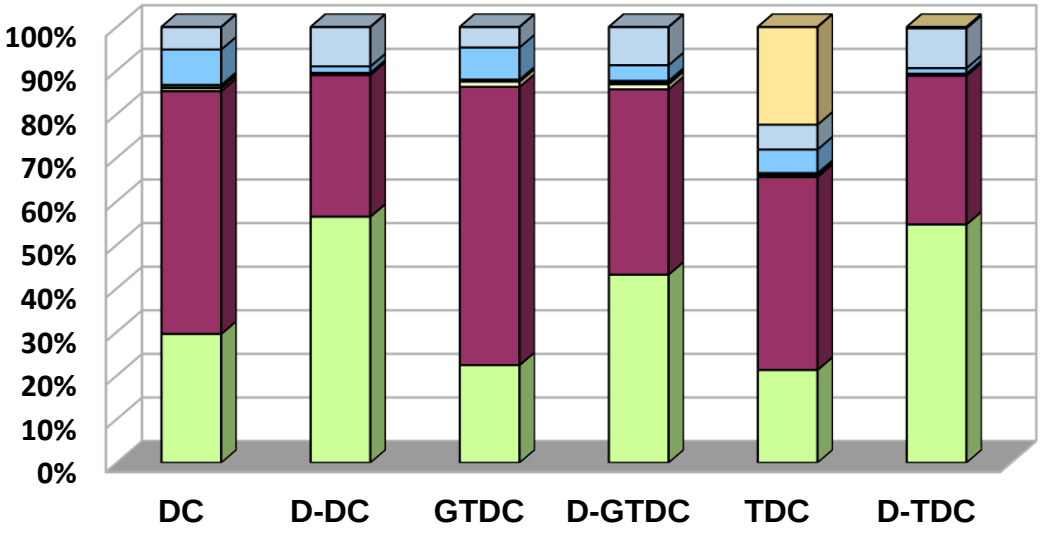
→ **Molecular interactions**

Bioaccessibility

The capacity of polyphenols to reach unmodified the intestinal tract after digestion, where they can carry out their antiproliferative activity against colon-rectal cancer cells.

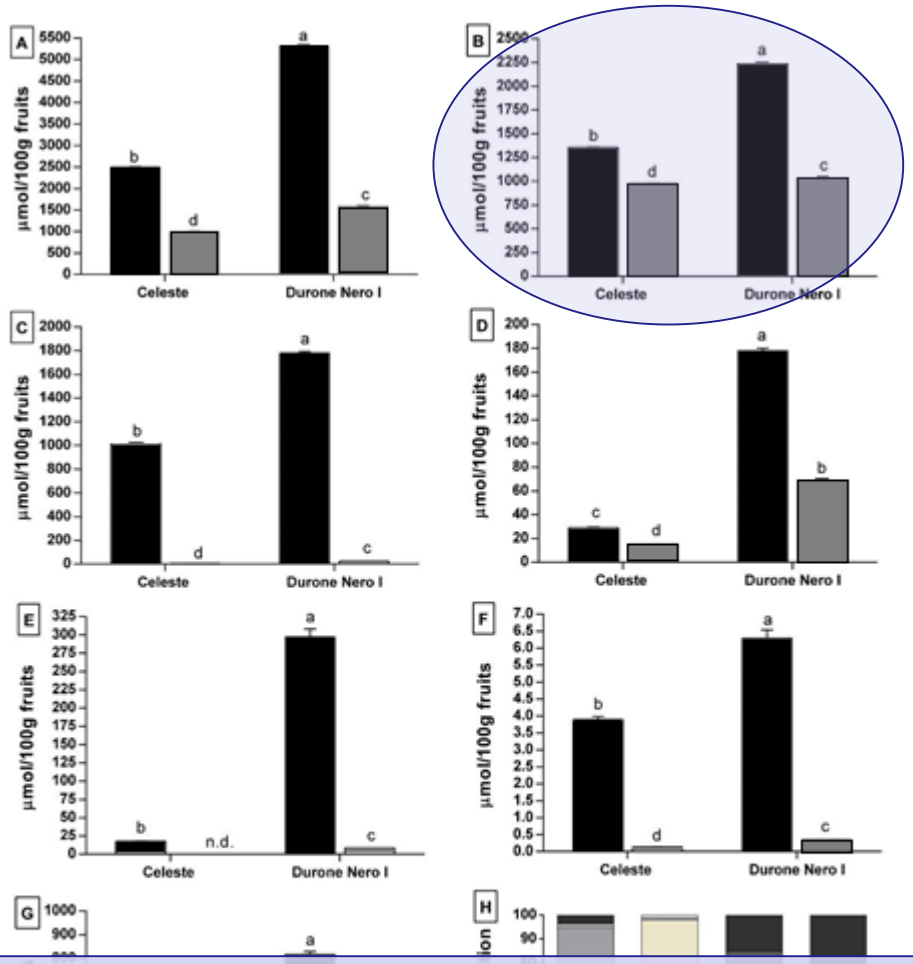


The bioaccessibility is strictly related to the food matrix and to the phenolic classes themselves.



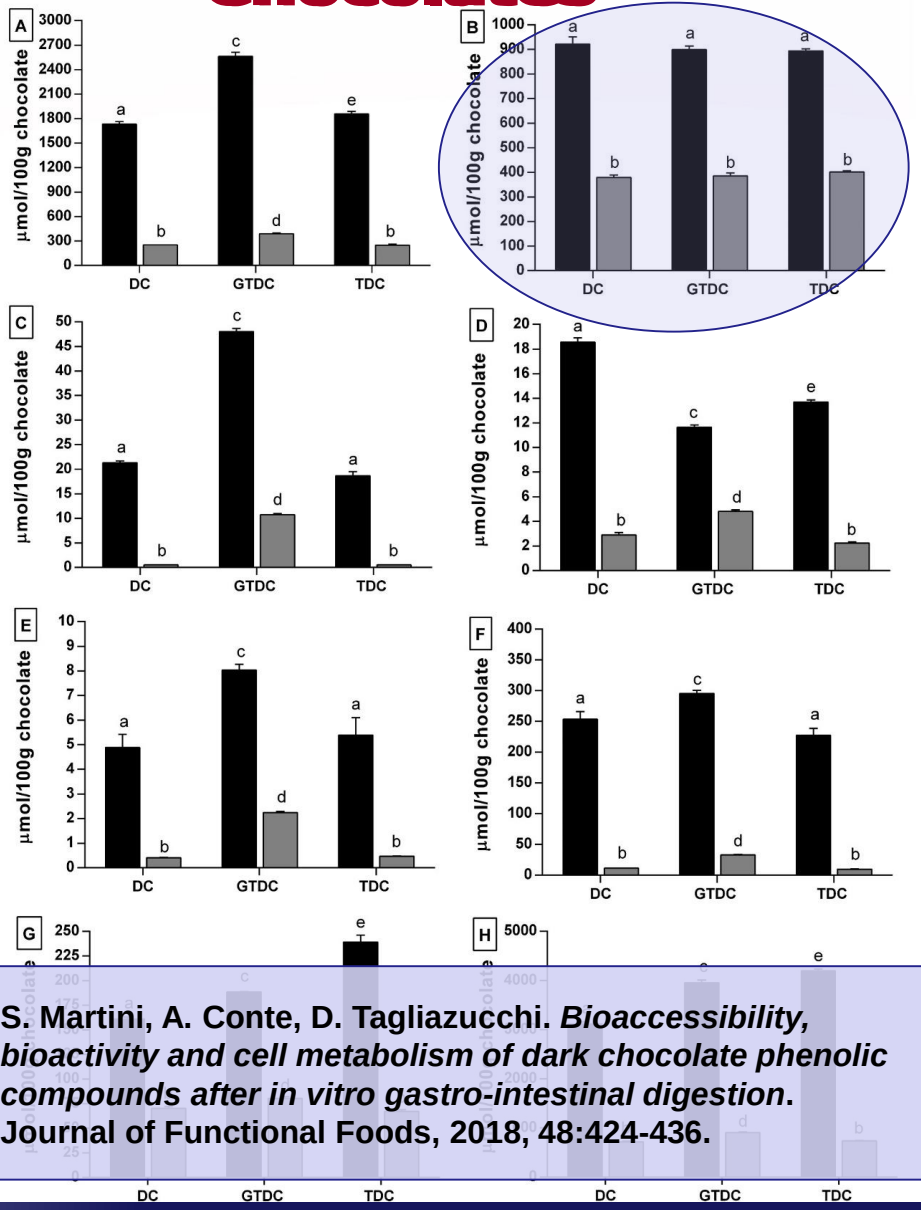
Bioaccessibility of phenolic classes

Cherries



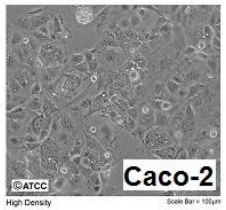
S. Martini, A. Conte, D.Tagliazucchi. ***Bioactivity and cell metabolism of in vitro digested sweet cherry (Prunus avium) phenolic compounds.*** International Journal of Food Sciences and Nutrition, ***In Press***. https://doi.org/10.1080/09637486.2018.1513996.

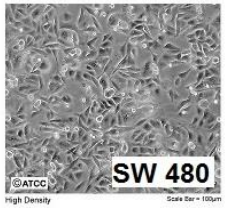
Chocolates



S. Martini, A. Conte, D. Tagliazucchi. ***Bioaccessibility, bioactivity and cell metabolism of dark chocolate phenolic compounds after in vitro gastro-intestinal digestion***. Journal of Functional Foods, 2018, 48:424-436.

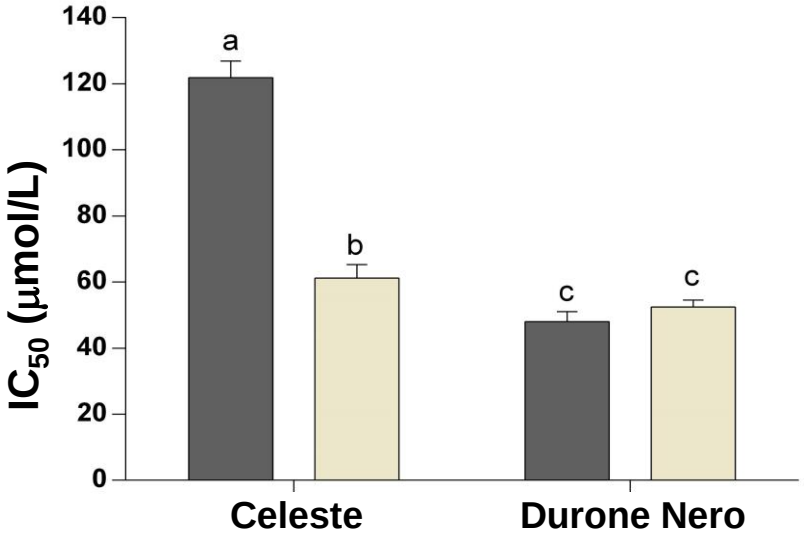
Anti-proliferative activities

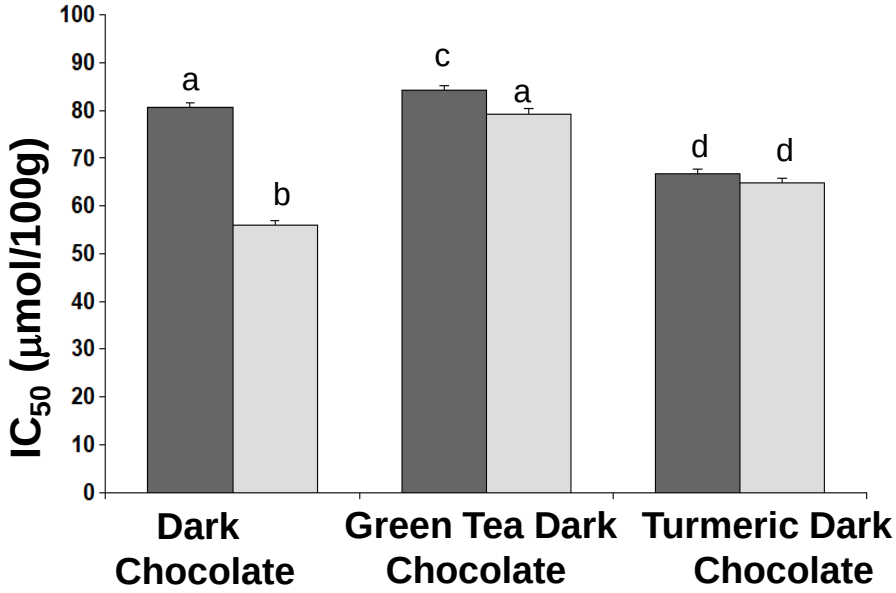




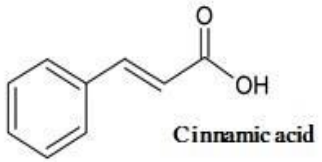
Anti-proliferative activity of phenolic-rich fractions extracted before and after *in vitro* digestion on SW 480.

Anti-proliferative activity of phenolic-rich fractions extracted at the end of the *in vitro* digestion on Caco-2 and SW 480.

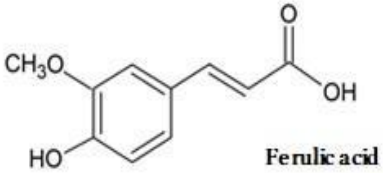




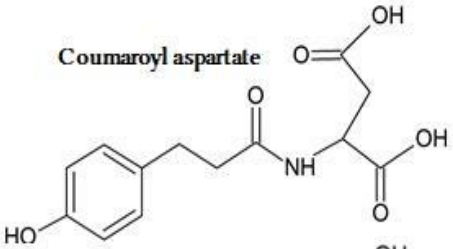
Hydroxycinnamic acids selection and extraction

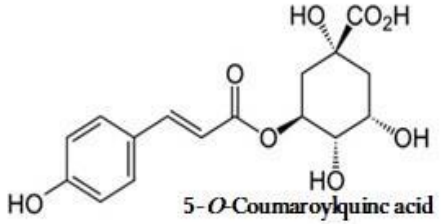


Cinnamic acid

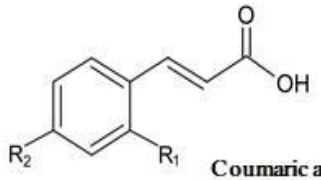


Ferulic acid

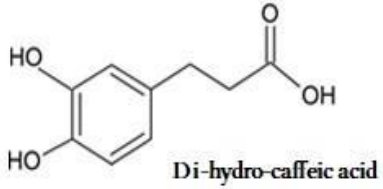




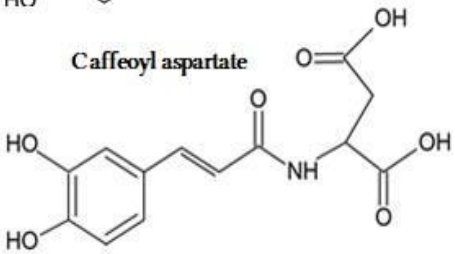
5-*O*-Coumaroylquinc acid

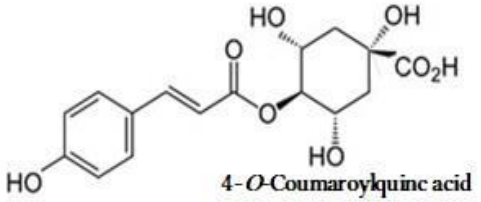


Coumaric acid

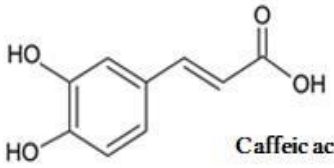


Di-hydro-caffeic acid

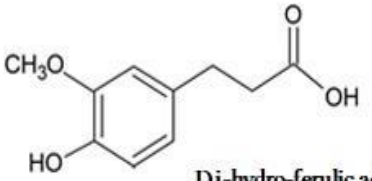




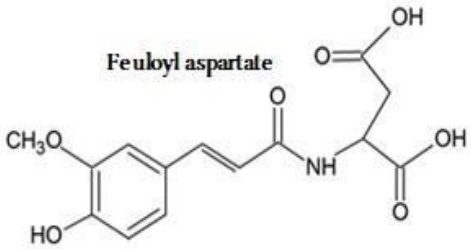
4-*O*-Coumaroylquinc acid

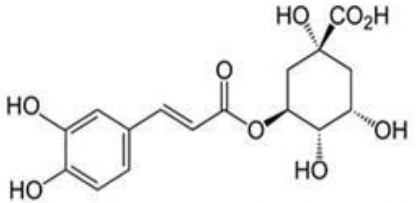


Caffeic acid



Di-hydro-ferulic acid





5-*O*-Caffeoylquinc acid

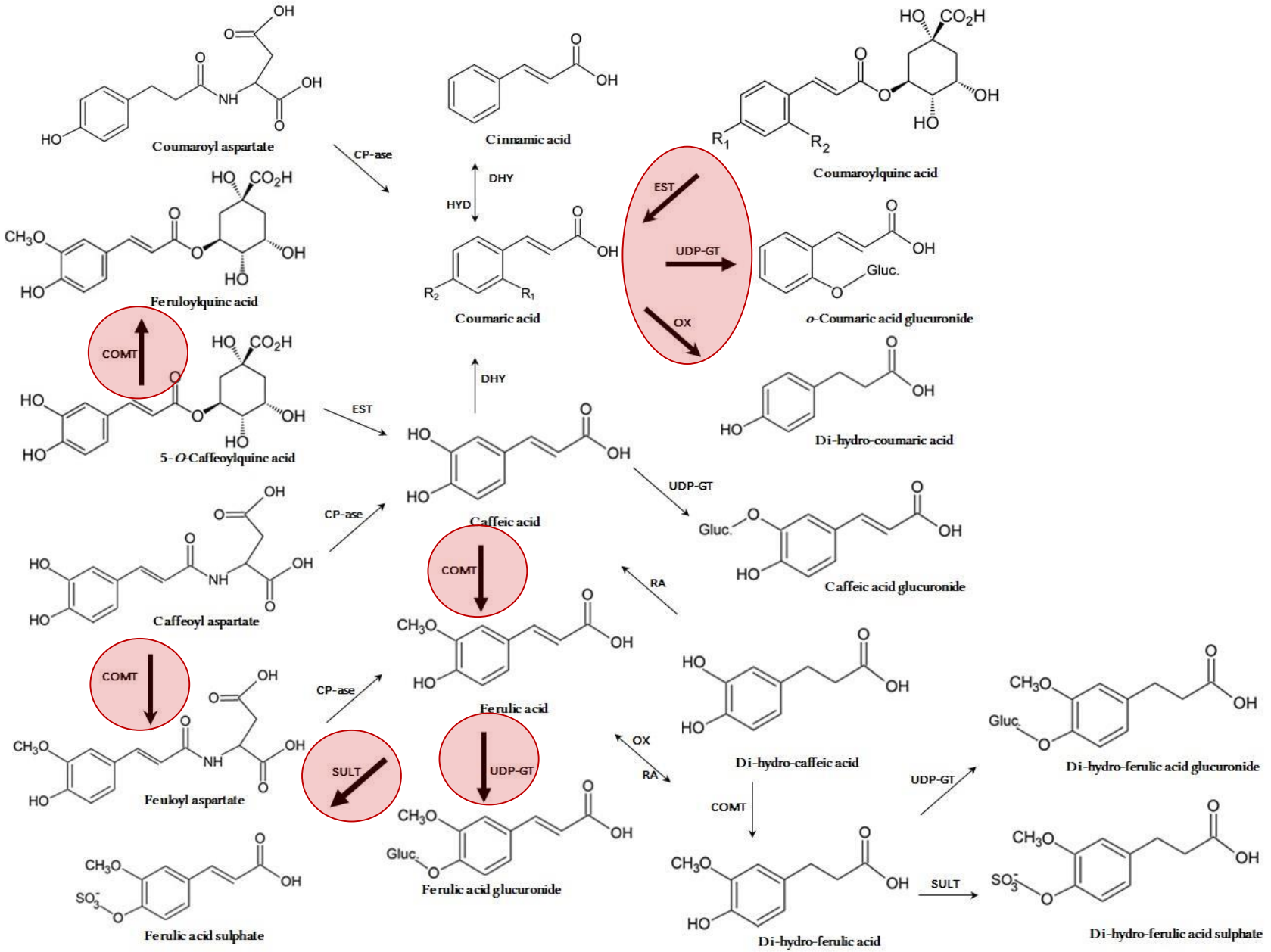
Anti-proliferative activities

Table. Effect of hydroxycinnamic acids and coumaroylquinic acids and hydroxycinnamoyl aspartates enriched fractions on the proliferation of colon adenocarcinoma human cell lines

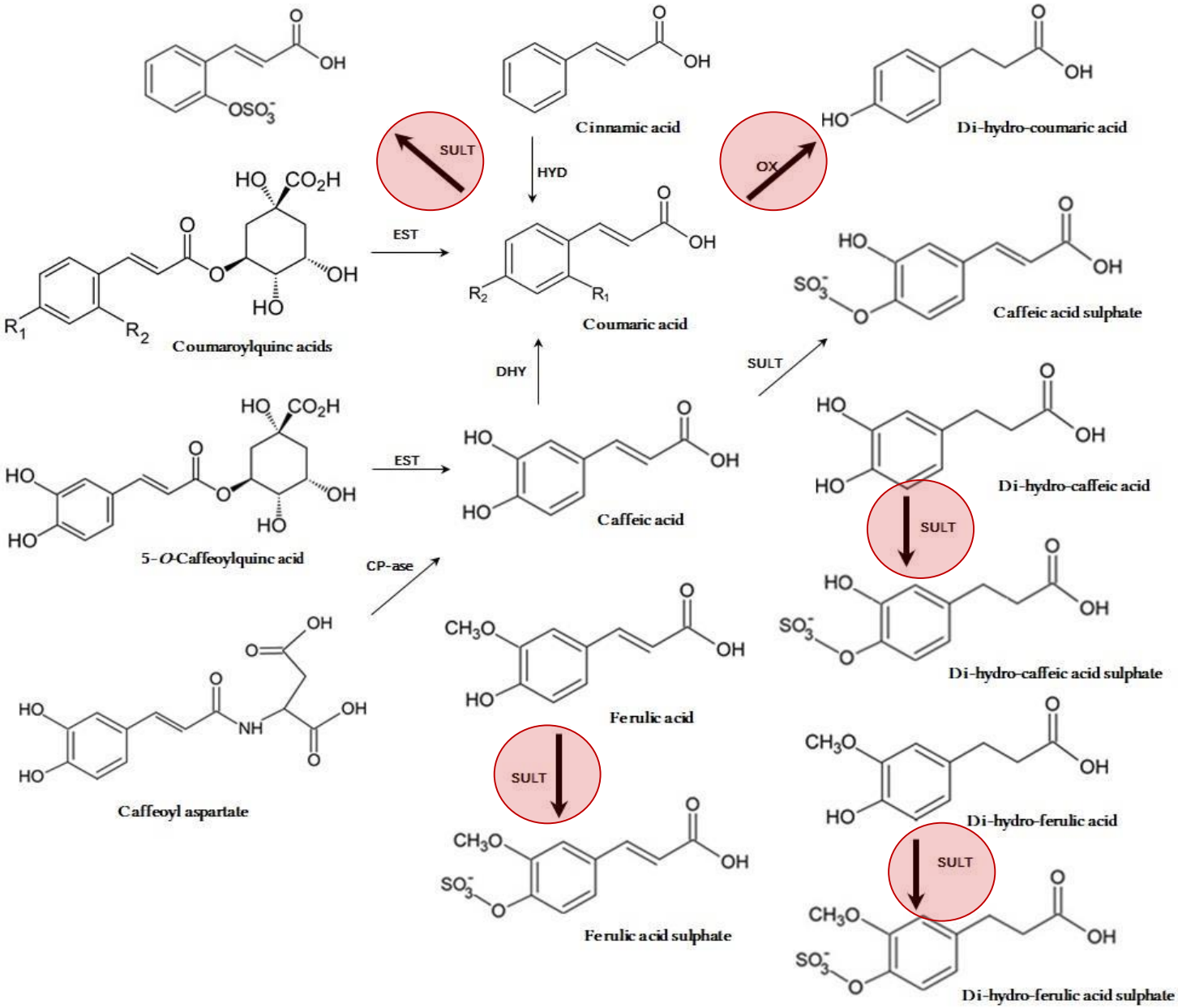
Compound	*Caco-2* *% inhibition*	*SW 480* *% inhibition*
Cinnamic acid	n.a.	n.a.
p-Coumaric acid	n.a.	n.a.
o-Coumaric acid	n.a.	7.2 ± 3.9^{e}
Caffeic acid	n.a.	40.8 ± 1.6^{c}
Ferulic acid	n.a.	59.9 ± 1.3^{f}
5-*O*-Caffeoylquinic acid	n.a.	n.a.
Di-hydro-caffeic acid	100.9 ± 1.7^{a}	46.8 ± 1.0^{b}
Di-hydro-ferulic acid	100.1 ± 2.1^{a}	37.3 ± 1.2^{cd}
Coumaroylquinic acids enriched fraction	n.a.	41.4 ± 3.3^{bc}
Hydroxycinnamoyl aspartates enriched fraction	10.7 ± 1.9^{e}	34.2 ± 1.6^{d}

Hydroxycinnamic acids were tested at 200 µmol/L. Coumaroylquinic acids and hydroxycinnamoyl aspartates enriched fractions were tested after two-fold dilution in cell media. Coumaroylquinic acids concentration in the cell media were 36.2 and 13.9 µmol/L for 4-*O*-coumaroylquinic and 5-*O*-coumaroylquinic acids, respectively. Hydroxycinnamoyl aspartates concentration in the cell media were 224.1, 55.2 and 33.1 µmol/L for coumaroyl-aspartate, caffeoyl-aspartate and feruloyl-aspartate, respectively.

Caco-2 hydroxycinnamates metabolic fate



SW 480 hydroxycinnamates metabolic fate



Conclusions

1. • MS-characterization extends the current knowledge on these food matrices phytochemistry providing the broadest phenolic profiling so far
2. • Bioactivity of phenolic compounds is primarily conditioned by their bioaccessibility. Hydroxycinnamic acids are the most bioaccessible phenolic compounds
3. • Digested fractions showed higher anti-proliferative activity against Caco-2 and SW 480 cell lines than the undigested ones
4. • Hydroxycinnamic acids are the actually responsible of the anti-proliferative effect
5. • Di-hydro-caffeic (DHC) and di-hydro-ferulic (DHF) acids were the most active tested compounds and could be the real active molecules involved in the reported biological effect
6. • This study allowed us to understand the molecules and mechanisms responsible for the specific biological effects of phenolic compounds and metabolites. Major metabolic pathways were identified

SINCERE ACKNOWLEDGEMENTS

WORK TEAM
Dr. **Davide Tagliazucchi**
Prof. **Angela Conte**

Prof. **Davide Malagoli**
Cell-Lab 'Paolo Buffa'

...and EVERYONE of the KennedyLab!

Biochemically, love is just like eating large amounts of chocolate.

John Milton, "The Devil's Advocate"

Thanks for your kind attention