A decorative border of small, colorful icons representing various food items (like bread, eggs, fruits, vegetables) and scientific symbols (like a microscope, a beaker, a leaf, a target) surrounds the central text.

# Extraction procedures and determination of bioactivities from various food sources

**Lea POGAČNIK**

University of Ljubljana, Biotechnical Faculty, Department of Food Science and Technology  
Jamnikarjeva 101, Ljubljana, Slovenia

# Where do I come from?

I FEEL  
SLOVENIA

*Slovenija*

Eslovenia



Olá = Živjo



## *Slovenija (slo)*

- 2,095,861 prebivalcev
- 78 pričakovana življenjska doba
- 20,271 km<sup>2</sup> območje
- 1382 km meje
- 46,6 km obale
- 58,1 % gozdov
- 2,864m najvišji vrh
- 10,200 podzemnih jam
- 3,320 ekoloških kmetij
- 37 % območja zavarovanega kot Narava 2000



## *Slovenia (eng)*

- 2,095,861 inhabitants
- 78 life expectancy
- 20,271 km<sup>2</sup> area
- 1382 km border
- 46,6 km coastline
- 58,1 % forests
- 2,864 m highest peak
- 10,200 underground caves
- 3,320 organic farms
- 37 % of territory protected as Natura 2000







## *Slovenija (slo)*

- 76,728 študentov
- 3,500 športnih organizacij in klubov
- 388 raziskovalnih organizacij
- 111 muzejev
- 153 galerij
- 577,544 družin
- 1,04 M žensk
- 1,02 M moških
- 18,631 zagonskih podjetij
- 70 % ločevanja odpadkov

## *Slovenia (eng)*

- 76,728 students
- 3,500 sport societies and clubs
- 388 research organisations
- 111 museums
- 153 galleries
- 577,544 families
- 1,04 M female residents
- 1,02 M male residents
- 18,631 number of start-up companies
- 70 % separate collection of waste





I FEEL  
SLOVENIA

Zgodbo Slovenije govori  
znamka I feel Slovenia, ki  
temelji na trajnostnem razvoju  
in zeleni barvi. „Slovenska  
zelena“ je barva slovenskega  
gozda.

The brand I feel Slovenia  
speaks the story  
of Slovenia. The brand is based  
on sustainable development  
and green colour. It's  
„Slovenian green“ or the colour  
of Slovenian forest.

2/3 Slovenije prekrivajo  
gozdovi; smo tretja najbolj  
gozdnata država v Evropi.

Two thirds of Slovenia are  
covered with forests; we  
are the third most forested  
country in Europe.



## *Ali ste vedeli? (slo)*

- Slovenija je edina država, ki v svojem imenu skriva besedo "love"
- Slovenci doma radi obujemo copate, kar se morda komu zdi nenavadno
- Slovenci obožujemo glasbo, smo tudi veliki ljubitelji zborovske glasbe
- Slovenija ima glede na svojo velikost največje število jam na svetu. Slovenske jame so poleg tega tudi dom številnih močerilov - človeških ribic



## *Did you know? (eng)*

- Slovenia is the only country in the world with „love“ in its name
- Slovenians love to wear slippers at home; it might seem unusual to some people
- Slovenians love music, we especially love to sing
- Slovenia has one of the highest numbers of underground **caves** in the world in terms of country area. Number of endemic animals called **proteus** or „human fish“ live in them



# Where do I come from?

Univerza v Ljubljani  
*Biotehniška* fakulteta





## Known Slovenians?



Primož Roglič and Tadej Pogačar



Jan Oblak



Aleksander Čeferin



## What can you do in Slovenia?

- Study at University of Ljubljana
- Enjoy life in Ljubljana:
  - small but great city
- Sport activities
- Learning Slovenian language



[https://www.youtube.com/watch?v=qIjwWUujyig&ab\\_channel=UniverzavLjubljani%2FUniversityofLjubljana](https://www.youtube.com/watch?v=qIjwWUujyig&ab_channel=UniverzavLjubljani%2FUniversityofLjubljana)

[https://www.youtube.com/watch?v=jKNpo4okbQE&ab\\_channel=StudyinSlovenia](https://www.youtube.com/watch?v=jKNpo4okbQE&ab_channel=StudyinSlovenia)

## 50.009 ESTUDANTES (2018/19)

20.750

Inscritos em Licenciatura  
(1.º Ciclo)

13.969

Inscritos em Mestrados  
Integrados (1.º e 2.º Ciclo)

10.791

Inscritos em Mestrados (2.º  
Ciclo)

4.499

Inscritos em  
Doutoramentos (3.º Ciclo)

- University of Ljubljana was founded in 1919
- large university, with more than 40,000 graduate and postgraduate students and approximately 5,600 employees
- 23 faculties and 3 arts academies (Art, Fine Arts, Natural Science, Technology and Engineering, Social Sciences, Humanities, Medicine, Health Sciences, Sport)
- UL is listed amongst the top 500 universities in the world according to the ARWU Shanghai, Times THSE-QS and Webometrics Ranking.
- In the period 2007-2013 UL cooperated in 745 EU projects, including 163 FP7 projects.



Univerza v Ljubljani  
*Biotehniška* fakulteta



2687 BSc, MSc students + 194 PhD students

Agronomy

Biology

Forestry

Landscape  
Architecture

Wood technology



Animal Science

**Food Science and  
Technology**

Biotechnology

Microbiology

## Programs for exchange students

- **Erasmus+:** the highest % of exchange students; call each year for exchange for studies and traineeships
- **CEEPUS:** call each year; 1 month – 10 months
- **Bilateral Scholarships:** call each year; 1 month – 10 months

### Organisation of work at BF and at UL:

Each study programme has its own **academic coordinator**; **faculty coordinator**; **university (institutional) coordinator**.

### Academic coordinators for exchange Students

- **Tutoring** system at BF





## Erasmus+ at BF

### Courses offered to exchange students

- <http://arhiv.bf.uni-lj.si/en/deans-office/international-activities/information-for-exchange-students/course-catalogue/academic-year-20202021/>

### Departmental coordinators

- <http://arhiv.bf.uni-lj.si/en/deans-office/international-activities/information-for-exchange-students/departmental-coordinators-for-exchange-students/>

## Department of Food Science and Technology

- Chair of Biochemistry and Food Chemistry
- Chair of Biotechnology, Microbiology and Food Safety
- Chair of Microbiology
- Chair of Meat Technology And Food Assessment
- Chair of Plant Food Technologies, Human Nutrition and Enology



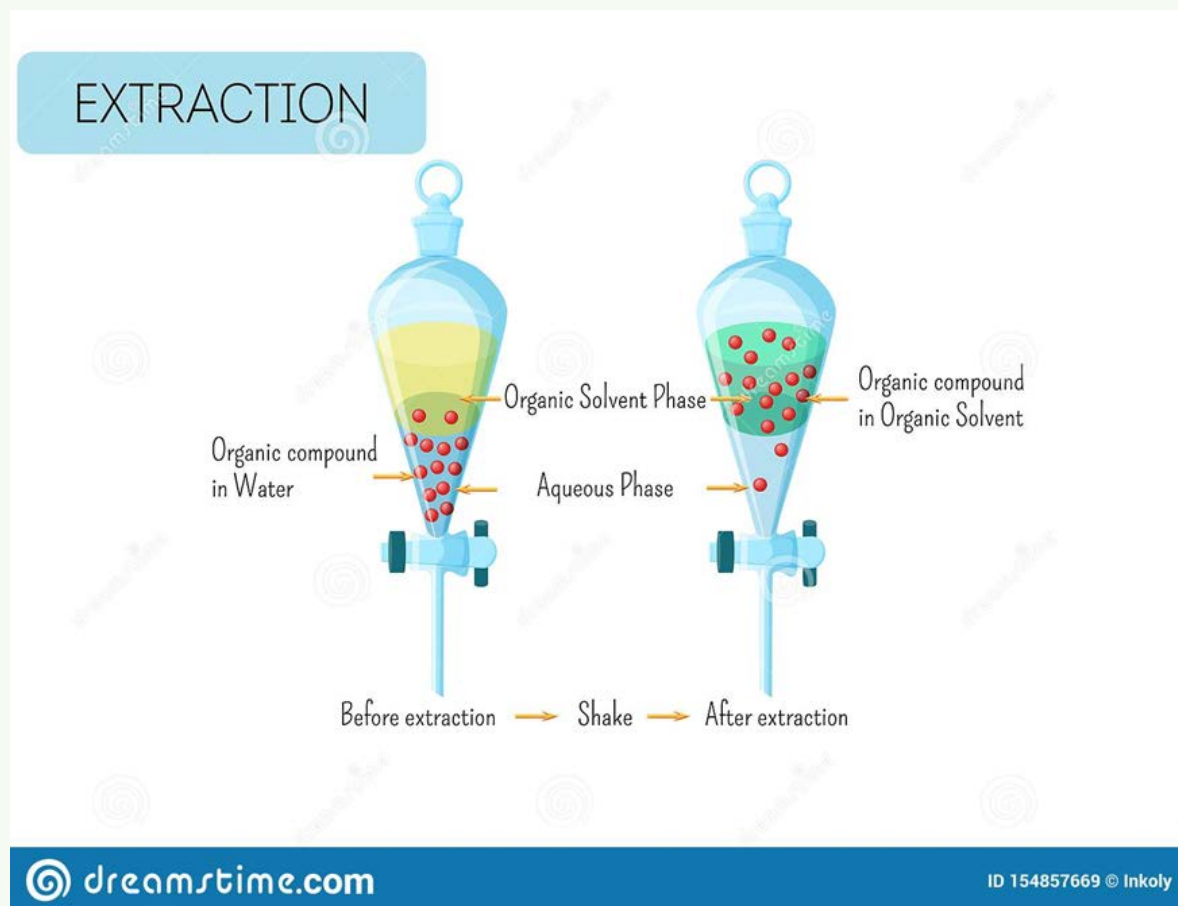


# Extraction procedures and determination of bioactivities from various food sources

Extraction methods:

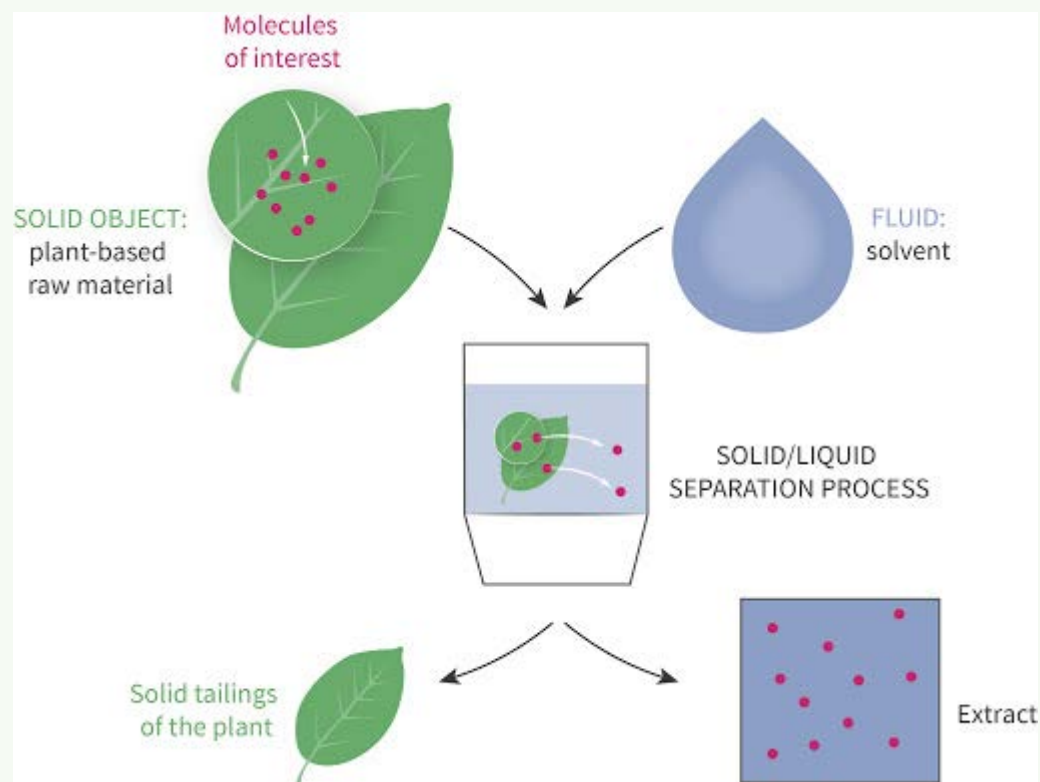
- solvent extraction,
- distillation method,
- pressing and
- sublimation.

## Solvent extraction – liquid/liquid extraction



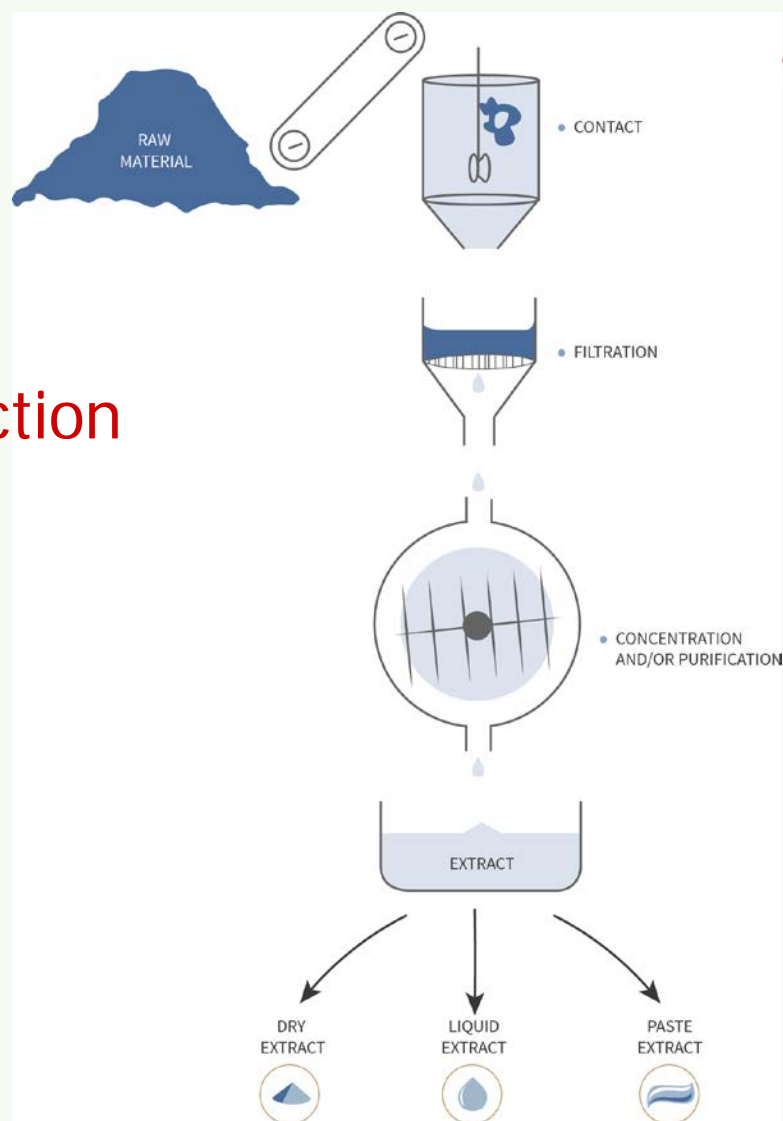


# Solid/liquid extraction



<http://www.berkem.com/en/expertise-en/plant-extraction>

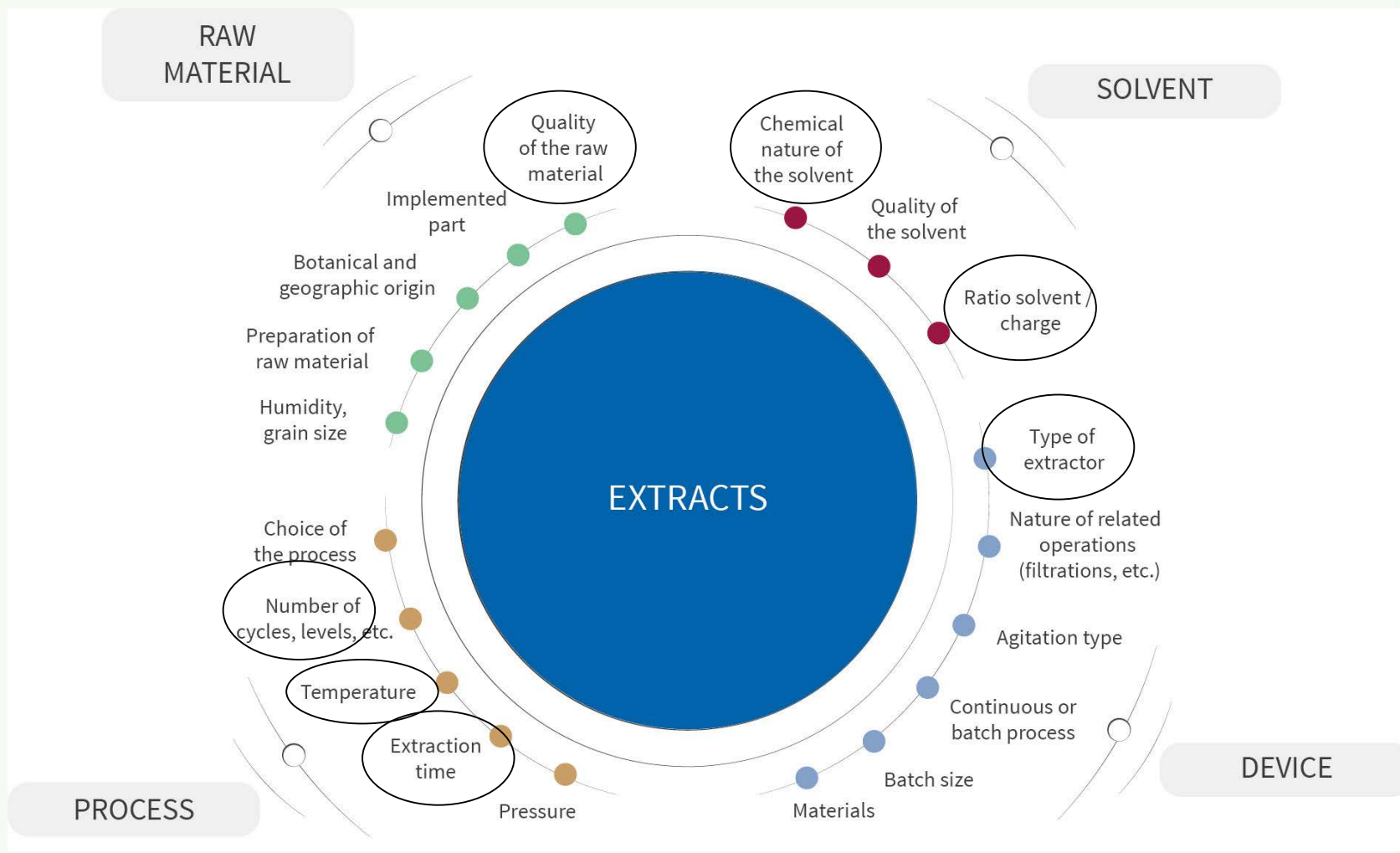
## Solid/liquid extraction





# What has to be optimised?

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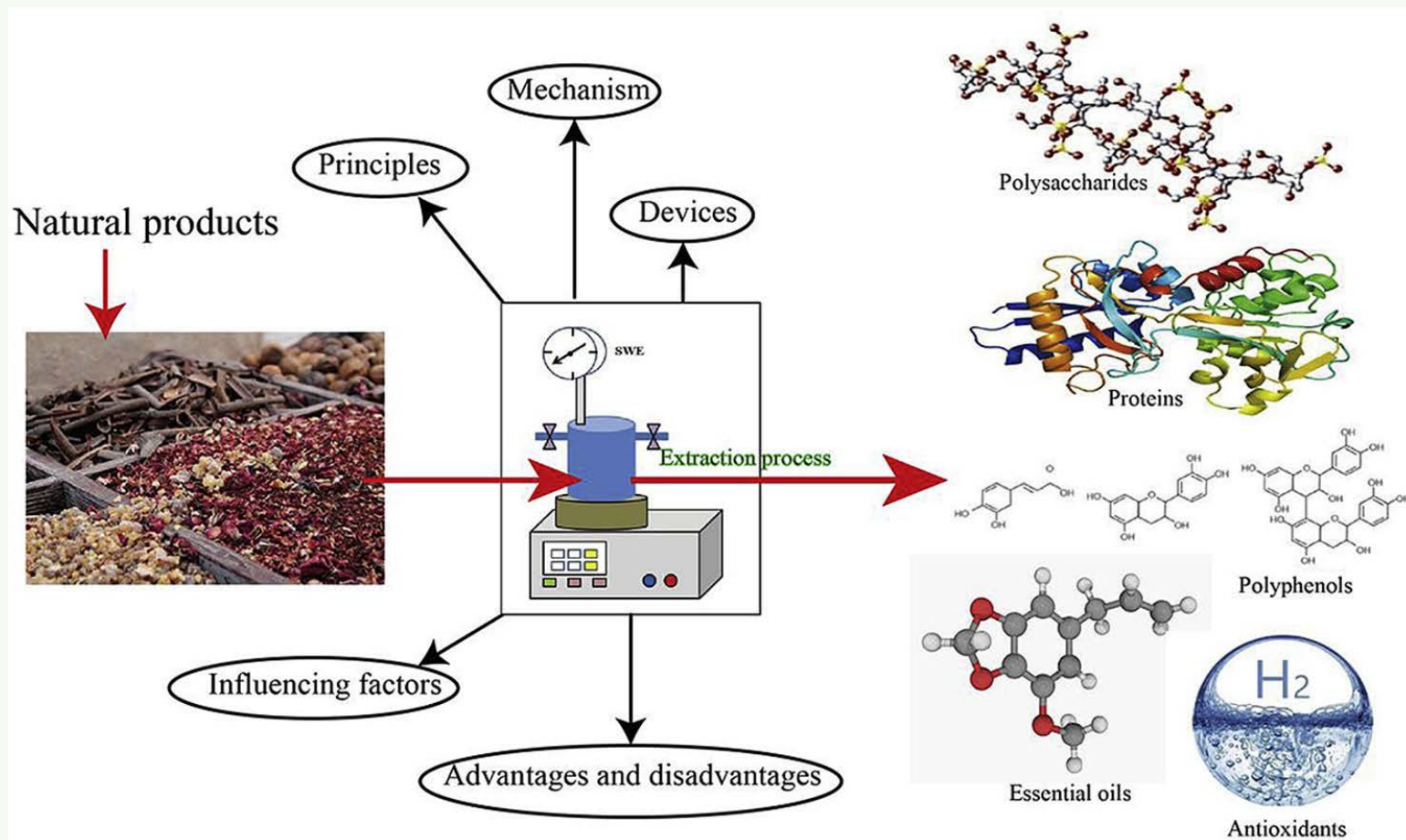


## What has to be optimised?

- Plant tissue
- Solvent (polar, non-polar, semi-polar)
- Extraction temperature
- Duration of extraction
- Type of extraction (Conventional maceration extraction, Ultrasound-assisted extraction)
- Solid-to-liquid ratio



# What do we extract?



# What happens after extraction?

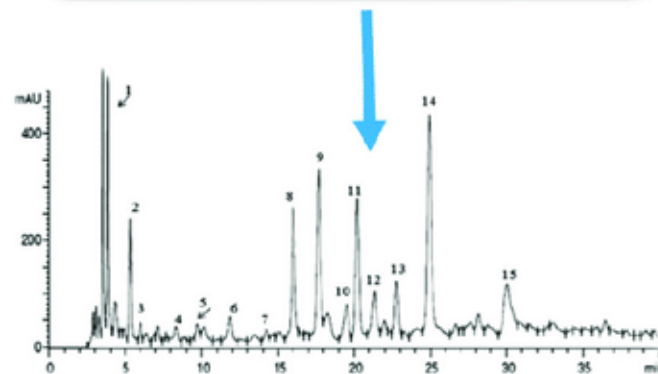
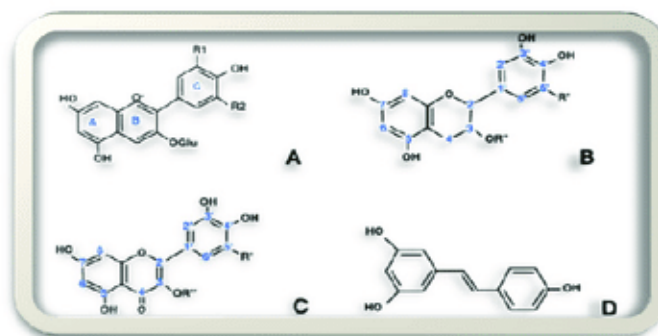
Plant material: selection and botanical identification



Extraction conditions

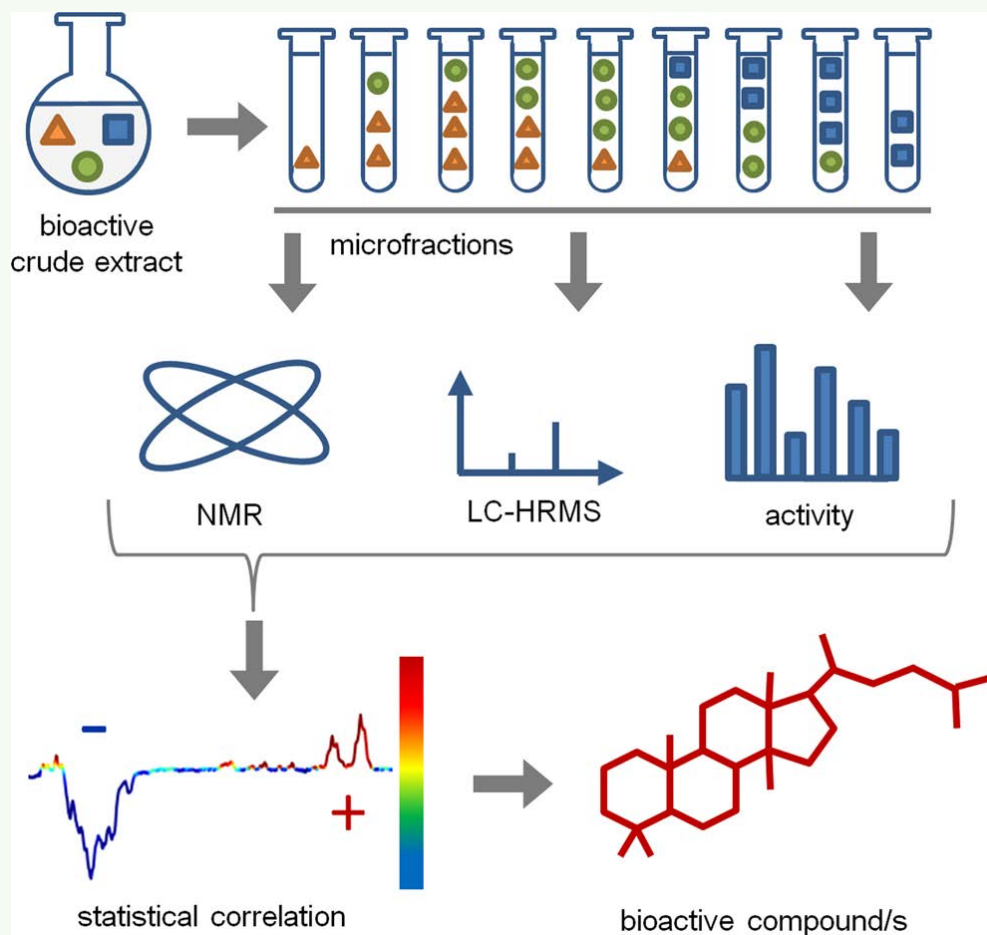


Identification and quantification of bioactive compounds





## What happens after extraction?



# Extraction of polyphenols from different plants/algae

- Knotweed
- Microalgae spirulina
- Onion skin



© chengyucheng/Getty Images

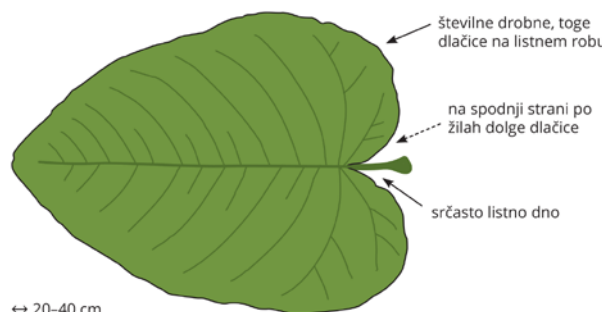


# What is knotweed?


- Knotweed is the member of the buckwheat family (Polygonaceae), it originates from Asia.
- It has hollow, upright, bamboo like stems, growing to 1 to 5 meters.
- It is listed by the World Conservation Union as one of the world's most invasive species.
- Three taxons:
  - Japanese knotweed (*Fallopia japonica*)
  - Sakhalin knotweed (*Fallopia sachalinensis*)
  - Bohemian knotweed (*Fallopia x bohemica*)

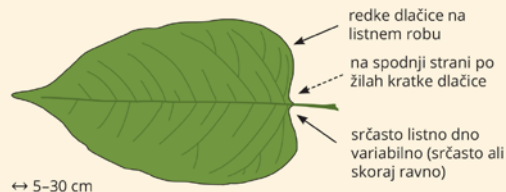
# Three different taxons

Sahalinski dresnik (*Fallopia sachalinensis*)  



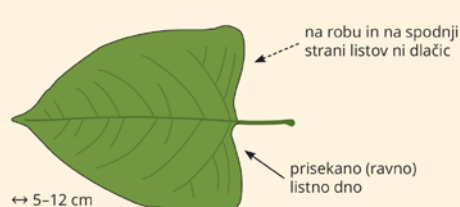
*Fallopia  
sachalinensis*

Češki dresnik (*Fallopia x bohemica*) 



*Fallopia x  
bohemica*

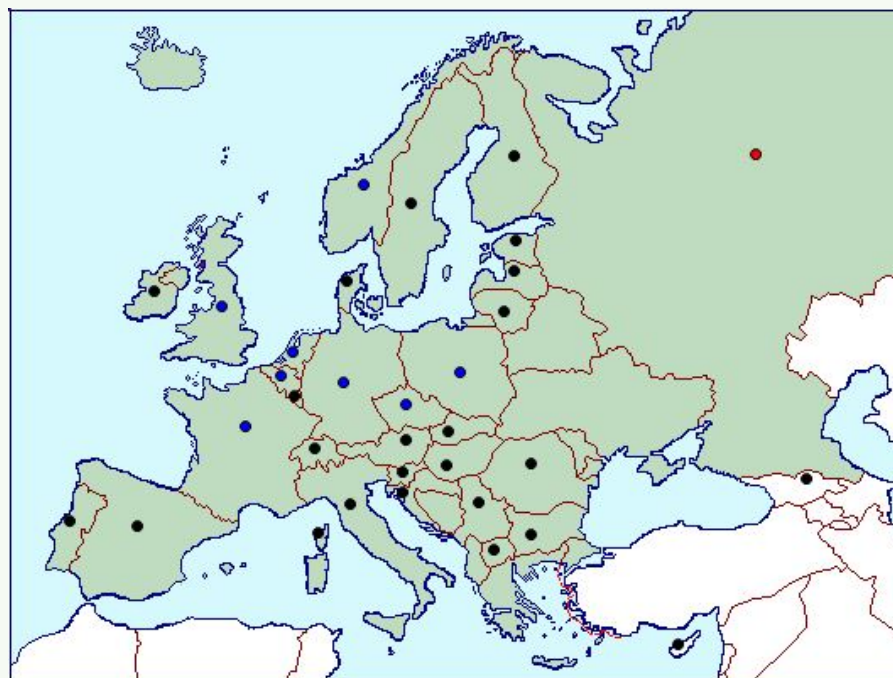
Japonski dresnik (*Fallopia japonica*) 



*Fallopia  
japonica*



## Distribution of knotweed in Europe



- = Present, no further details  
 ● = Widespread  
 ● = Localised  
 ● = Confined and subject to quarantine  
 ● = Occasional or few reports  
 ● = Evidence of pathogen  
 ● = Last reported  
 ● = Presence unconfirmed  
 ● = See regional map for distribution within the country

<http://www.cabi.org/isc/datasheet/23875#20057025705> (The Invasive Species Compendium (ISC), January 2017)

## Why is knotweed good?

- It is a good source of vitamins A and C, manganese, zinc, potassium, and a number of phenolic compounds.
- According to the study Burns et al. (2002) the Itadori tea is a good dietary source of resveratrol and a suitable replacement for red wine. People, who avoid wine because of alcohol, may benefit from resveratrol by drinking.





# Knotweed is a very good source of antioxidants

## 5 Amazing Food Sources of RESVERATROL



Red Grapes



Itadori Tea



Peanuts



Dark Chocolate



Blueberries

Resveratrol is a powerful antioxidant with benefits for muscle strength, anti-inflammatories, metabolism, neurodegenerative diseases, diabetes, cardiovascular disease and even cancer.

## Knotweed as a source of antioxidants

- Resveratrol, which is isolated from the roots of Japanese knotweed, is already often used as a dietary supplement, in cosmetics and medicine
- Young shoots contain similar components as the roots, but they are present in lower concentrations.
- Due to the large amounts of biomass that knotweed produces, its wide distribution, invasiveness, content of stilbenes and other metabolites they can represent an excellent source for the isolation of antioxidants.
- One hectare can produce between 20 and 30 tons of dry matter, which contains more than 80 kg stilbenes.





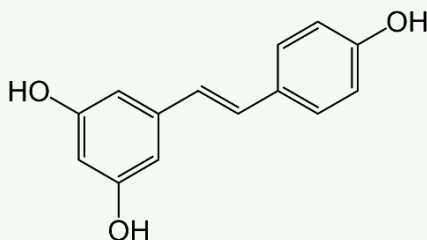
# Preparation of extracts



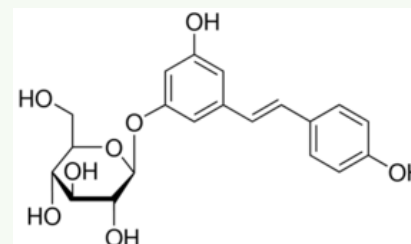
# Extraction

- 50% ethanol + 50% acidified water (2% acetic acid)
- Two-phase extraction
- Ultrasound extraction

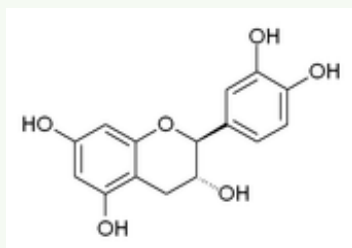
resveratrol



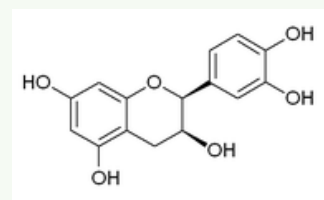
polydatin



catechin



epicatechin

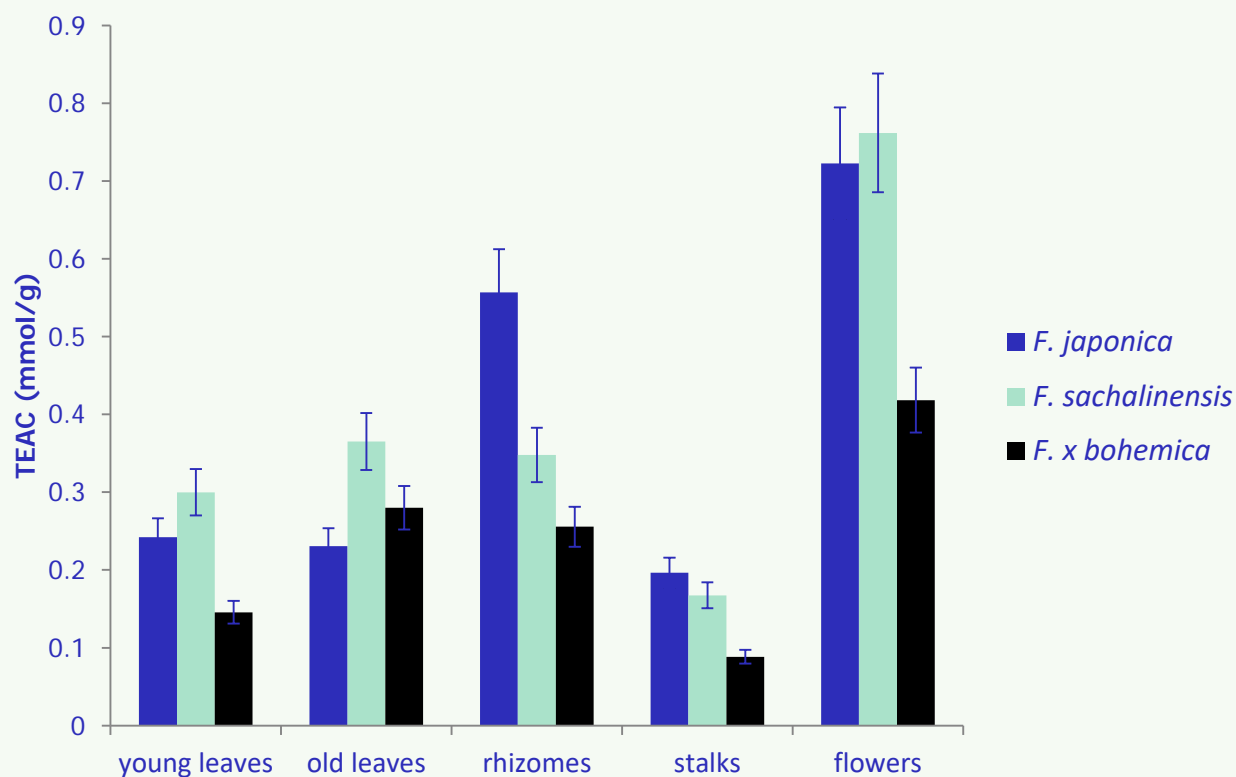




## Analyses

- Determination of antioxidant capacity (DPPH, ABTS, Folin–Ciocalteu, Chemiluminescence assay, cellular antioxidant capacity – yeast)
- Determination of prooxidant activity
- Identification and quantification of selected polyphenols (HPLC/DAD)
- Evaluation of anticancer and antidiabetic activity
- Determination of antimicrobial activity
- Lipid peroxidation studies

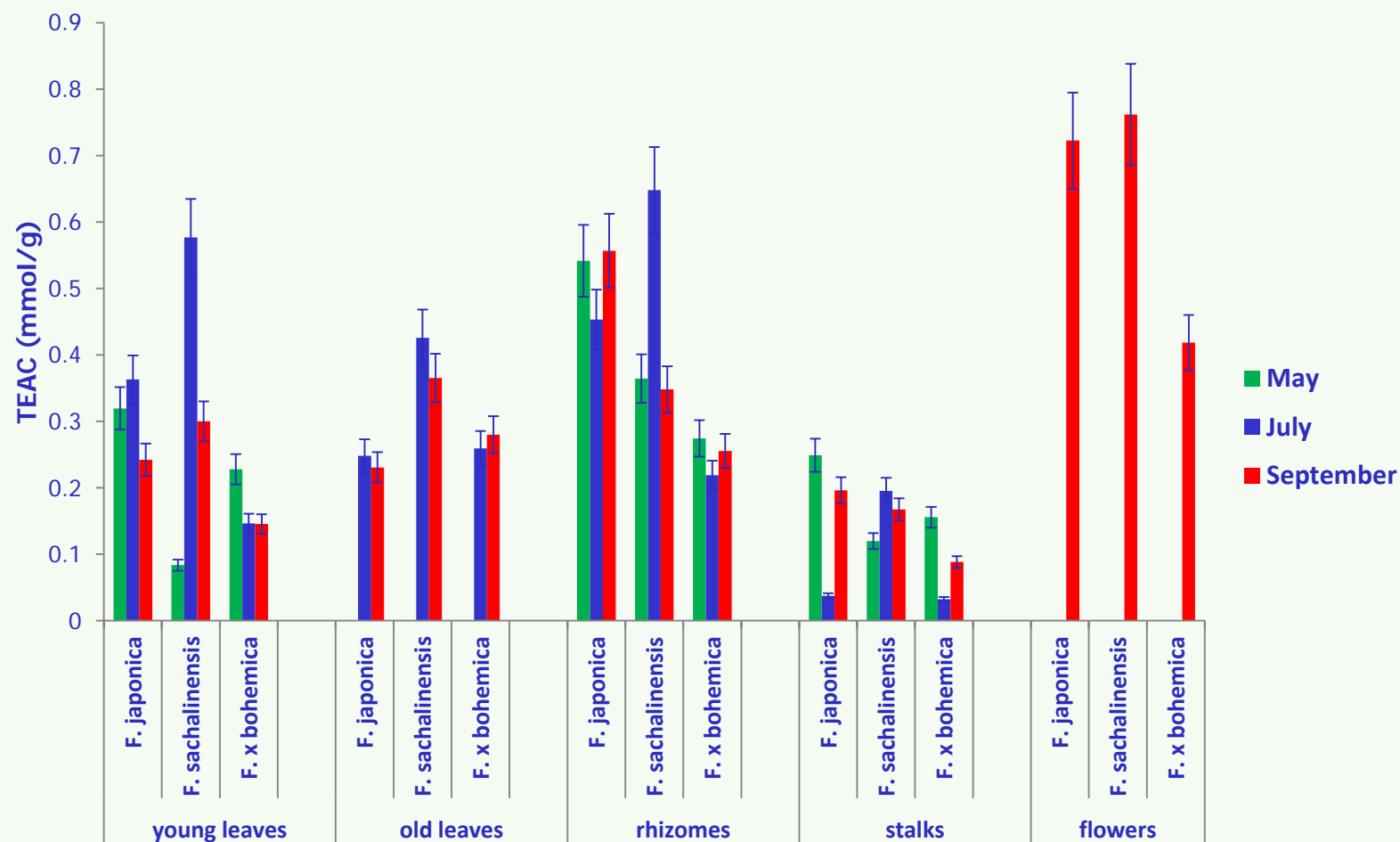
## AOC in different knotweed species



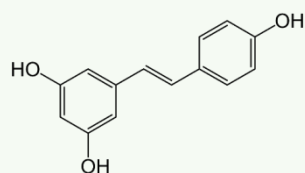
The results are expressed as averages of TEAC (trolox equivalent antioxidant capacity), determined by 4 methods (chemiluminescence, DPPH, ABTS, Folin-Ciocalteu)



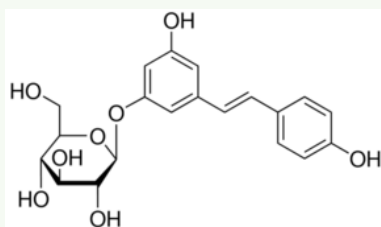
# Influence of picking season on overall AOC



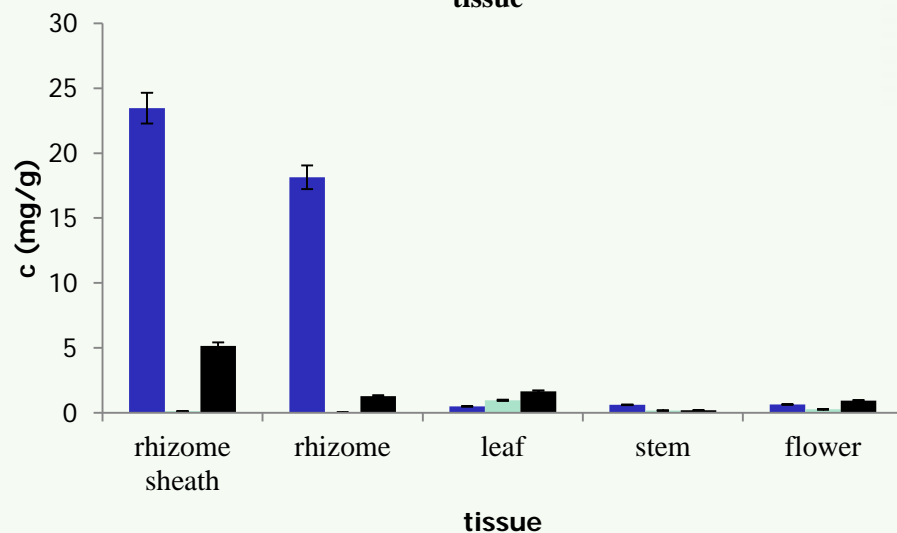
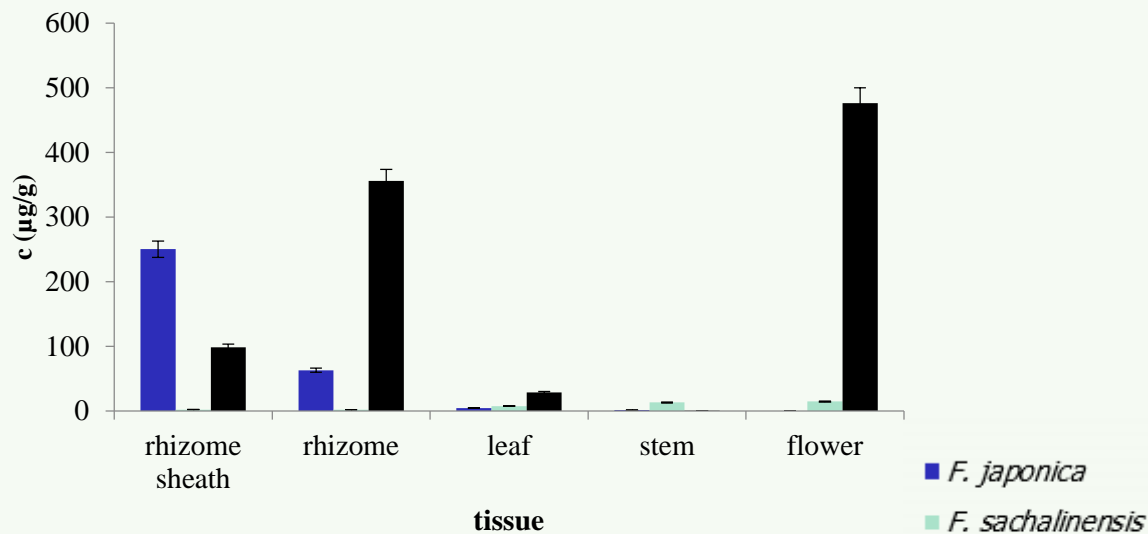
resveratrol



polydatin

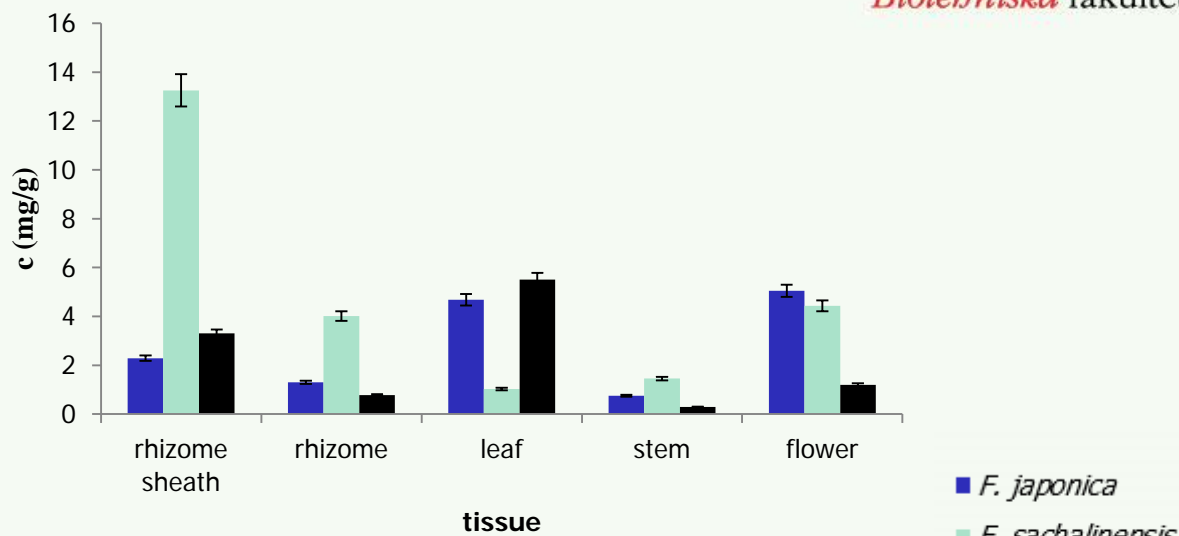
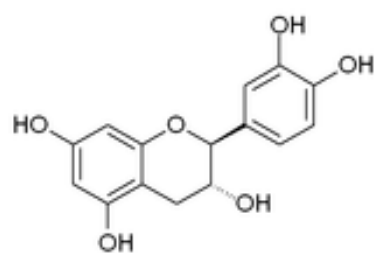


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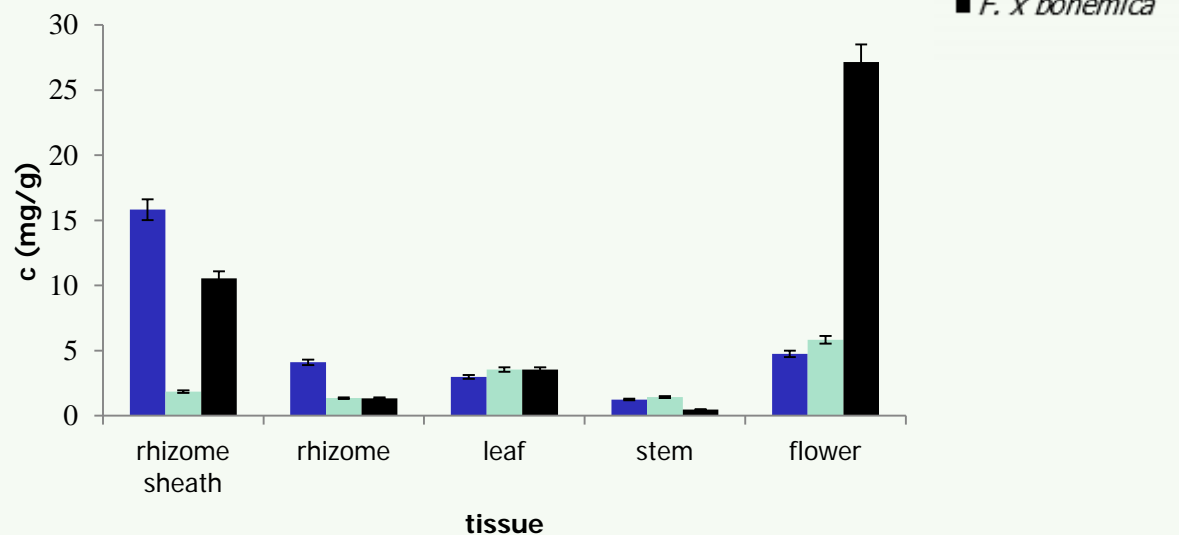
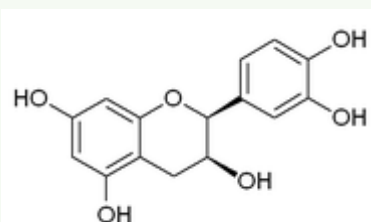




catechin



epicatechin



## Anticancer activity

### Method:

- Preparation of concentrated (100 mg/mL) ethanol extracts of rhizome and flowers from *F. japonica* and *F. x bohemica*

### Cell lines:

- Hek- non cancer kidney cell line
- HepG2 – liver cancer cell line
- HeLa – cervical cancer cell line
- PaTu – pancreatic cancer cell line



## Anticancer activity

Concentration [ $\mu\text{g/mL}$ ] that halved the viability ( $\text{IC}_{50}$ ) of cancer (PaTu, HeLa, HepG2) and control (HEK 293T cell line).

	rhizome extract		flower extract	
	F. japonica	F. x bohemica	F. japonica	F. x bohemica
HEK 293T	$36.7 \pm 4.9^{\text{ab}}$	$24.9 \pm 7.6^{\text{a}}$	$92.4 \pm 8.5^{\text{c}}$	$54.7 \pm 8.2^{\text{b}}$
PaTu	$182.2 \pm 48.5^{\text{a}}$	$228.0 \pm 37.3^{\text{a}}$	$430.6 \pm 135.8^{\text{b}}$	$311.7 \pm 58.9^{\text{ab}}$
HeLa	$36.9 \pm 4.2^{\text{a}}$	$45.8 \pm 7.0^{\text{a}}$	$90.2 \pm 13.9^{\text{b}}$	$59.0 \pm 6.1^{\text{a}}$
HepG2	$63.0 \pm 9.0^{\text{a}}$	$63.7 \pm 7.6^{\text{a}}$	$277.3 \pm 41.9^{\text{c}}$	$174.7 \pm 26.9^{\text{b}}$

Hek – non-cancer kidney cell line

HepG2 – liver cancer cell line

HeLa – cervical cancer cell line

PaTu – pancreatic cancer cell line

# Antidiabetic activity

Concentration [ $\mu\text{g/mL}$ ] that halved the  $\alpha$ -amylase activity ( $\text{IC}_{50}$ ).

plant tissue	species	$\text{IC}_{50}$ [ $\mu\text{g/mL}$ ]
RHIZOME	F. japonica	$0.17 \pm 0.02^a$
	F. x bohemica	$6.5 \pm 2.5^b$
FLOWER	F. japonica	$8.2 \pm 0.4^{bc}$
	F. x bohemica	$25.1 \pm 13.3^c$



# Antimicrobial activity

## Method:

- Preparation of concentrated (600 mg/mL) ethanol extracts of rhizome sheaths
- Broth dilution method
- Determination of the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of antimicrobial substances

## Minimal inhibitory concentration (MIC) determination



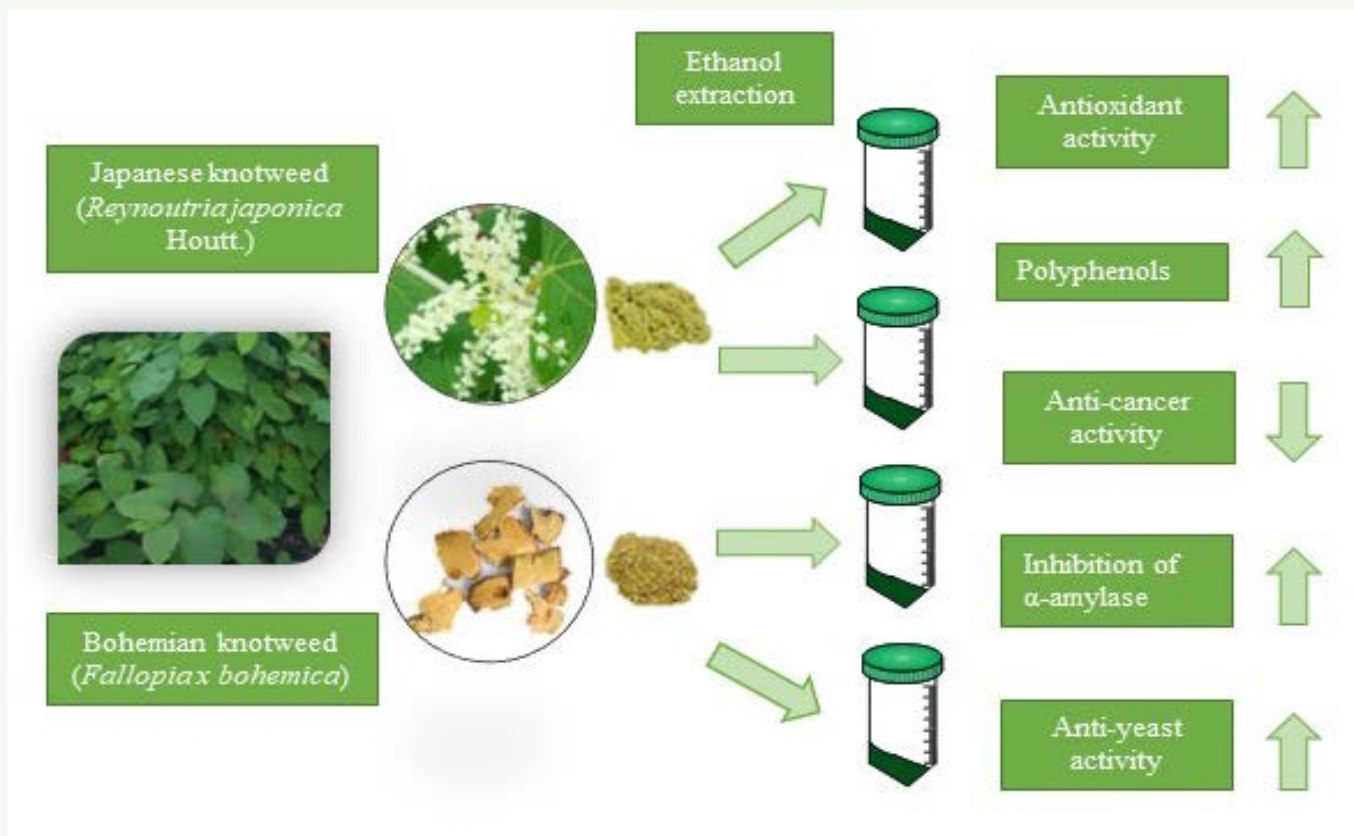
- 1-8: different concentrations of extracts
- 9-11: positive control (bacteria + colouring reagent INT)
- 12: negative control (no cells + colouring reagent INT)

## Antimicrobial activity - results

	<i>Escherichia Coli</i> ŽM370  gram-negative bacteria		<i>Listeria Monocytogenes</i> ŽM509  gram-positive bacteria		<i>Candida Albicans</i> ZIM2202  fungi	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
<i>F. japonica</i>	19	75	-	12	75	-
<i>F. sachalinensis</i>	19	-	-	23	37	-
<i>F. x bohemia</i>	19	-	-	23	75	-

minimal inhibitory concentration (MIC)  
 minimal bactericidal concentration (MBC)







Article

# In Vitro Comparison of the Bioactivities of Japanese and Bohemian Knotweed Ethanol Extracts

Lea Pogačnik <sup>1,\*</sup> , Tina Bergant <sup>1</sup>, Mihaela Skrt <sup>1</sup>, Nataša Poklar Ulrih <sup>1,2</sup> , Jitka Viktorová <sup>3</sup> and Tomáš Ruml <sup>3</sup>

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<sup>2</sup> The Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins, 1000 Ljubljana, Slovenia

<sup>3</sup> Department of Biochemistry and Microbiology, University of Chemistry and Technology Prague, Technická 3, Prague 6, Czech Republic; jitka.prokesova@vscht.cz (J.V.); tomas.ruml@vscht.cz (T.R.)

\* Correspondence: lea.pogacnik@bf.uni-lj.si; Tel.: +386-1-3203781

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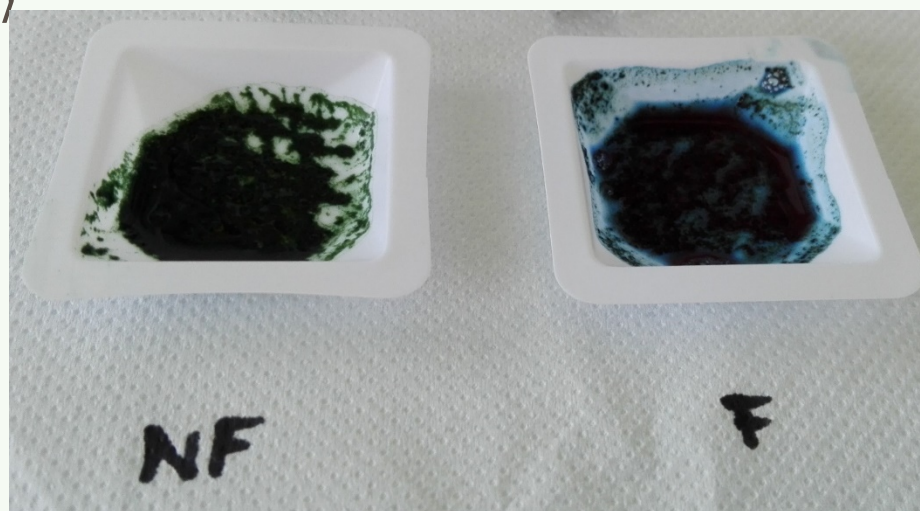
<https://www.mdpi.com/2304-8158/9/5/544>

# Preparation and characterisation of cianobacteria species *Arthrospira platensis* (*spirulina*) extracts before and after the lactic acid fermentation

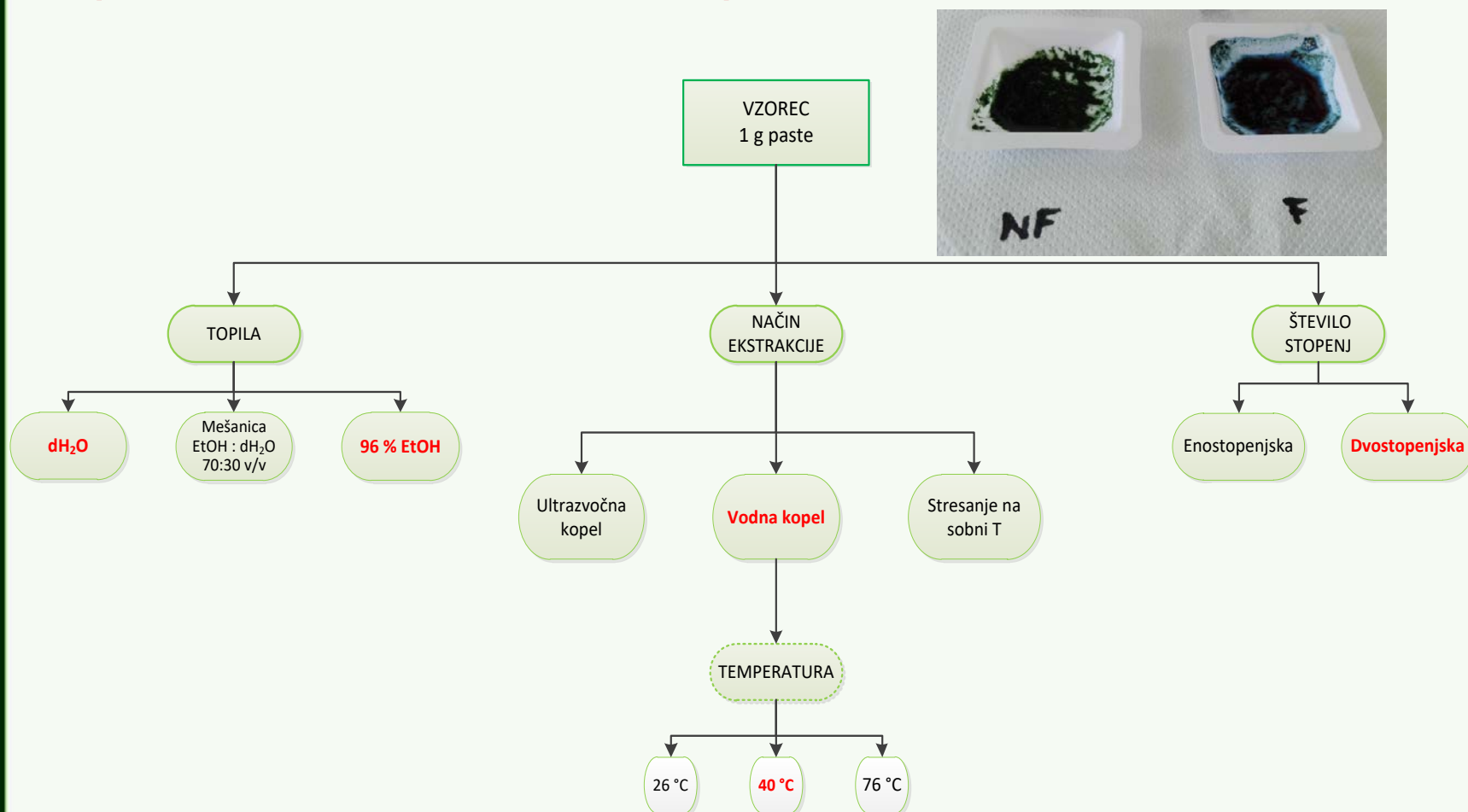


## Materials

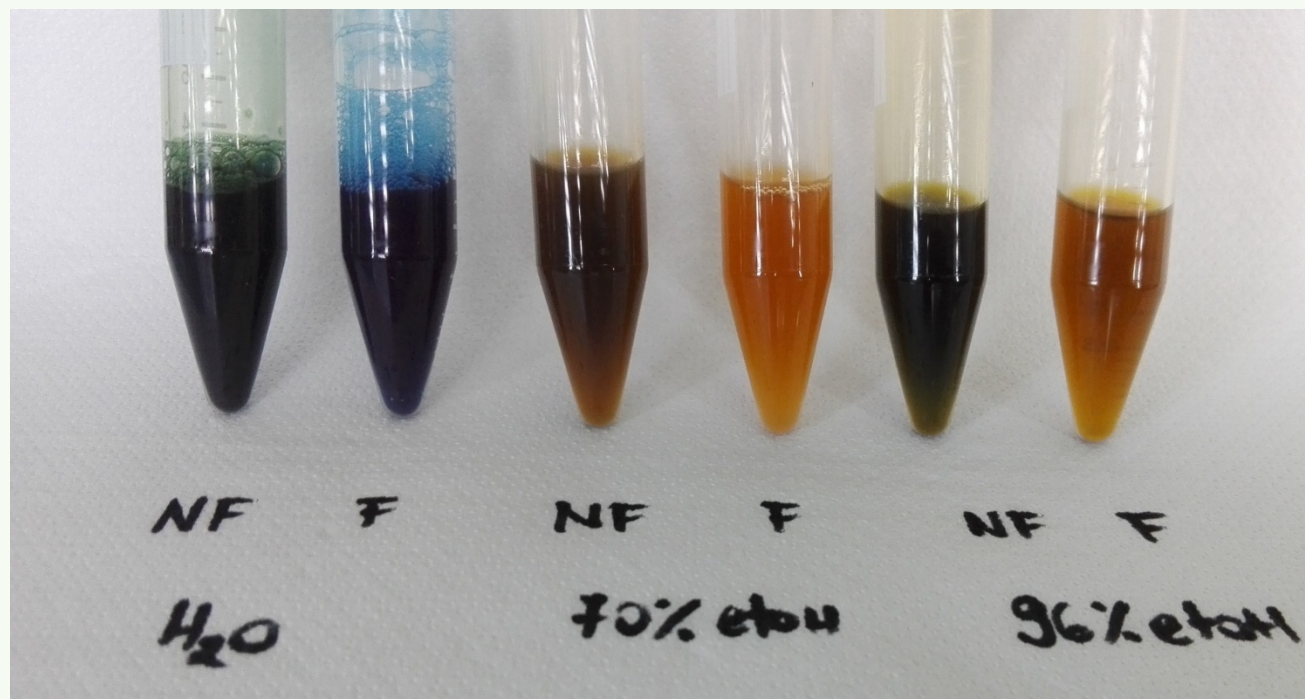
- PASTA (10 g spirulina + 10 mL physiological solution)
- MIKROORGANISMS
  - *Lactobacillus plantarum*
  - *Lactobacillus brevis*
- Fermentation time
  - Not fermented (NF)
  - Fermented (F):
    - 0 h, 24 h, 48 h, 72 h



# Optimisation of extraction procedure

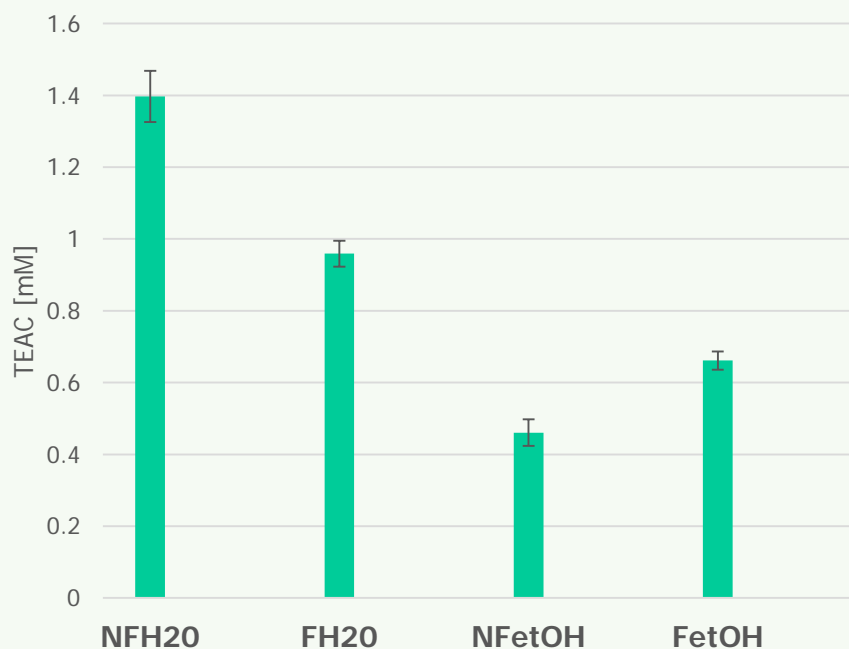


## Extracts





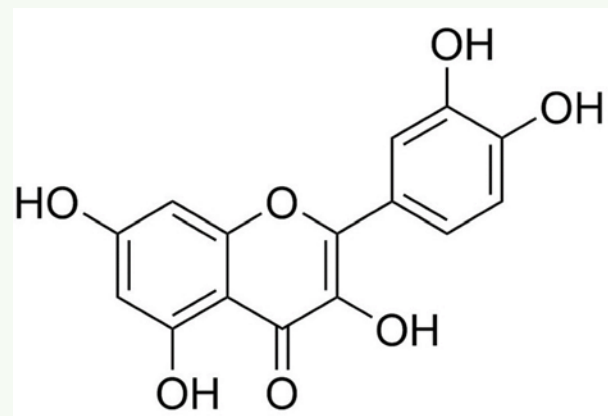
## Antioxidant capacity of non-fermented (NF) and fermented (F) Spirulina extracts



H2O – water extracts  
etOH – ethanol extracts

Rezultati so povprečja vseh pogojev (biomasa, čas fermentacije, mikroorganizem)

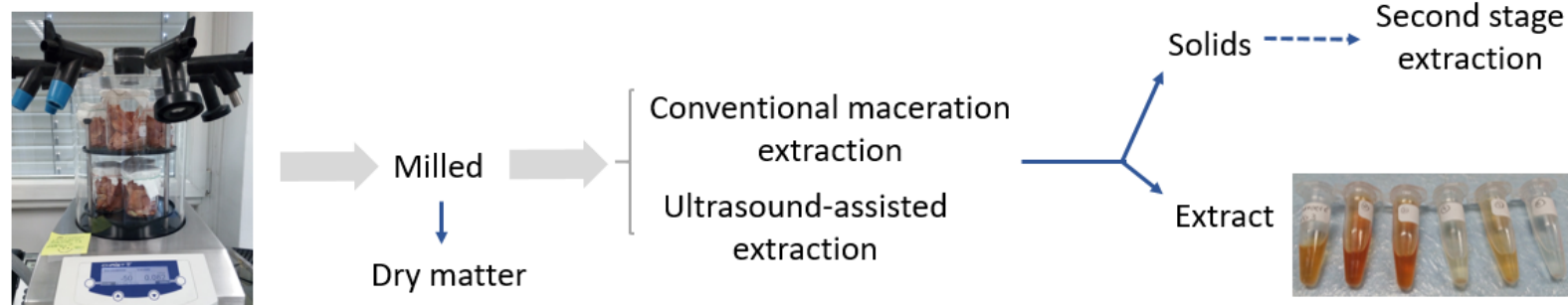
# Enhanced yield of bioactivities from onion (*Allium cepa* L.) skin and their antioxidant and anti- $\alpha$ -amylase activities



quercetin

# Postopek ekstrakcije

## 2.1. Extraction conditions



Freeze-drying

### Solvent

- 2% acetic acid
- 50% ethanol
- 70% ethanol
- 100% ethanol
- 100% methanol
- 100% ethyl acetate

### Mass-to-liquid ratio

- 1:10
- 1:20
- 1:50
- 1:100

### Time

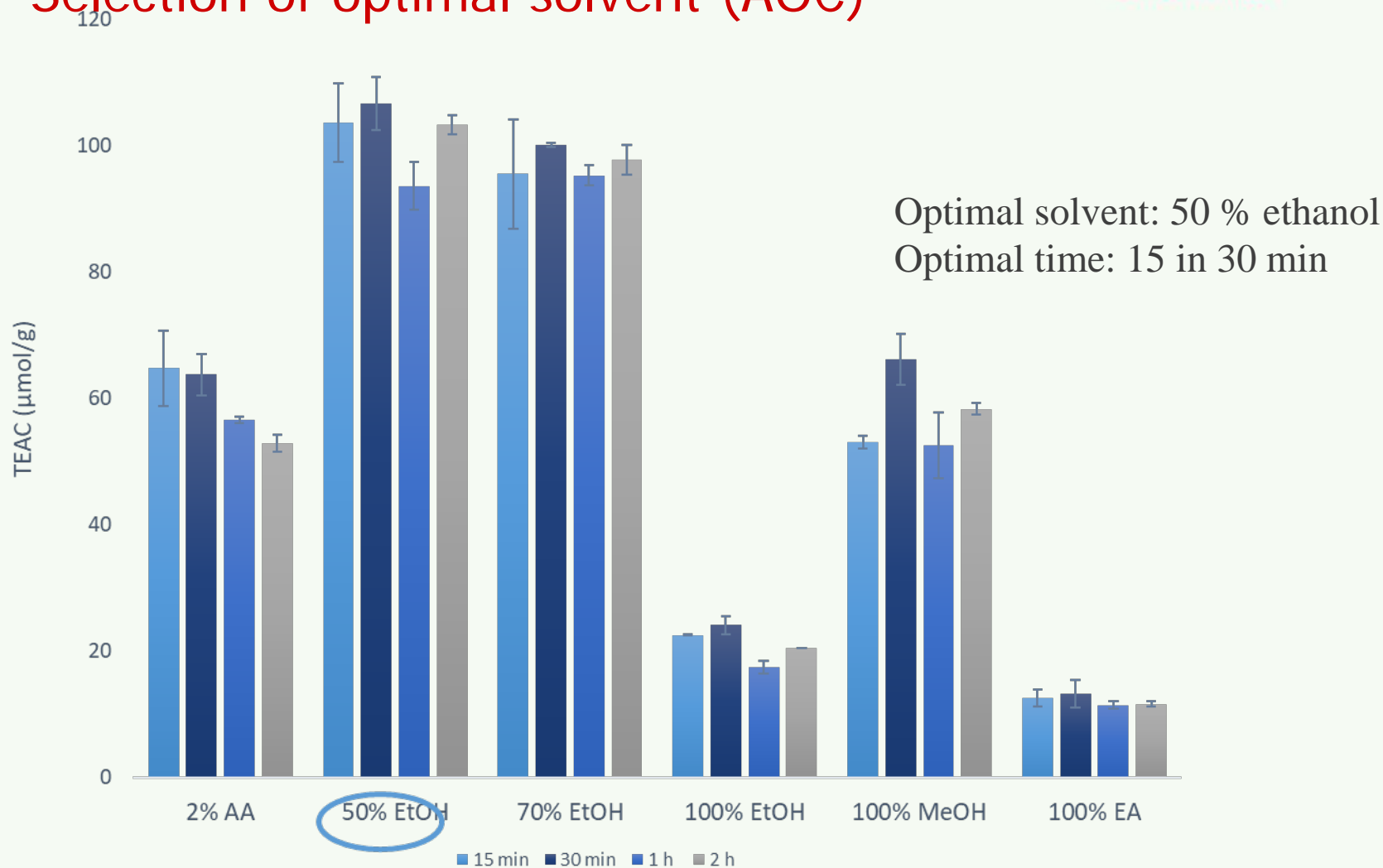
- 15 min
- 30 min
- 1 h
- 2 h

### Temperature

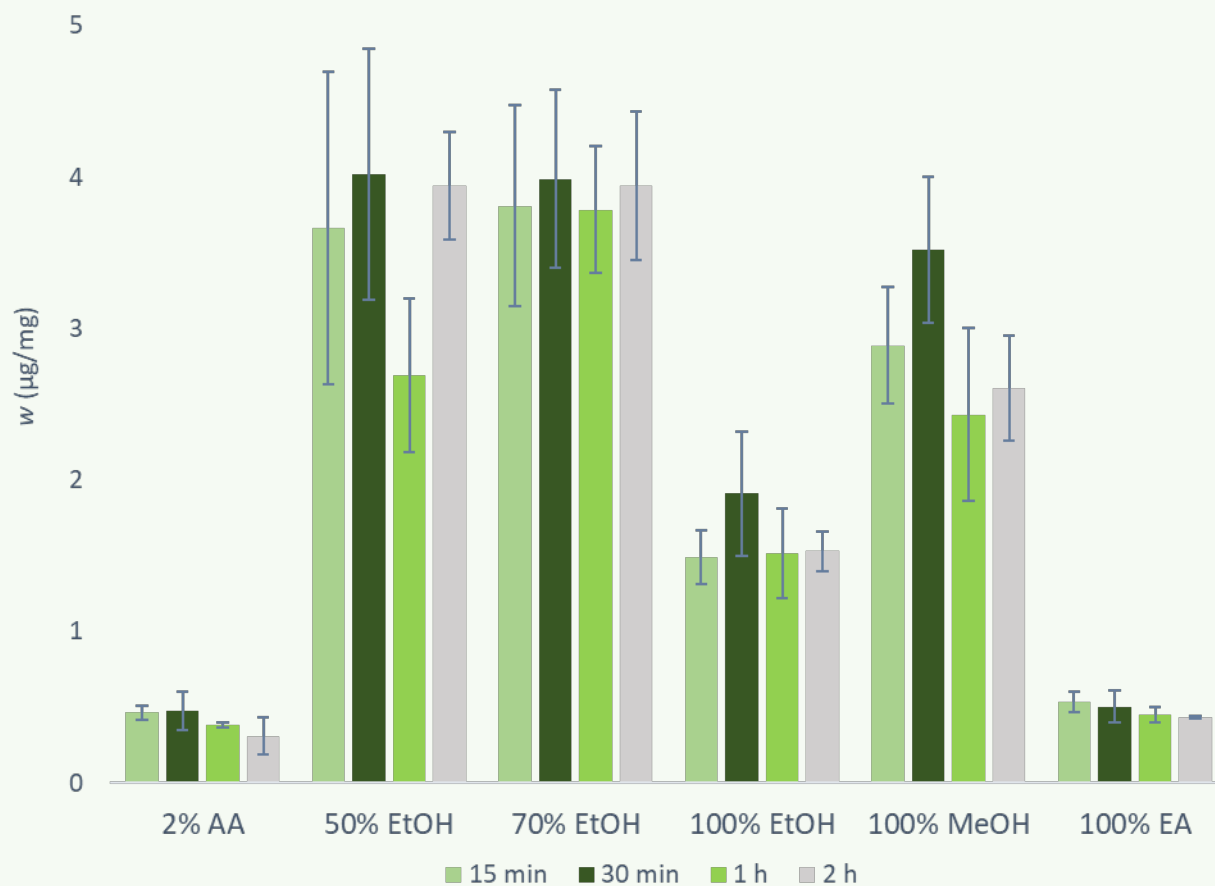
- 25 °C
- 40 °C
- 60 °C



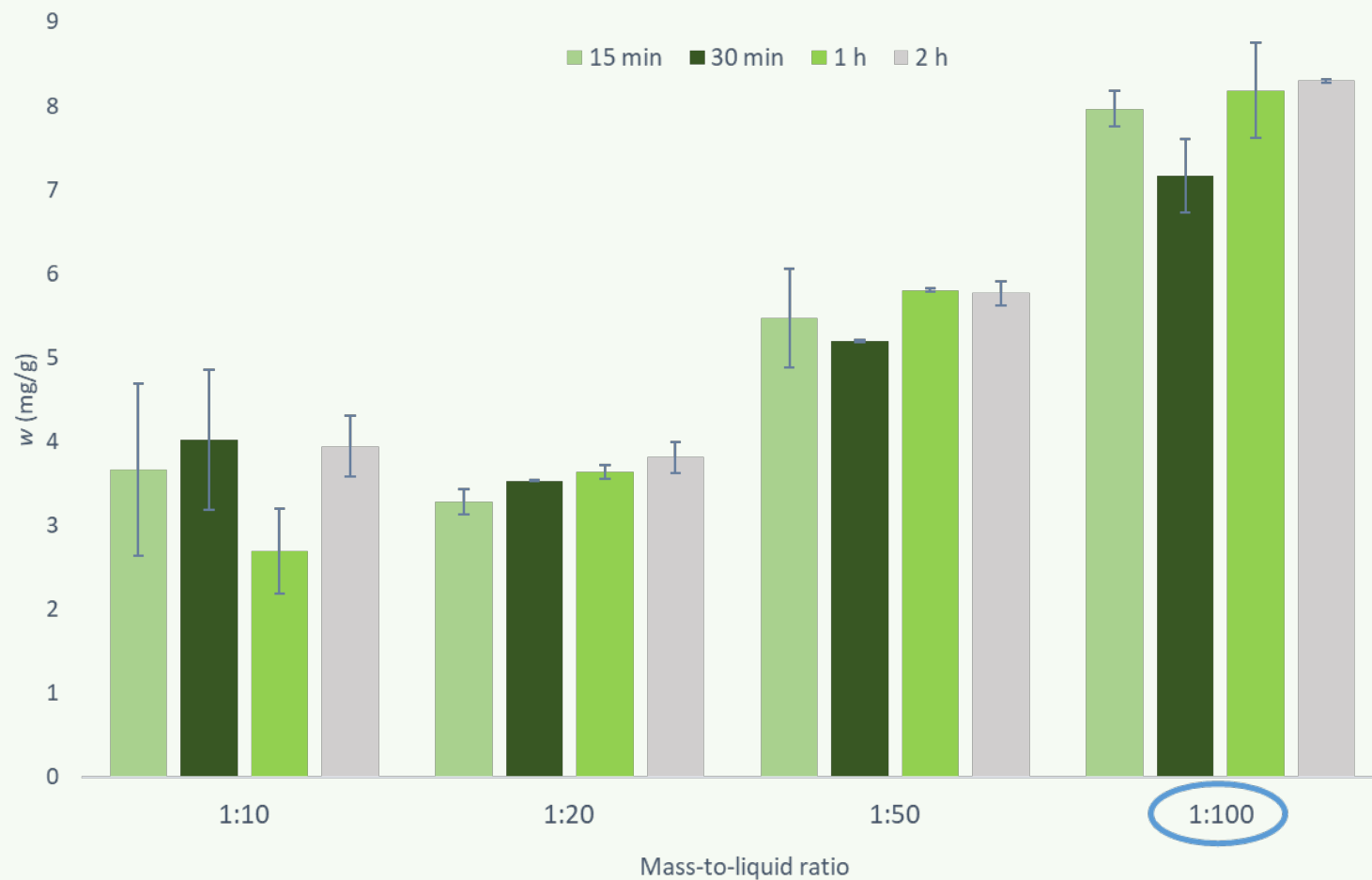
## Selection of optimal solvent (AOC)



## Selection of optimal solvent (quercetin)

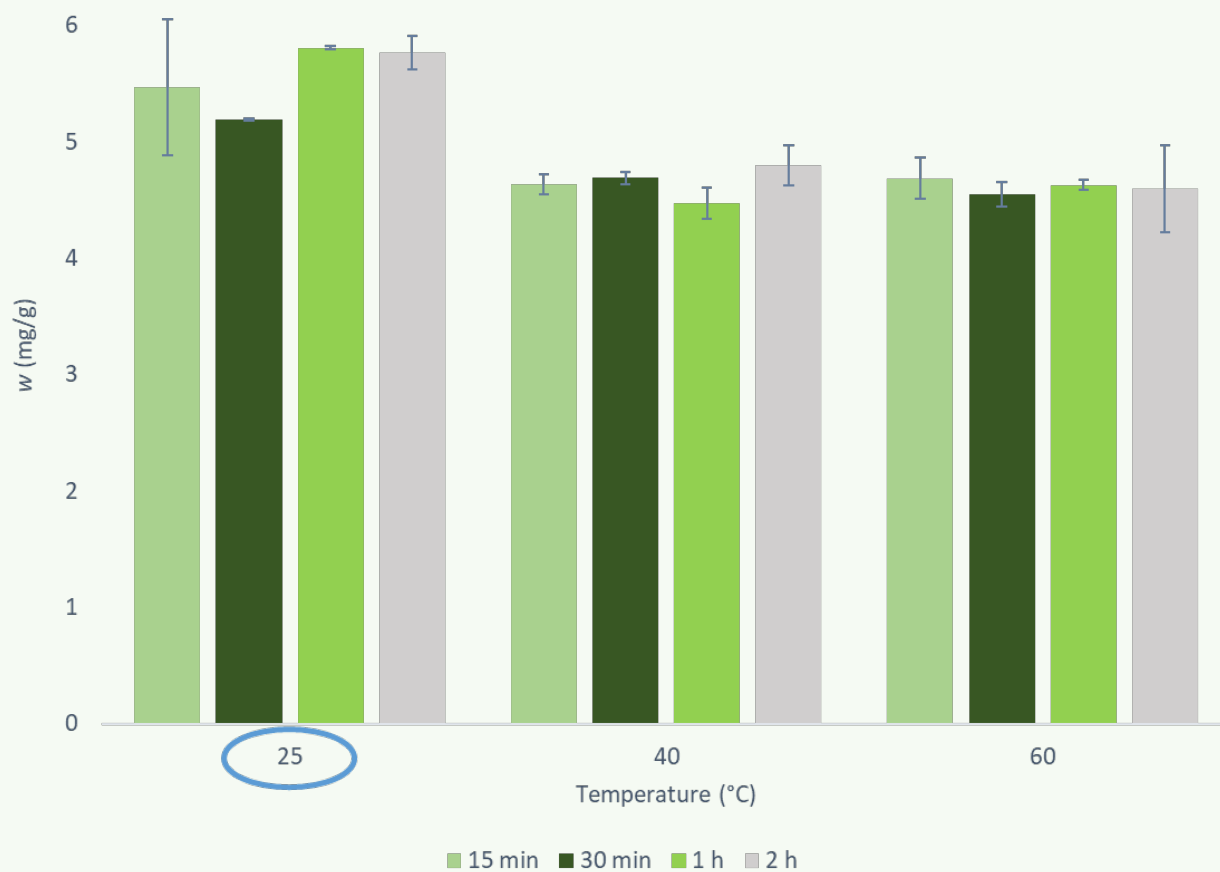


# Optimisation of mass-to-liquid ratio



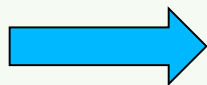


## Optimisation of temperature



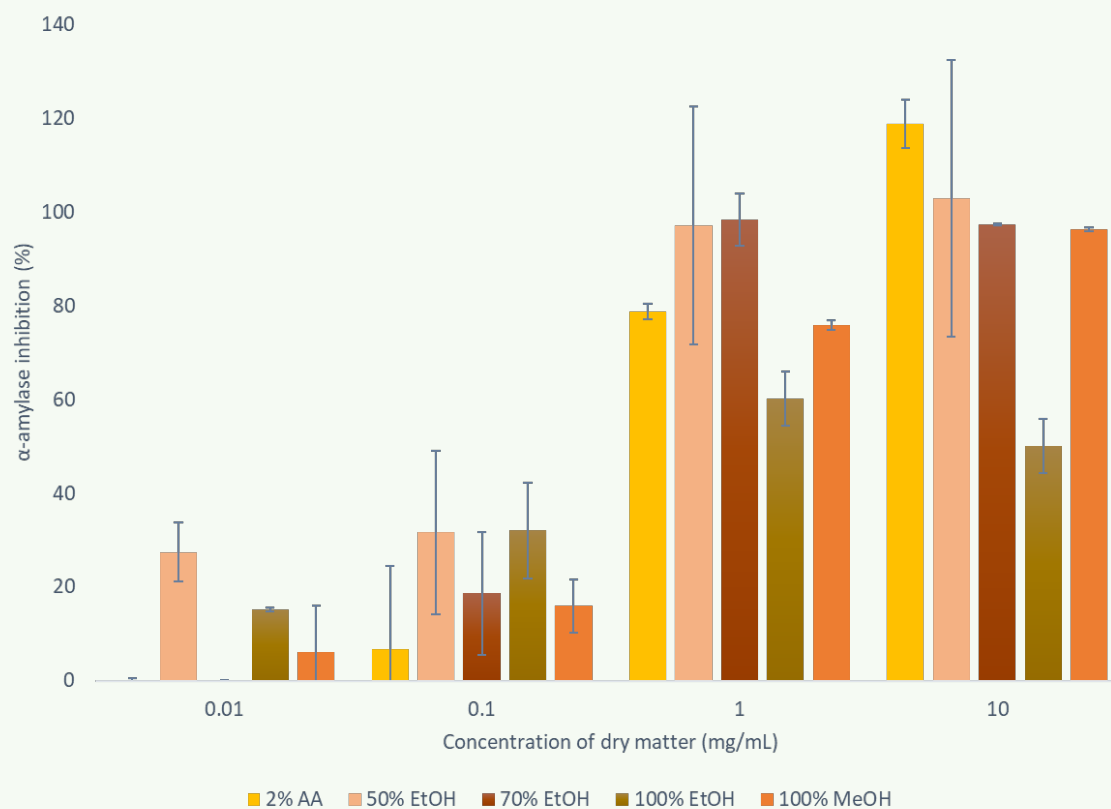
## Optimal extraction conditions

- 50 % ethanol
- 1:100 mass-to-liquid ratio
- 25 °C
- 15 min

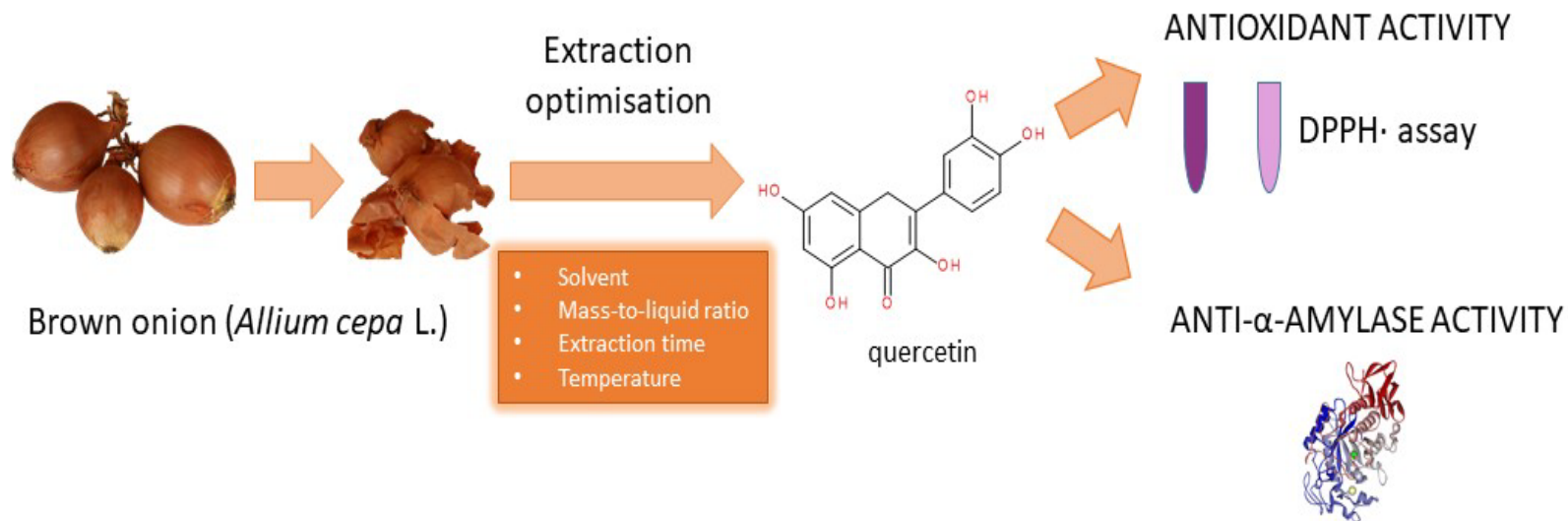


- $c_{\text{TEAC}}$  in dry matter = 104.5  $\mu\text{mol/g}$
- $w_{\text{quercetin}}$  = 7.96 mg/g

# Anti-diabetic activity (inhibition of $\alpha$ -amilase)









Article

# Enhanced Yield of Bioactivities from Onion (*Allium cepa* L.) Skin and Their Antioxidant and Anti- $\alpha$ -Amylase Activities

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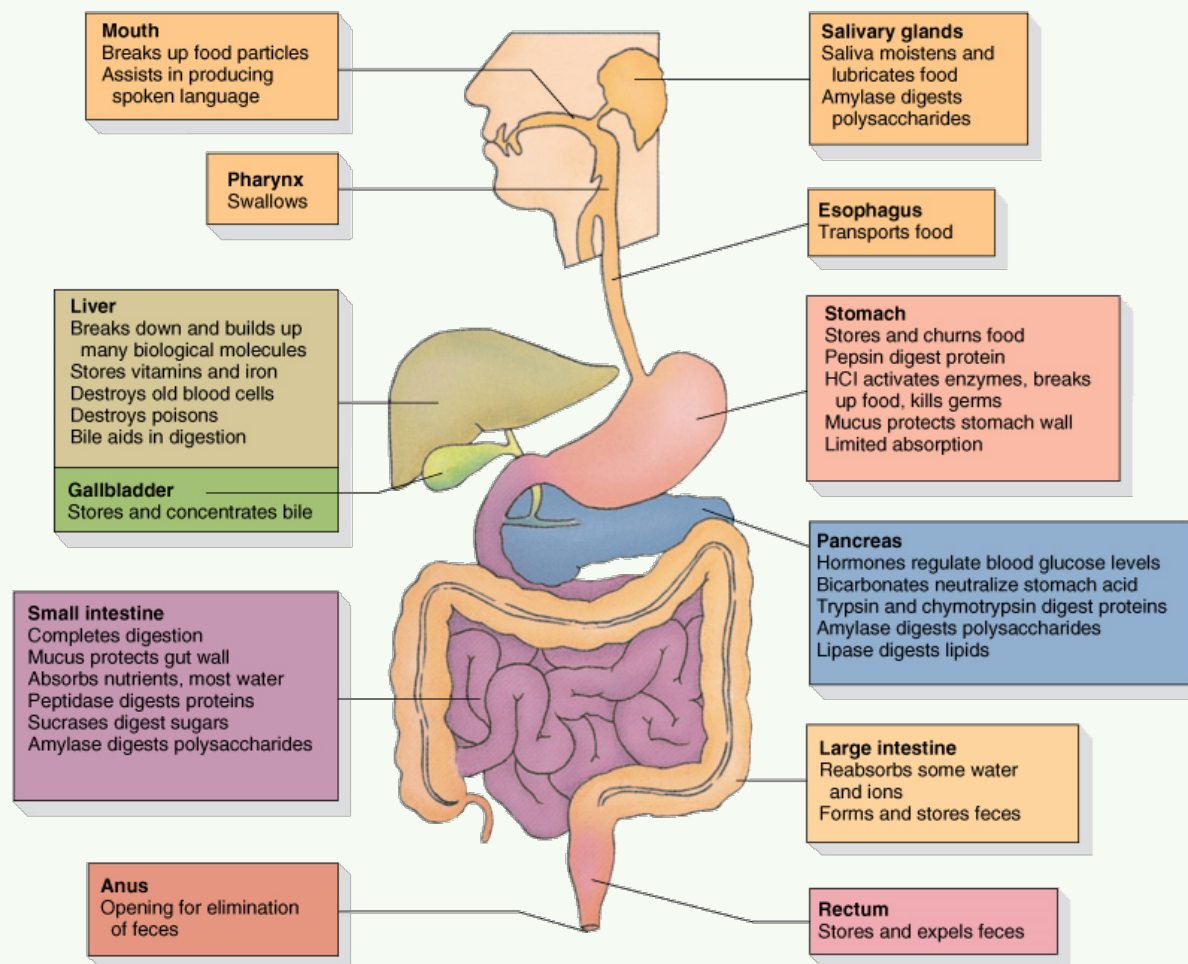
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<https://www.mdpi.com/1422-0067/21/8/2909>

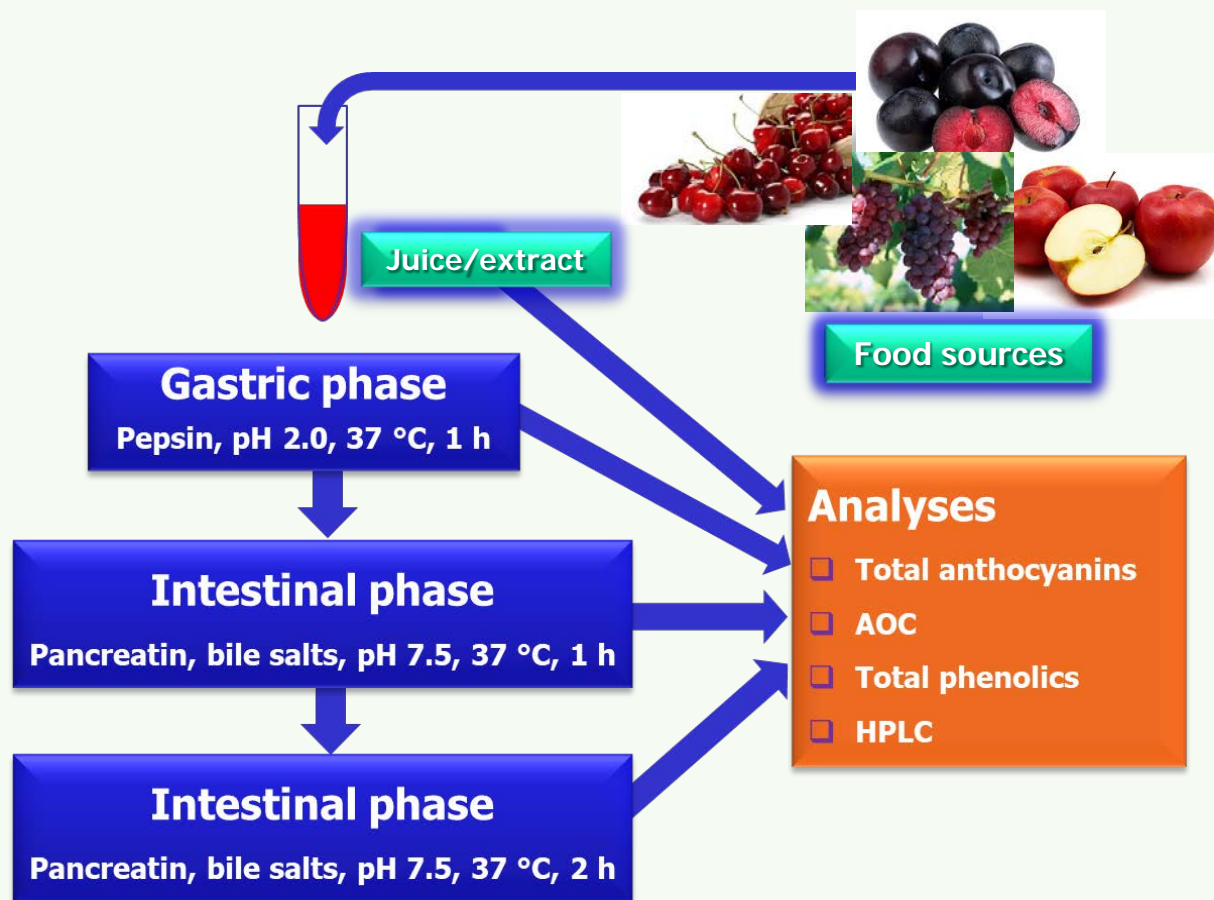
# Bioavailability of bioactive substances?

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# Digestion models *in vitro*



# Neuroprotection and brain availability of dietary polyphenols



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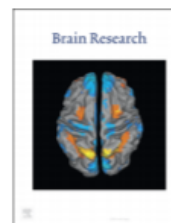
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### Research report

## Potential for brain accessibility and analysis of stability of selected flavonoids in relation to neuroprotection in vitro



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