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Budapest, May 21-24, 2023

Subcritical Water and Conventional Extraction of Phenolic compounds from Onion Skin Waste: Implications in Diabetes Disease.

E. Trigueros*,

Ó. Benito-Román, A.P. Oliveira, P.B. Andrade M.T. Sanz and S. Beltrán

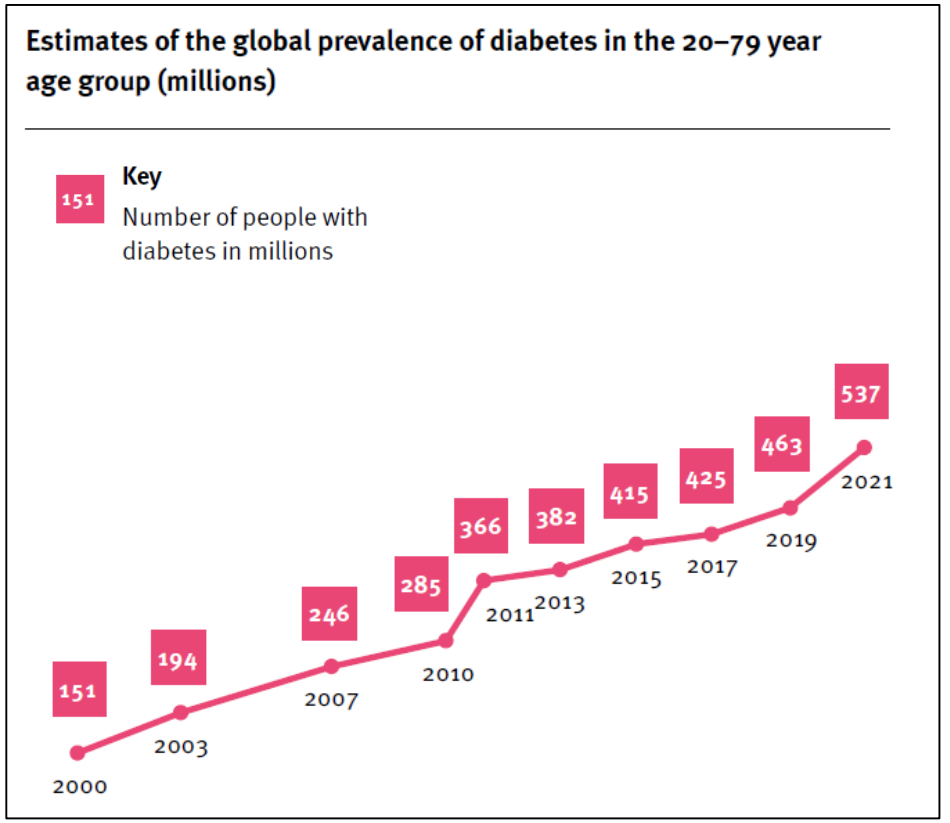
U.PORTO
FACULDADE DE FARMÁCIA
UNIVERSIDADE DO PORTO

LAQV requimte
LABORATÓRIO ASSOCIADO PARA A QUÍMICA VERDE

UNIVERSIDAD DE BURGOS

DIABETES

- One of the **most prevalent** diseases in the world.



(IDF Atlas, 10th Edition 2021)

DIABETES

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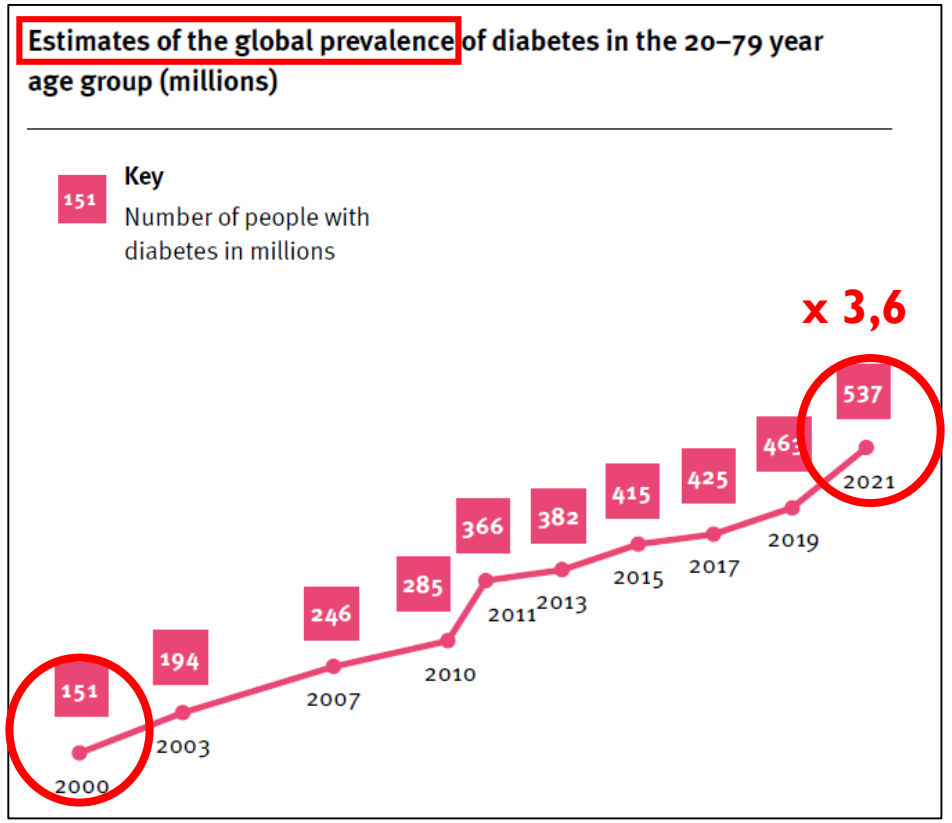

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Contextualization…

DIABETES

- One of the **most prevalent** diseases in the world.
- One of the **leading causes of death worldwide**.

DIABETES

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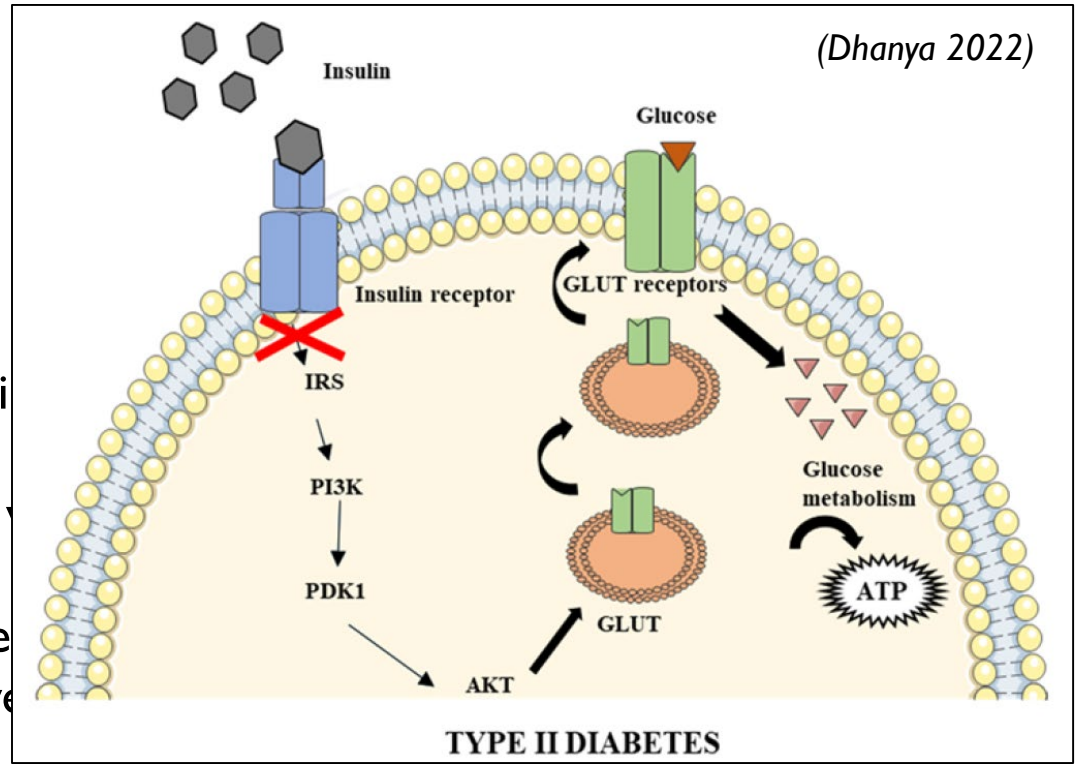
- One of the **leading causes of death worldwide**.
- **Multifactorial illness** characterized by dysregulation of proteins, lipids and carbohydrates metabolism and high levels of glucose in blood.

DIABETES

- One of the **most prevalent** diseases i
- One of the **leading causes of death**
- **Multifactorial illness** characterize carbohydrates metabolism and high leve
- **Three types**: diabetes type 1, diabetes type 2 (90%) and gestational diabetes.
- Non-insulin dependent or diabetes type 2 is caused by cells not being able to respond adequately to the action of the insulin, condition known as **insulin resistance**.



DIABETES

Treatment strategies for Diabetes type 2

Blood glucose levels CONTROL

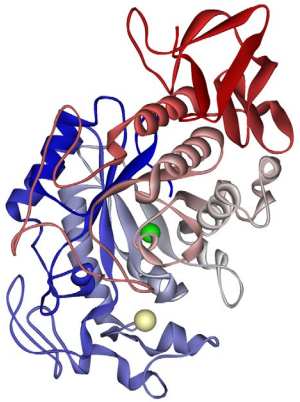
- Stimulating the secretion of **insulin**
  - → Medication
  - → Dietary control
- Interrupting/Slowing the **digestion of dietary carbohydrates** → Carbohydrate hydrolyzing enzymes ✗
  - ← Reduce the absorption of blood sugar

DIABETES

Treatment strategies for Diabetes type 2

Enzymes responsible for the carbohydrate's digestion:

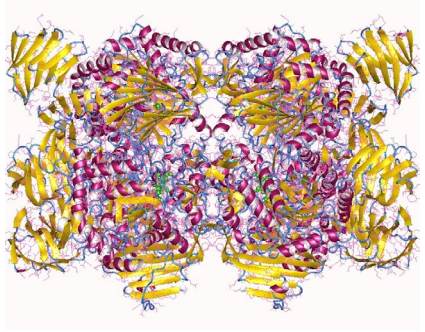
α-amylase



Mouth and the **small intestine**.

Hydrolyze the **α-(1→4)-glycosidic linkages of starch**

α-glucosidase



Small intestine.

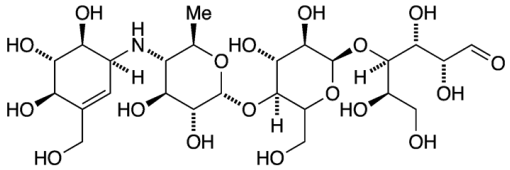
Hydrolyze **disaccharides** into monosaccharides

DIABETES

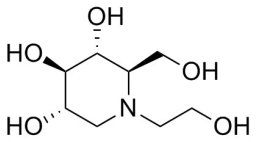
Treatment strategies for Diabetes type 2

Oral Antidiabetics

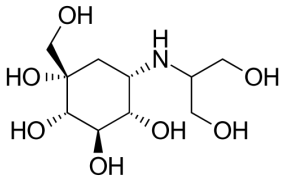
Acarbose

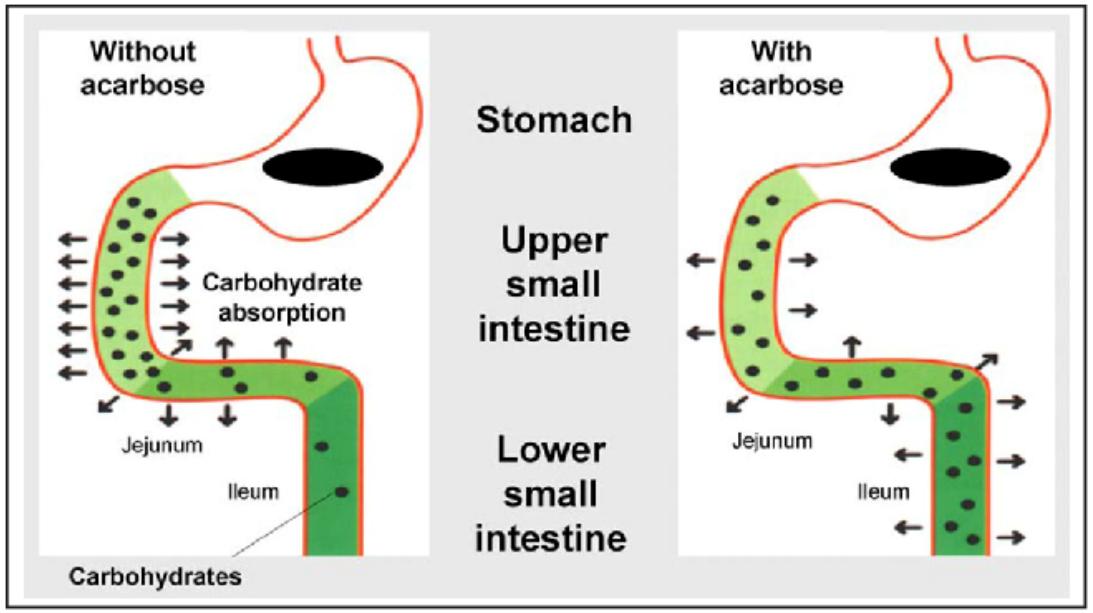


Miglitol



Voglibose





(He et al. 2014)

DIABETES

Treatment strategies for Diabetes type 2

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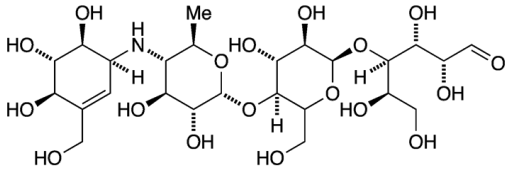

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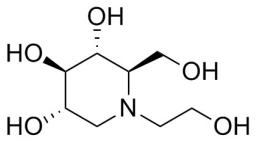

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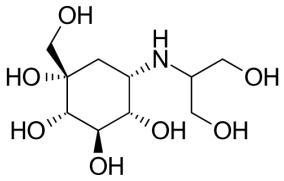


Strong α-amylase Inhibition

=

Many side effects !

- Digestive disorders
  (abd. distention, flatulence, diarrhoea…)
- Liver disorder

DIABETES

Treatment strategies for Diabetes type 2

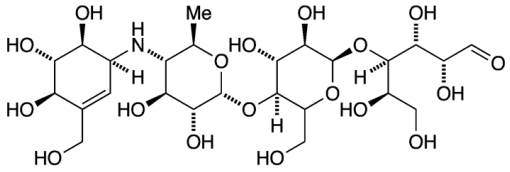
~~Oral Antidiabetics~~

Natural compounds

α-glucosidase

α-amylase

Acarbose



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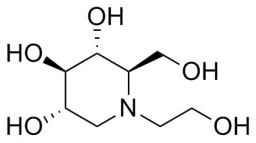

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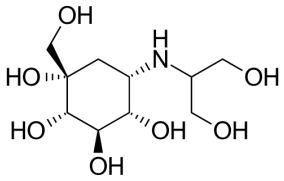

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SKIN ONION

Onion (*Allium cepa* L.)

Non-edible waste

0.6 million tons annually

Waste management: 40€/ton

Skin onion

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Non-edible waste

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Waste management: 40€/ton

Skin onion

Higher concentration of flavonoids:

- ++ Quercetin
- + Kaempferol
- + Protocatechuic acid
- +Quercetin glucosides
- …

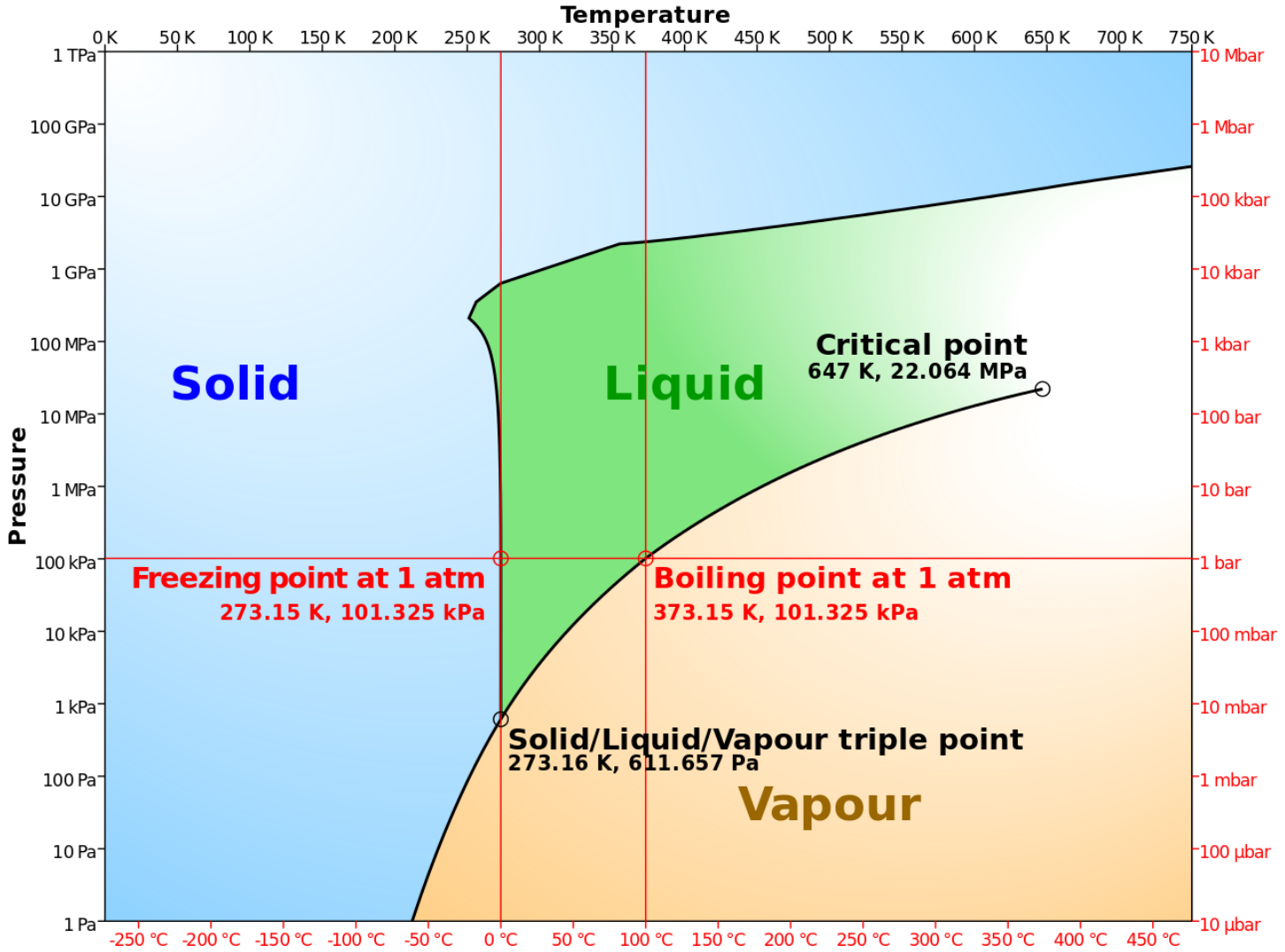
Antioxidant, anticáncer, antiobesity, **antidiabetes**, antibacterial, neuroprotective

Alternative to current pharmacology therapies

Safe solvents for human consumption !

SWE

Subcritical Water:



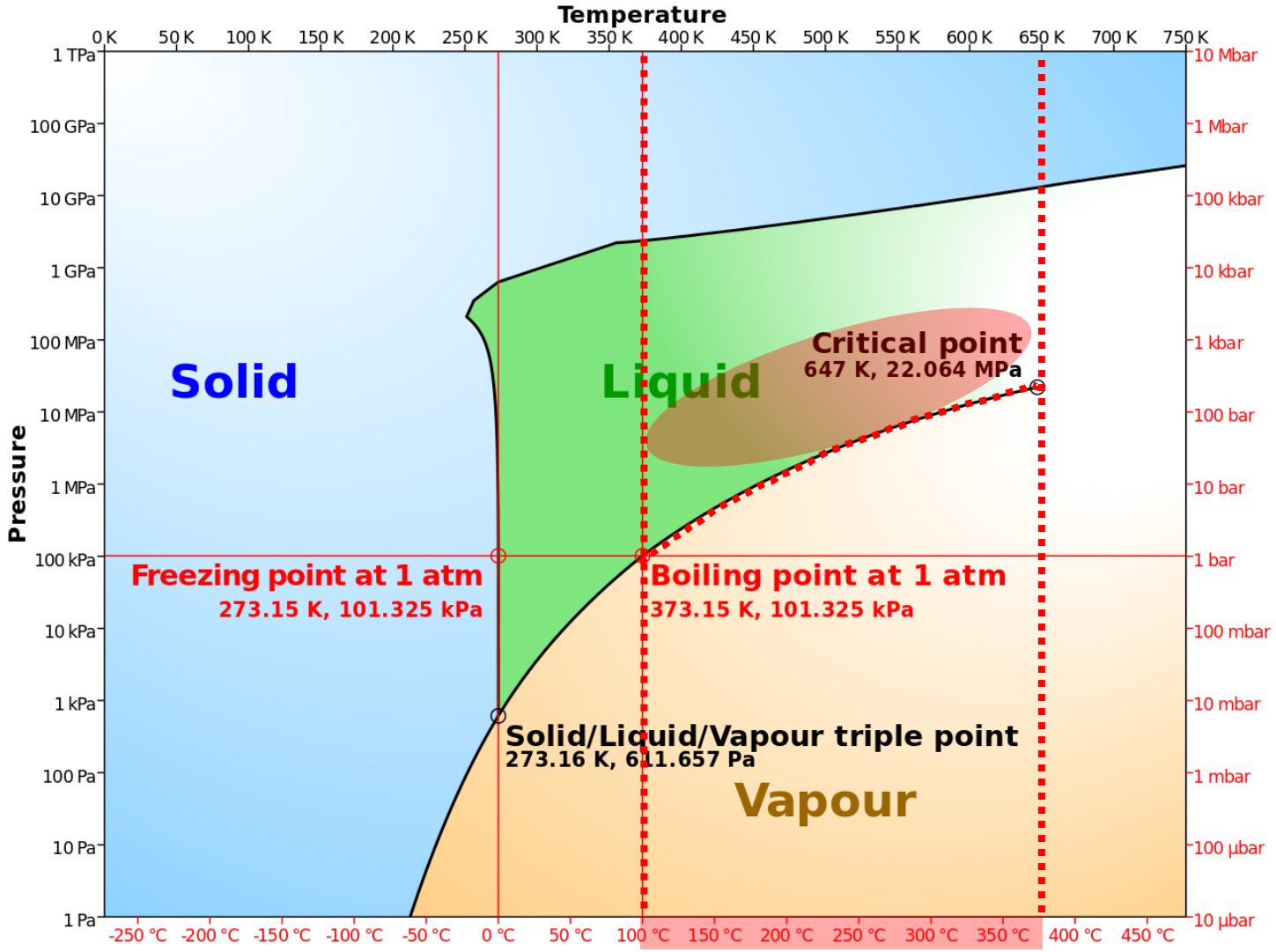
Adapted from (*Plaza & Turner et al. 2015*)

SWE

Subcritical Water:

Temperature
100 – 374°C

Pressure
> 50bar



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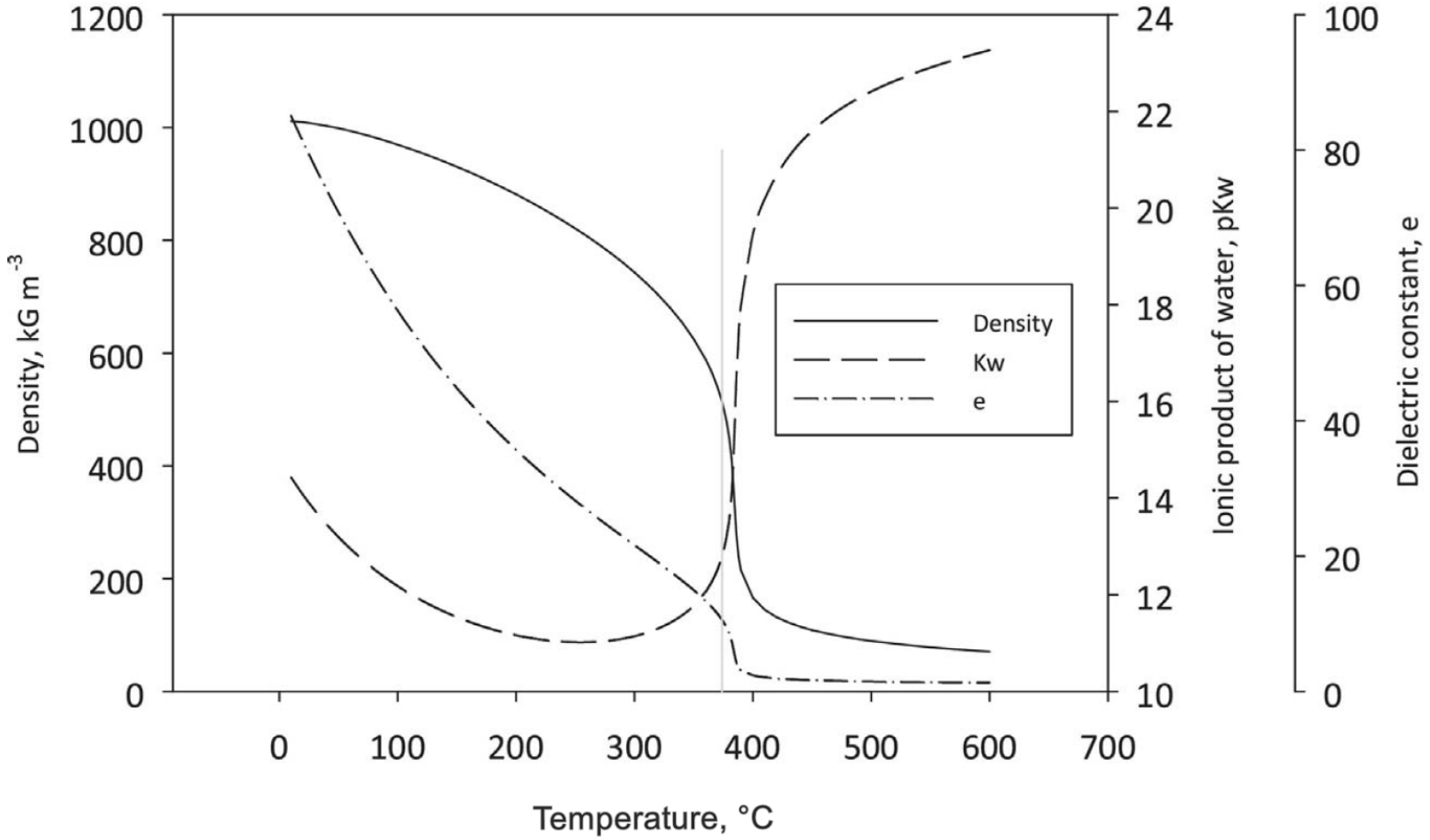
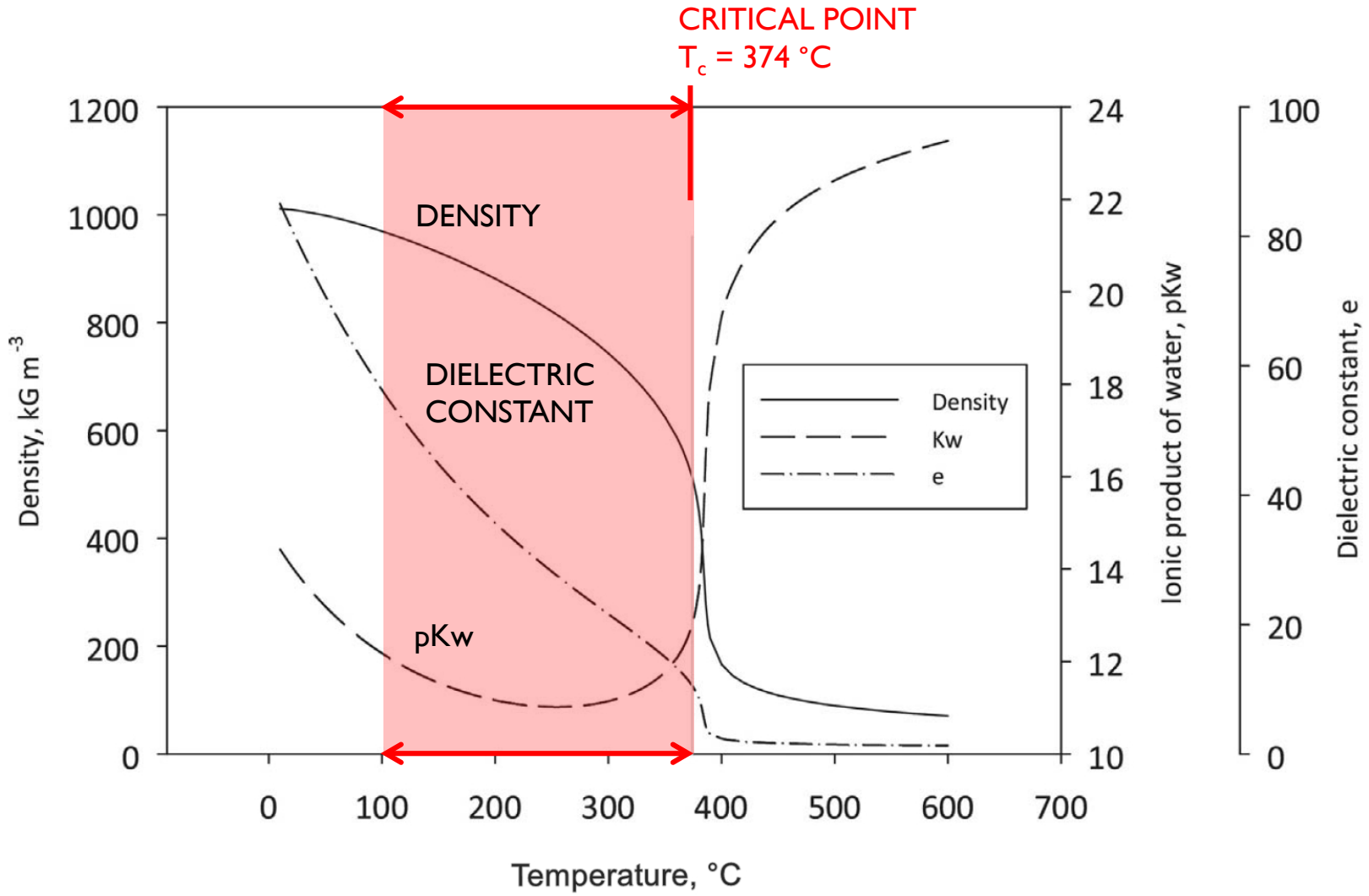


(Cocero et al. 2018)

SWE



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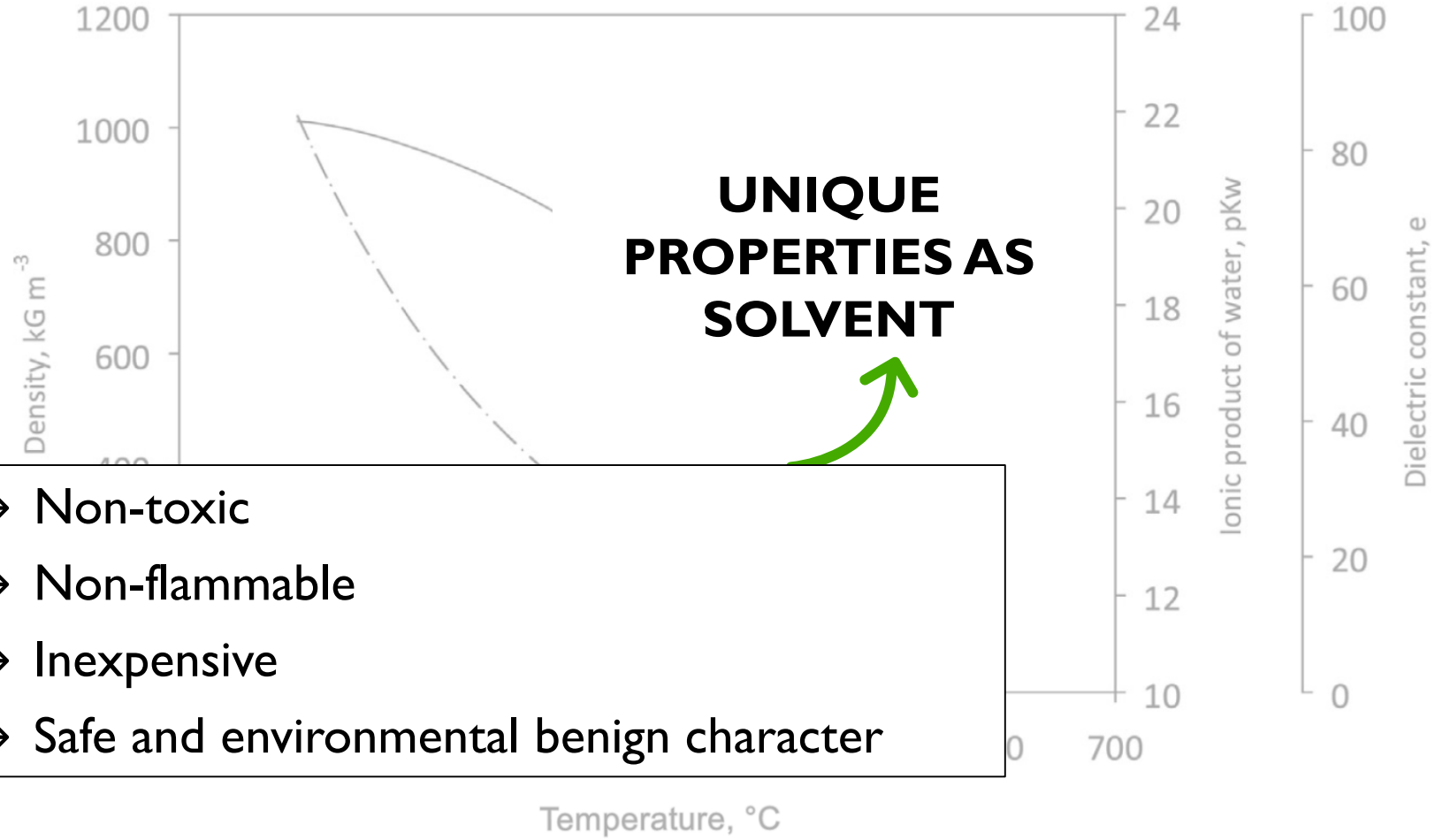


UNIQUE PROPERTIES AS SOLVENT

→ Non-toxic
→ Non-flammable
→ Inexpensive
→ Safe and environmental benign character

OBJECTIVES

- Study the **antidiabetic potential** of the **onion skin extract** obtained through **subcritical water extraction**.
- **Compare** the SWE extract with those obtained by **conventional extraction**.

EVALUATION OF
- Chemical composition
- Antidiabetic activity
- Antioxidant capacity

Onion (*Allium cepa* L.) skin wastes (OSW)

Cardeña desde 1925 food

- *Horcal*
- *Red*

→ Outermost skins

→ Drying (room temperature)

→ Milling (1mm)

Optimal conditions

(*Benito-Román et al. 2021*)

ethanol:water mixture (70%, v/v)

37°C, 60 min

Horcal Red

50g OSW + 400 mL solvent

Liquid

Solid

Rotary evaporator → etOH

Freeze-drying → H_2O

Dry-powder OSW
CE extract

Optimal conditions

(*Benito-Román et al. 2020*)

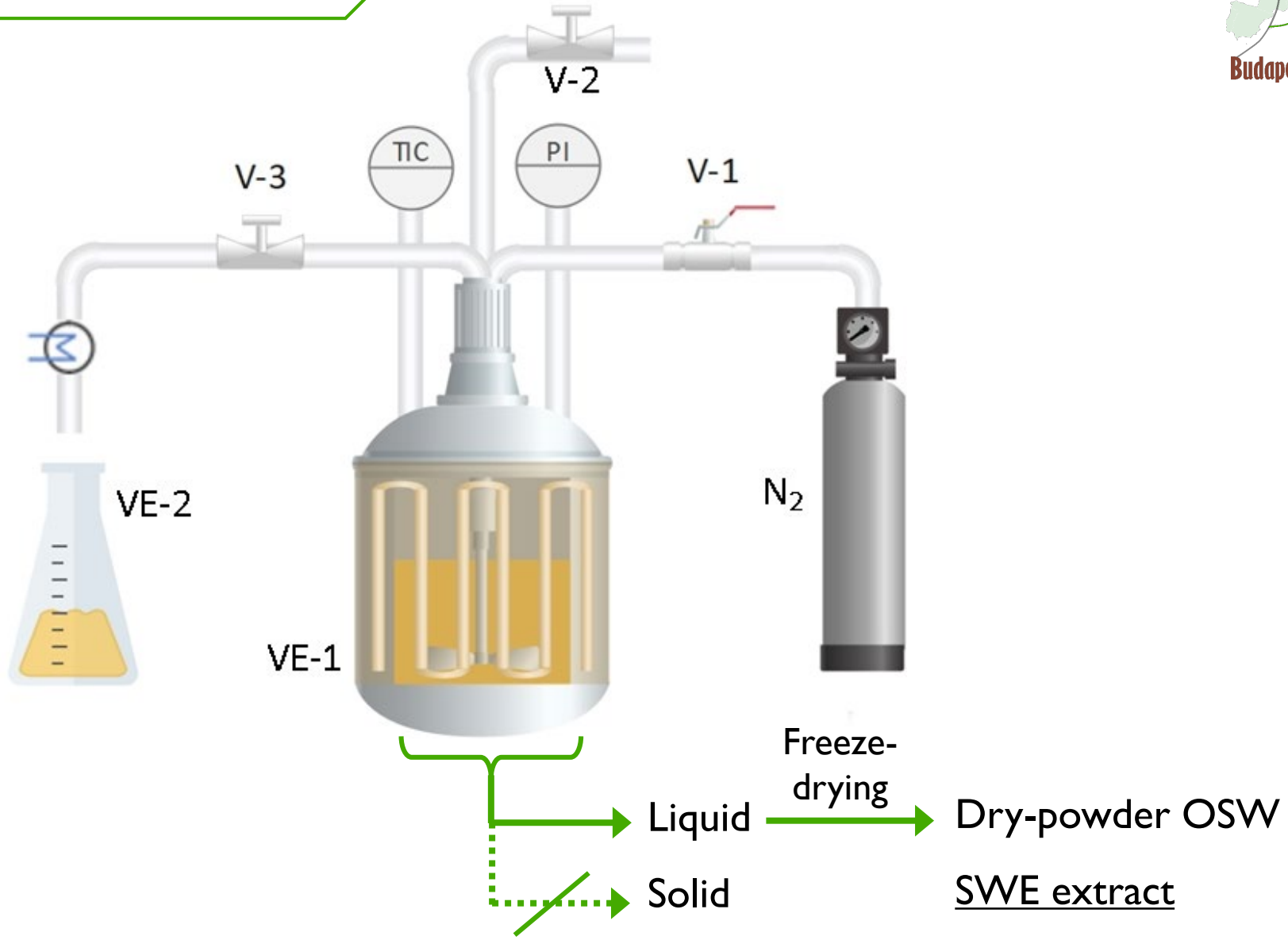
Horcal

15g OSW + 350 mL

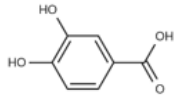
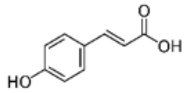
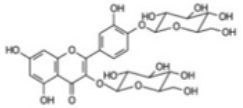
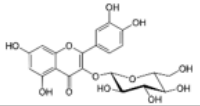
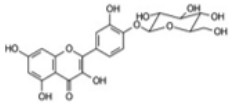
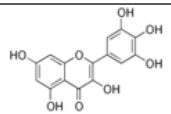
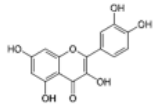
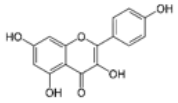
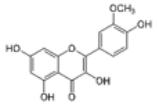
145°C

50 bar

50 min

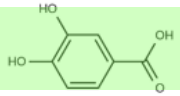
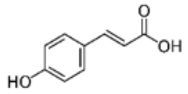
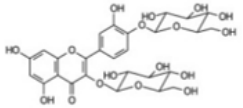
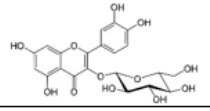
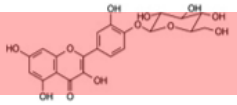
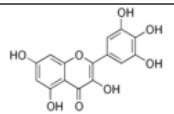
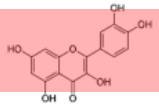
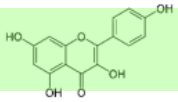
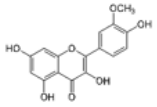


Phenolic profile

	Phenolic compounds:		Rt (min)	*Horcal* - CE	*Red* - CE	*Horcal* - SWE
1	Protocatechuic acid		9.7	18.0 ± 0.2^{a}	16.19 ± 0.01^{b}	20 ± 3^{ab}
2	*p*-Coumaric acid		39.9	12.8 ± 0.9^{a}	1.3 ± 0.1^{b}	0.10 ± 0.05^{c}
3	Quercetin-3,4′-*O*-diglucoside		47.8	2.7 ± 0.1^{a}	6 ± 3^{a}	–
4	Quercetin-3-*O*-glucoside		55.5	0.3 ± 0.0^{a}	0.22 ± 0.05^{a}	0.10 ± 0.05^{b}
5	Quercetin-4′-*O*-glucoside		62.6	51.5 ± 0.3^{a}	39 ± 1^{b}	7.5 ± 0.2^{c}
6	Myricetin		63.8	2.6 ± 0.3^{a}	2.0 ± 0.2^{b}	0.7 ± 0.1^{c}
7	Quercetin		67.5	52 ± 2^{a}	24 ± 1^{b}	3.2 ± 0.6^{c}
8	Kaempferol		70.1	12.6 ± 0.1^{a}	12.2 ± 0.2^{b}	0.20 ± 0.05^{c}
9	Isorhamnetin		70.6	2.3 ± 0.1^{a}	1.99 ± 0.01^{b}	0.20 ± 0.05^{c}
	Total Phenolic Compounds:			155 ± 2^{a}	103 ± 3^{b}	32 ± 3^{c}

Values with different letters in each column are significantly different when applying unpaired two-tailed Student *t*-test ($p < 0.05$).

Phenolic profile

	Phenolic compounds:		Rt (min)	*Horcal* - CE	*Red* - CE	*Horcal* - SWE
1	Protocatechuic acid		9.7	18.0 ± 0.2^{a}	16.19 ± 0.01^{b}	20 ± 3^{ab}
2	*p*-Coumaric acid		39.9	12.8 ± 0.9^{a}	1.3 ± 0.1^{b}	0.10 ± 0.05^{c}
3	Quercetin-3,4′-*O*-diglucoside		47.8	2.7 ± 0.1^{a}	6 ± 3^{a}	–
4	Quercetin-3-*O*-glucoside		55.5	0.3 ± 0.0^{a}	0.22 ± 0.05^{a}	0.10 ± 0.05^{b}
5	Quercetin-4′-*O*-glucoside		62.6	51.5 ± 0.3^{a}	39 ± 1^{b}	7.5 ± 0.2^{c}
6	Myricetin		63.8	2.6 ± 0.3^{a}	2.0 ± 0.2^{b}	0.7 ± 0.1^{c}
7	Quercetin		67.5	52 ± 2^{a}	24 ± 1^{b}	3.2 ± 0.6^{c}
8	Kaempferol		70.1	12.6 ± 0.1^{a}	12.2 ± 0.2^{b}	0.20 ± 0.05^{c}
9	Isorhamnetin		70.6	2.3 ± 0.1^{a}	1.99 ± 0.01^{b}	0.20 ± 0.05^{c}
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Values with different letters in each column are significantly different when applying unpaired two-tailed Student *t*-test ($p < 0.05$).

Total flavonoids content:

Red-CE : 81 mg QE/g

Horcal-CE: 115 mg QE/g

Phenolic profile

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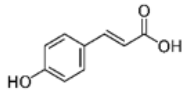
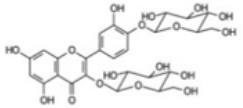
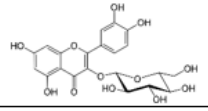
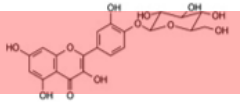
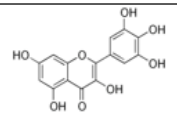
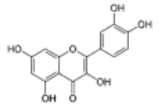
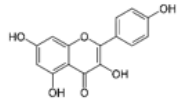
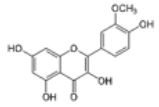
2	*p*-Coumaric acid		39.9	12.8 ± 0.9^{a}	1.3 ± 0.1^{b}	0.10 ± 0.05^{c}	
3	Quercetin-3,4′-*O*-diglucoside		47.8	2.7 ± 0.1^{a}	6 ± 3^{a}	–	
4	Quercetin-3-*O*-glucoside		55.5	0.3 ± 0.0^{a}	0.22 ± 0.05^{a}	0.10 ± 0.05^{b}	
5	Quercetin-4′-*O*-glucoside		62.6	51.5 ± 0.3^{a}	39 ± 1^{b}	7.5 ± 0.2^{c}	23%
6	Myricetin		63.8	2.6 ± 0.3^{a}	2.0 ± 0.2^{b}	0.7 ± 0.1^{c}	
7	Quercetin		67.5	52 ± 2^{a}	24 ± 1^{b}	3.2 ± 0.6^{c}	
8	Kaempferol		70.1	12.6 ± 0.1^{a}	12.2 ± 0.2^{b}	0.20 ± 0.05^{c}	
9	Isorhamnetin		70.6	2.3 ± 0.1^{a}	1.99 ± 0.01^{b}	0.20 ± 0.05^{c}	
	Total Phenolic Compounds:			155 ± 2^{a}	103 ± 3^{b}	32 ± 3^{c}	

Values with different letters in each column are significantly different when applying unpaired two-tailed Student *t*-test ($p < 0.05$).

Phenolic profile

	Phenolic compounds:	Rt (min)	*Horcal* - CE	*Red* - CE	*Horcal* - SWE
1	Protocatechuic acid	9.7	18.0 ± 0.2^{a}	16.19 ± 0.01^{b}	20 ± 3^{ab}
2	*p*-Coumaric acid	39.9	12.8 ± 0.9^{a}	1.3 ± 0.1^{b}	0.10 ± 0.05^{c}
3	Quercetin-3,4′-*O*-diglucoside	47.8	2.7 ± 0.1^{a}	6 ± 3^{a}	–
4	Quercetin-3-*O*-glucoside	55.5	0.3 ± 0.0^{a}	0.22 ± 0.05^{a}	0.10 ± 0.05^{b}
5	Quercetin-4′-*O*-glucoside	62.6	51.5 ± 0.3^{a}	39 ± 1^{b}	7.5 ± 0.2^{c}
6	Myricetin	63.8	2.6 ± 0.3^{a}	2.0 ± 0.2^{b}	0.7 ± 0.1^{c}
7	Quercetin	67.5	52 ± 2^{a}	24 ± 1^{b}	3.2 ± 0.6^{c}
8	Kaempferol	70.1	12.6 ± 0.1^{a}	12.2 ± 0.2^{b}	0.20 ± 0.05^{c}
9	Isorhamnetin	70.6	2.3 ± 0.1^{a}	1.99 ± 0.01^{b}	0.20 ± 0.05^{c}
	Total Phenolic Compounds:		155 ± 2^{a}	103 ± 3^{b}	32 ± 3^{c}

Values with different letters in each column are significantly different when applying unpaired two-tailed Student *t*-test ($p < 0.05$).

Total flavonoids content:

Horcal-SWE: 26 mg QE/g

***77% < Horcal*-CE**

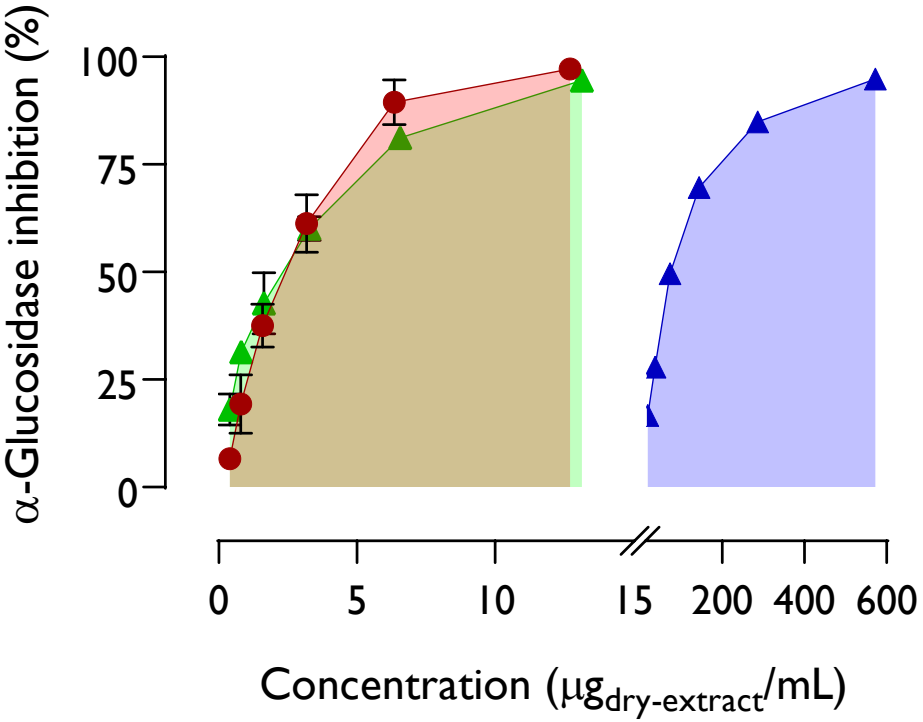
***68% < Red*-CE**

Antidiabetic potential

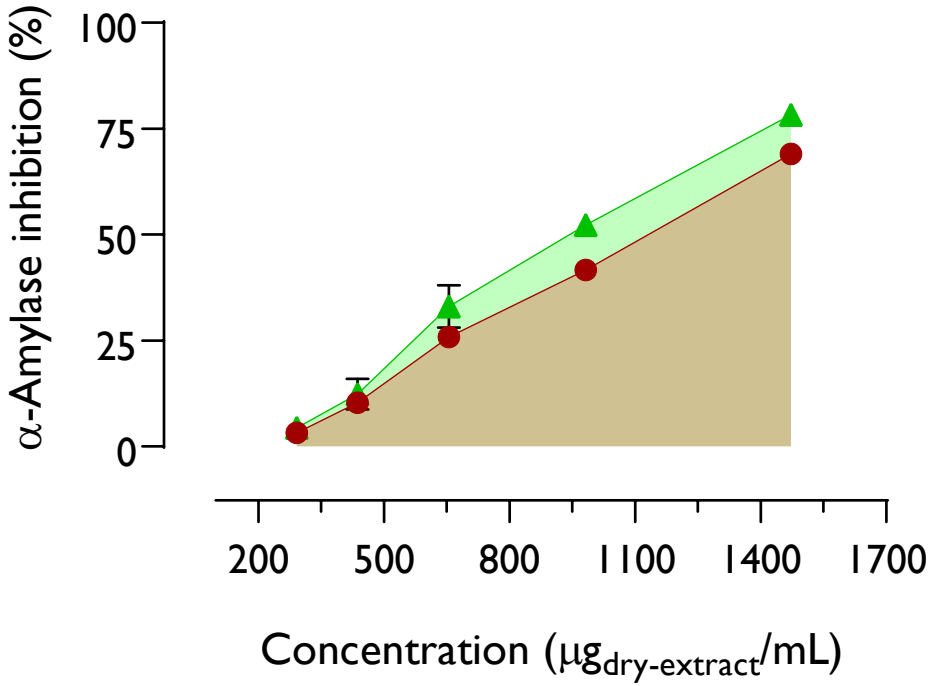
Red-CE
Horcal-CE
Horcal-SWE

Enzyme inactivation → concentration dependent manner

α-Glucosidase

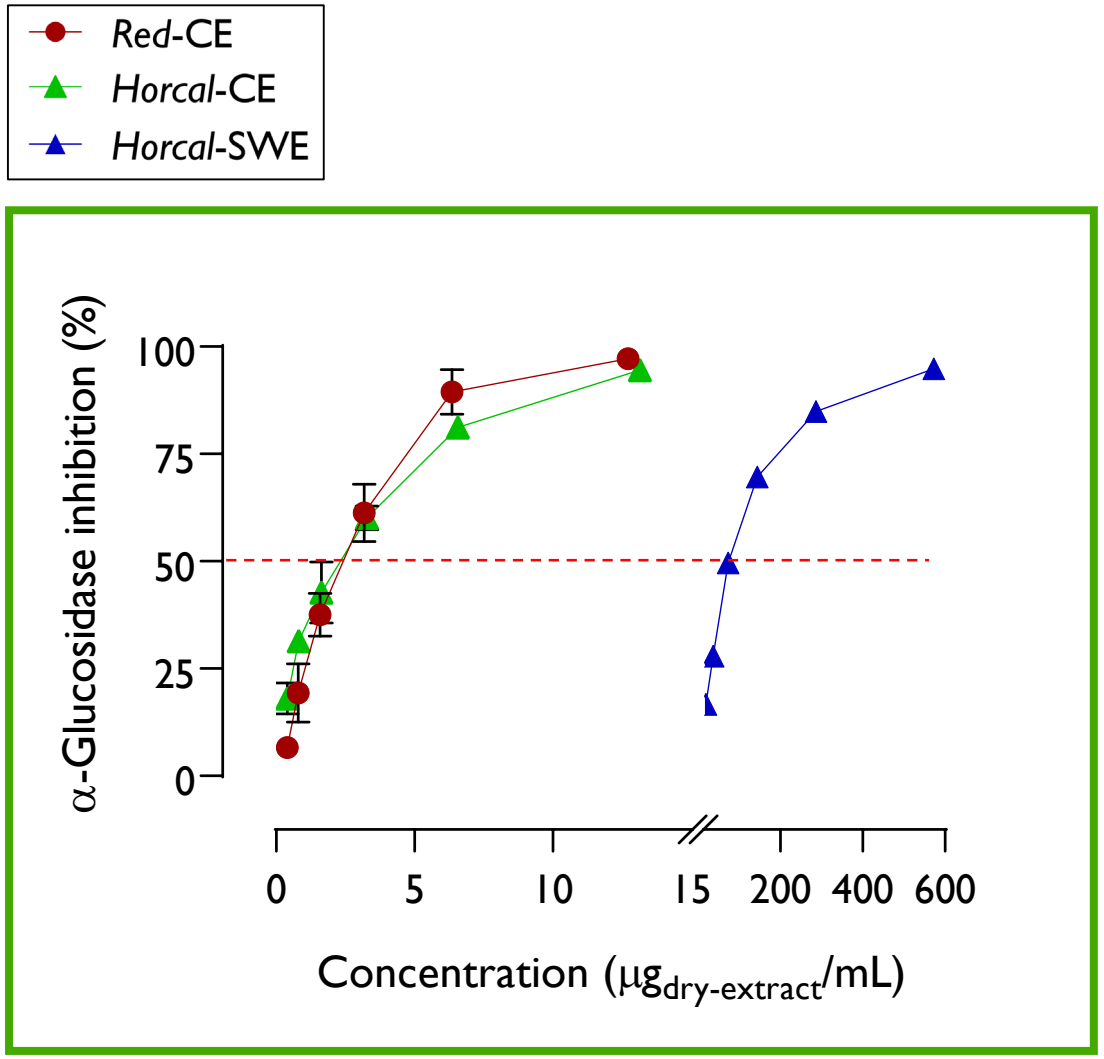


α-Amylase



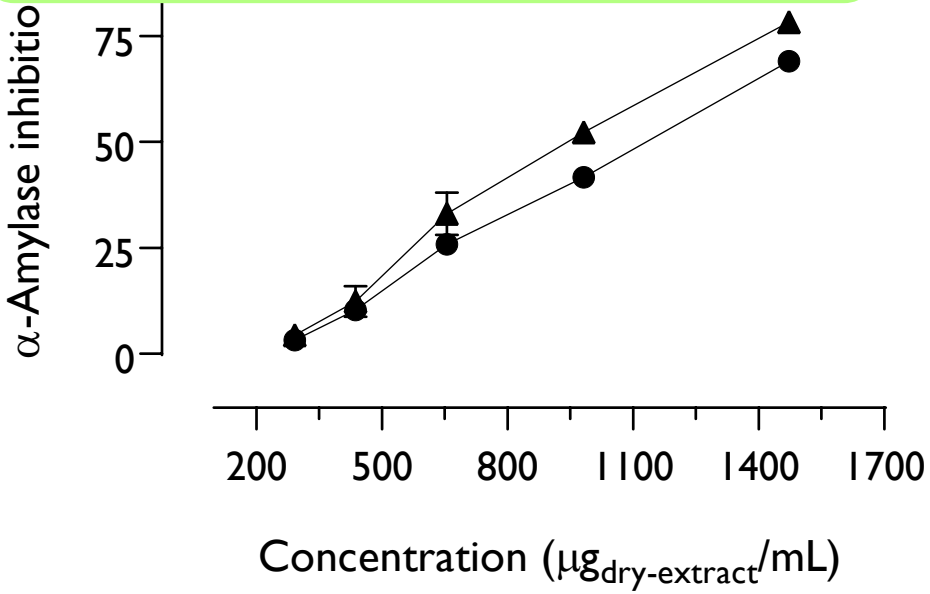
Antidiabetic potential

α-Glucosidase



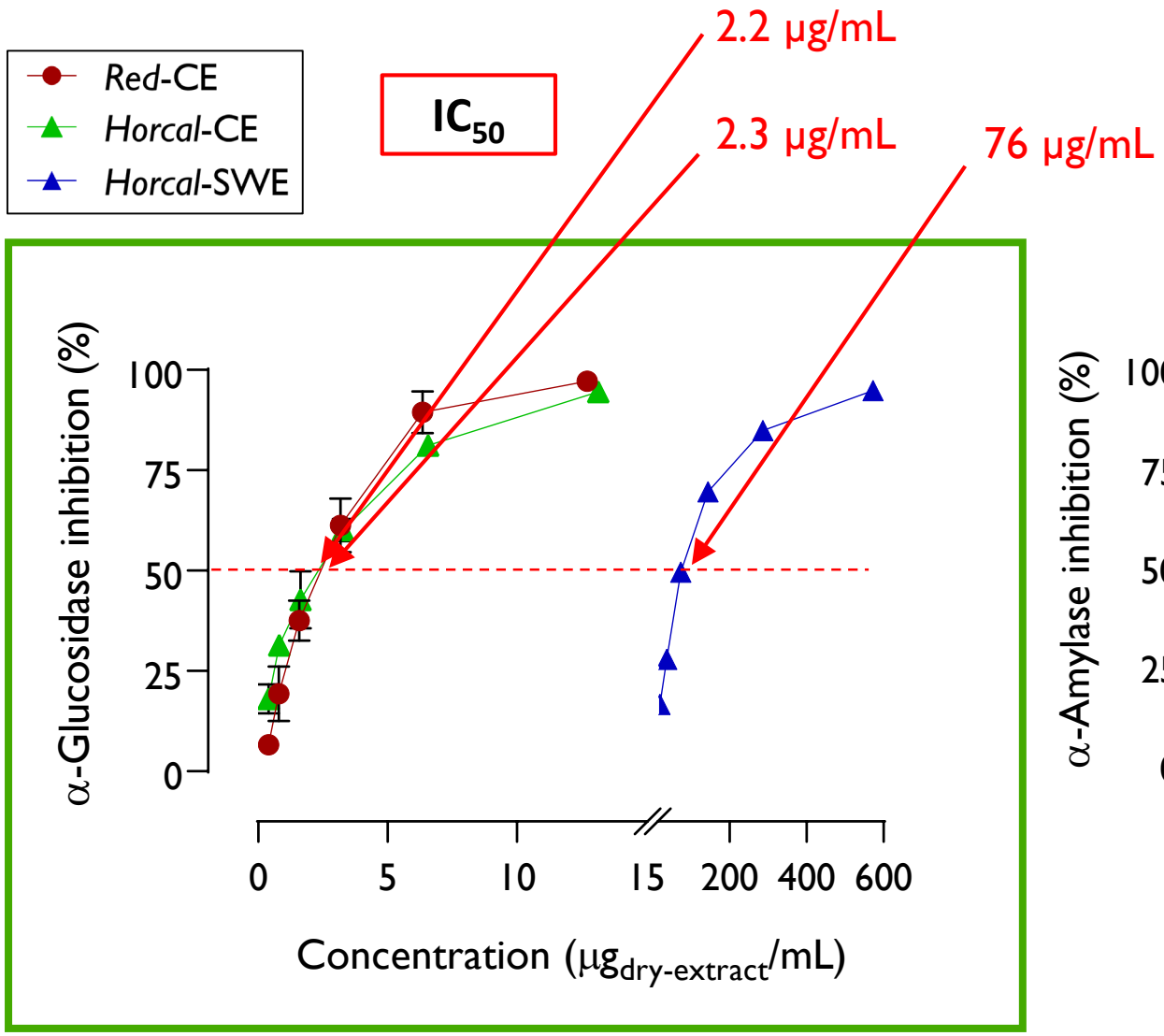
All the extracts inhibited α-glucosidase.

CE extracts more active tan SWE extract.



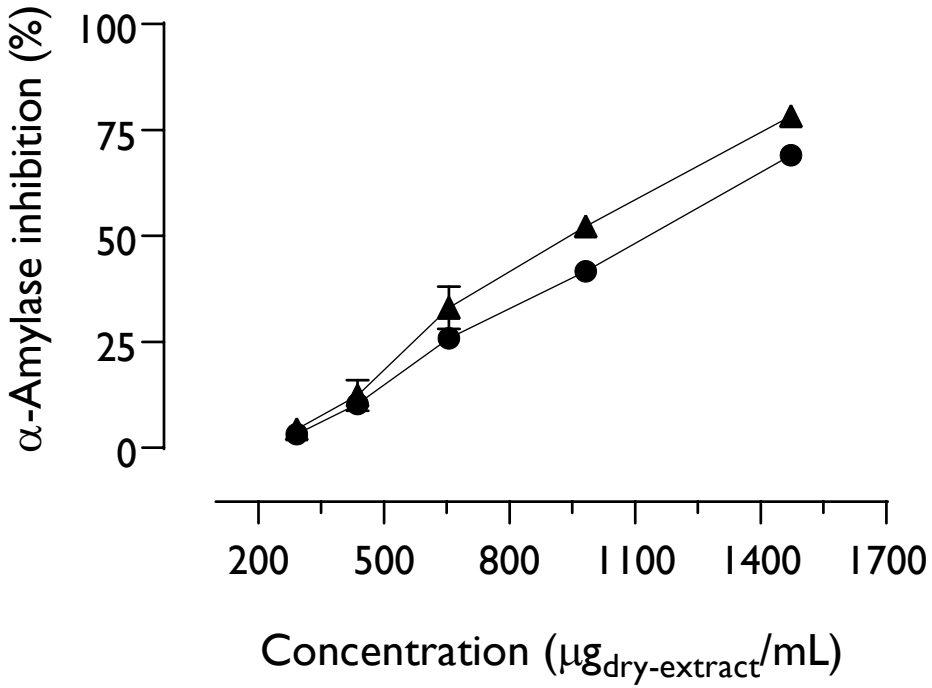
Antidiabetic potential

α-Glucosidase



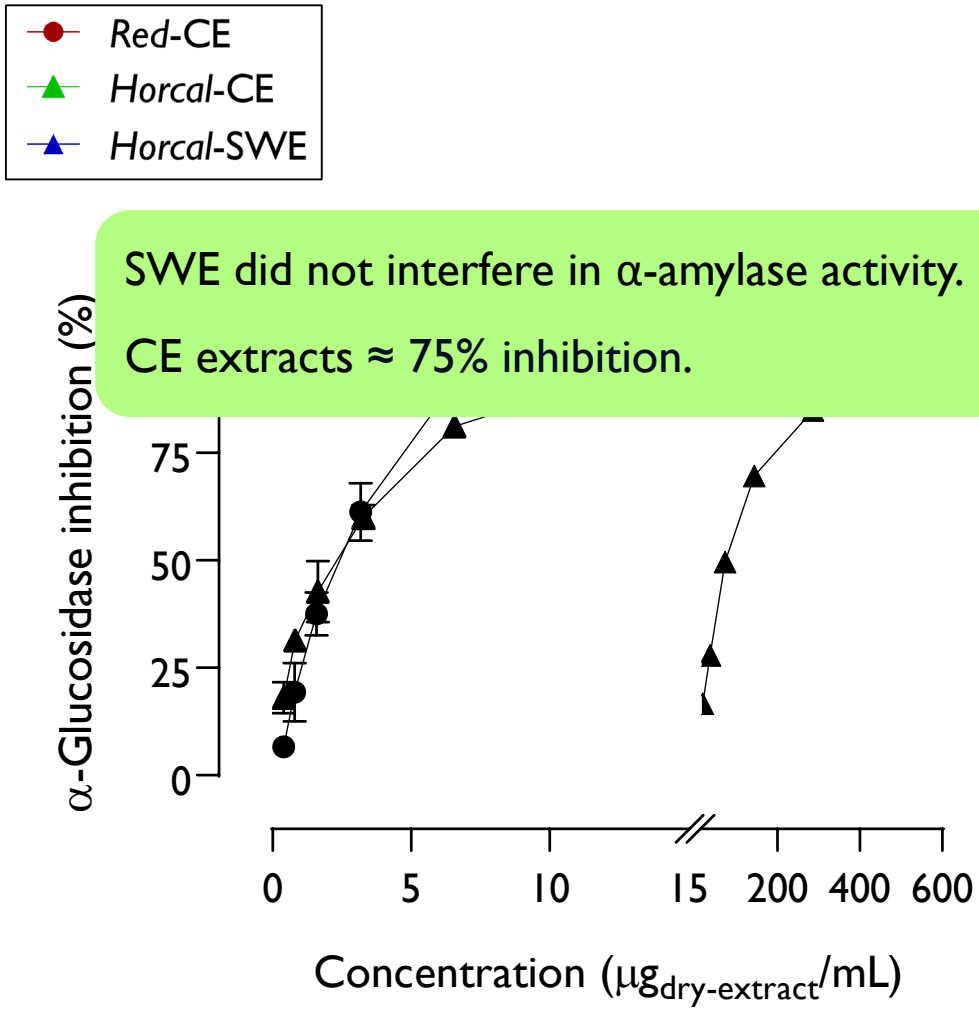
Acarbose: 136 µg/mL

($p < 0.01$)



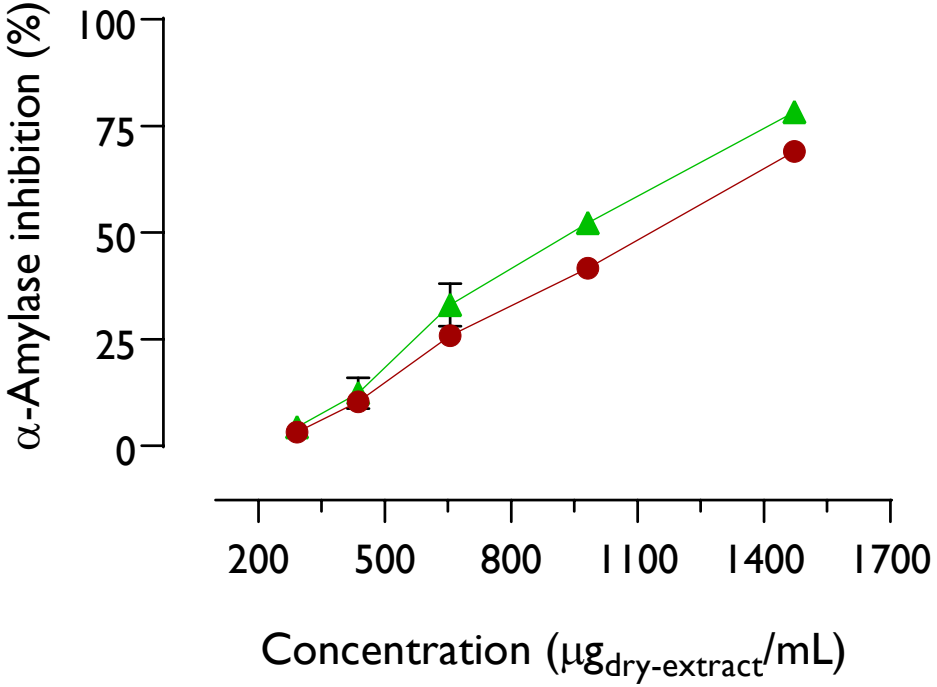
Antidiabetic potential

α-Amylase



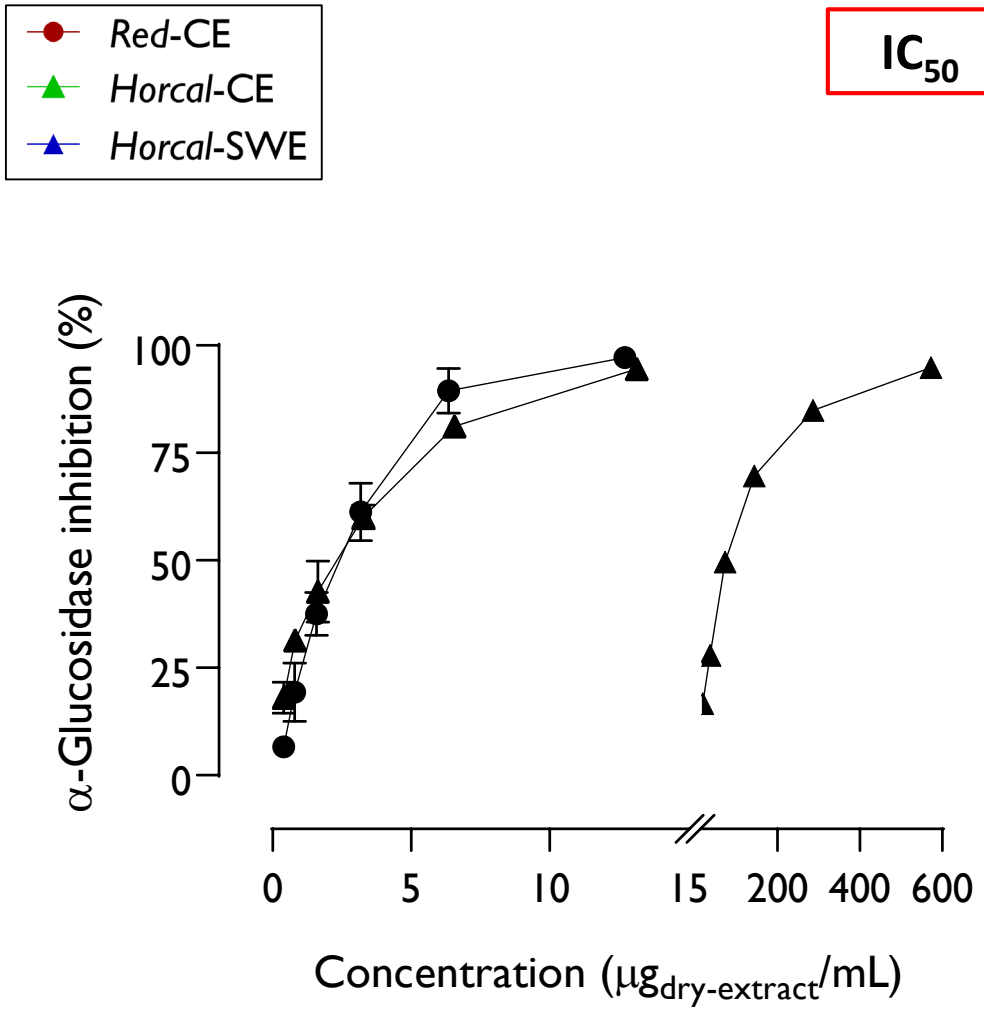
SWE did not interfere in α-amylase activity.

CE extracts ≈ 75% inhibition.



Antidiabetic potential

α-Amylase

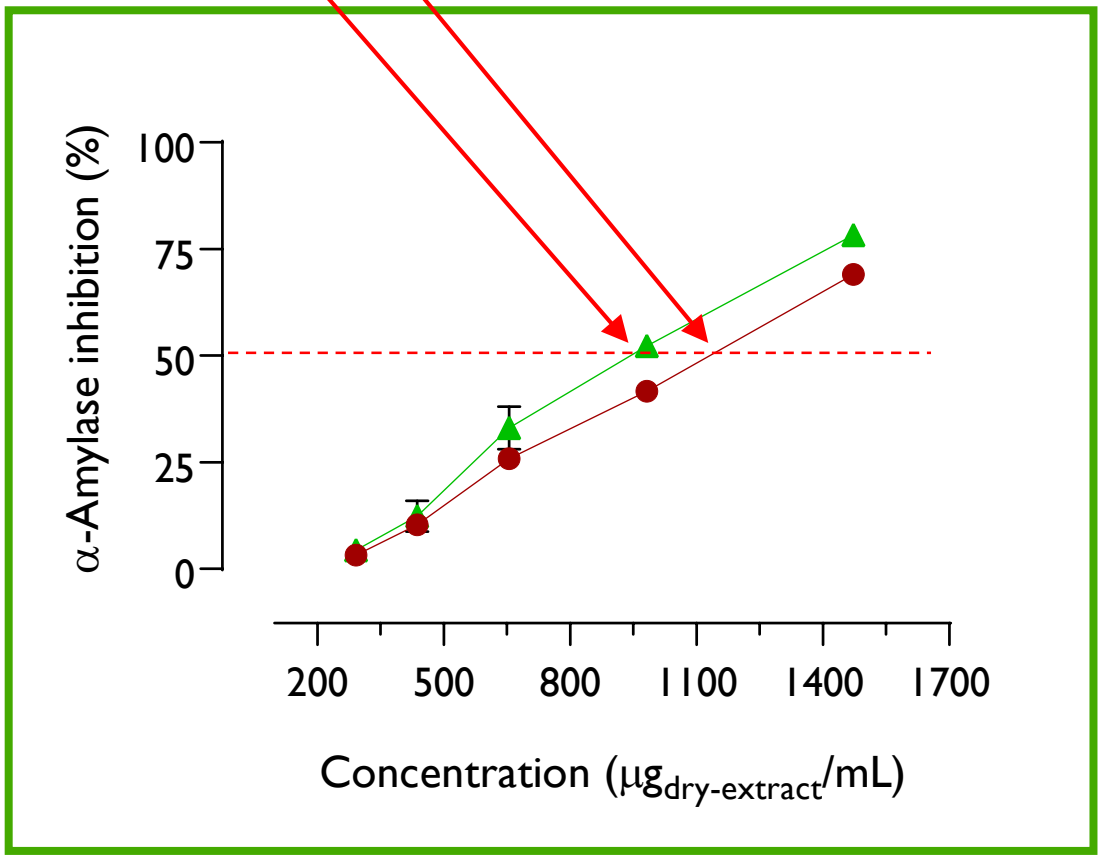


1126 µg/mL

932 µg/mL

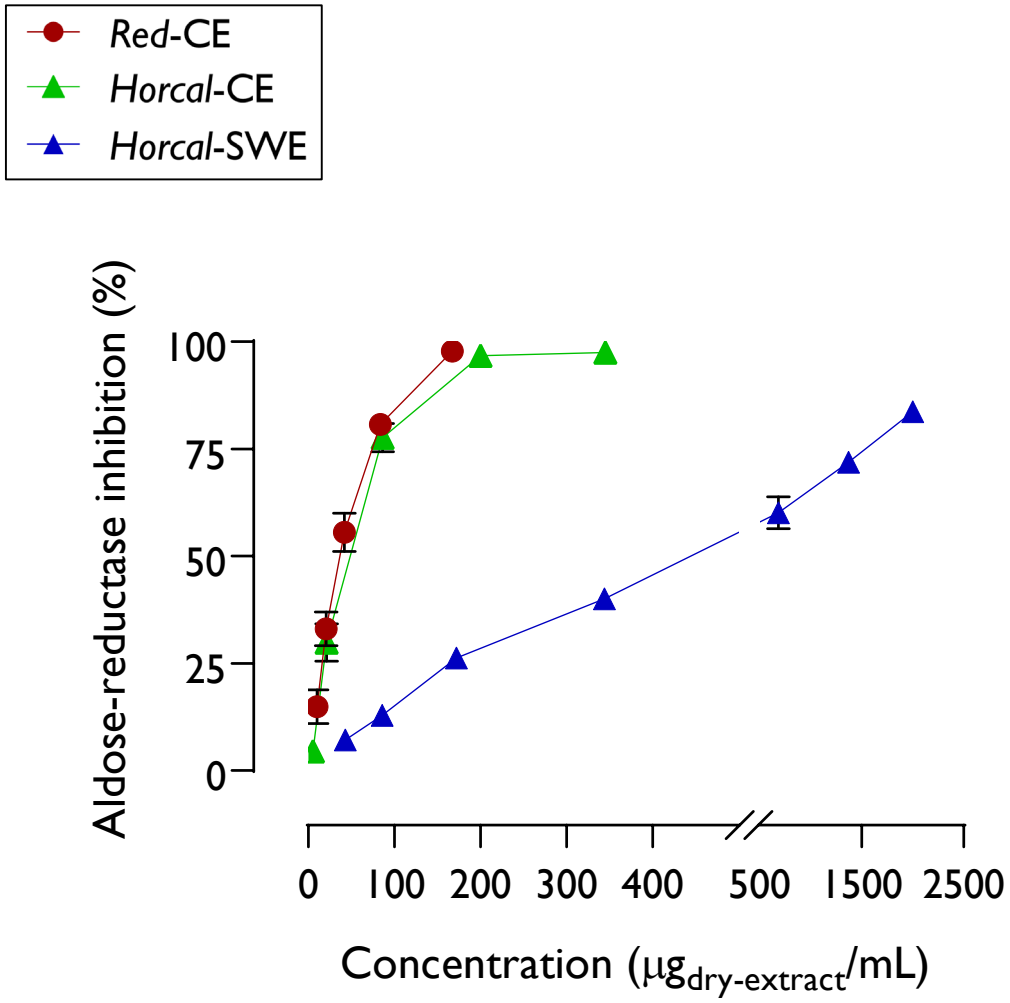
Acarbose: 83.4 µg/mL

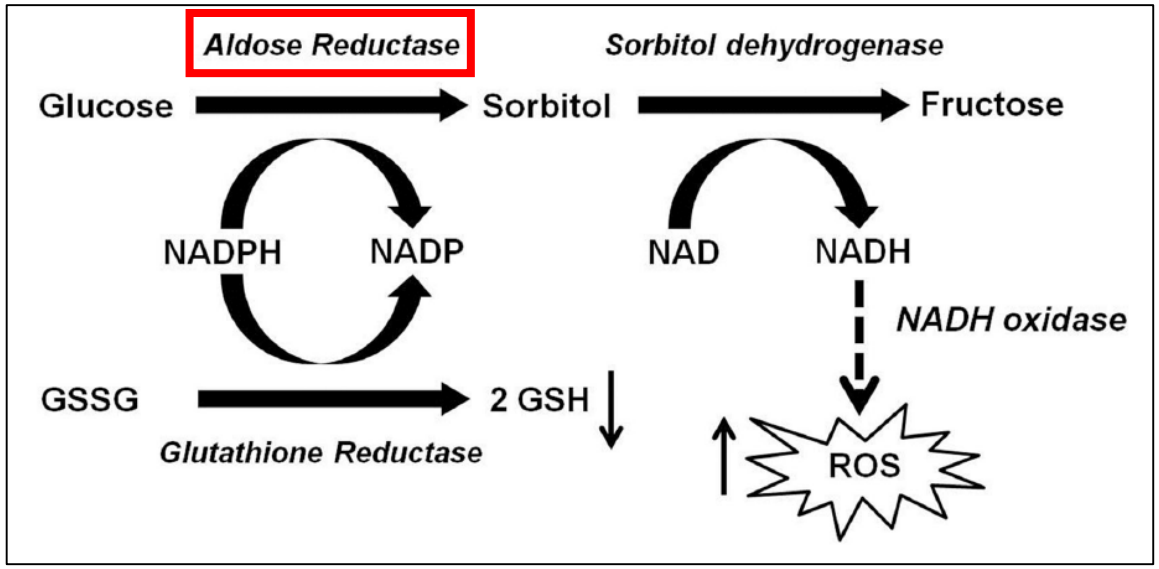
($p < 0.01$)



Antidiabetic potential

Aldose-reductase

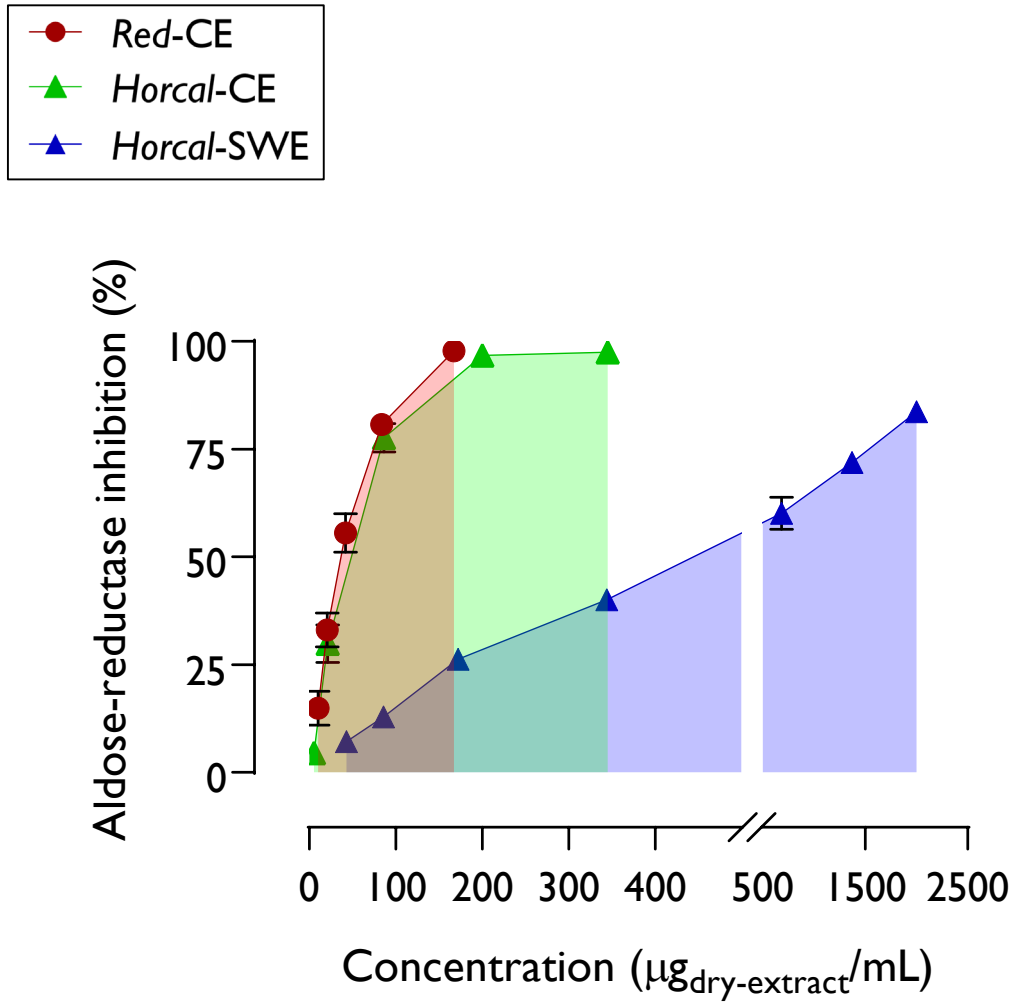




(Tang et al. 2012)

Antidiabetic potential

Aldose-reductase



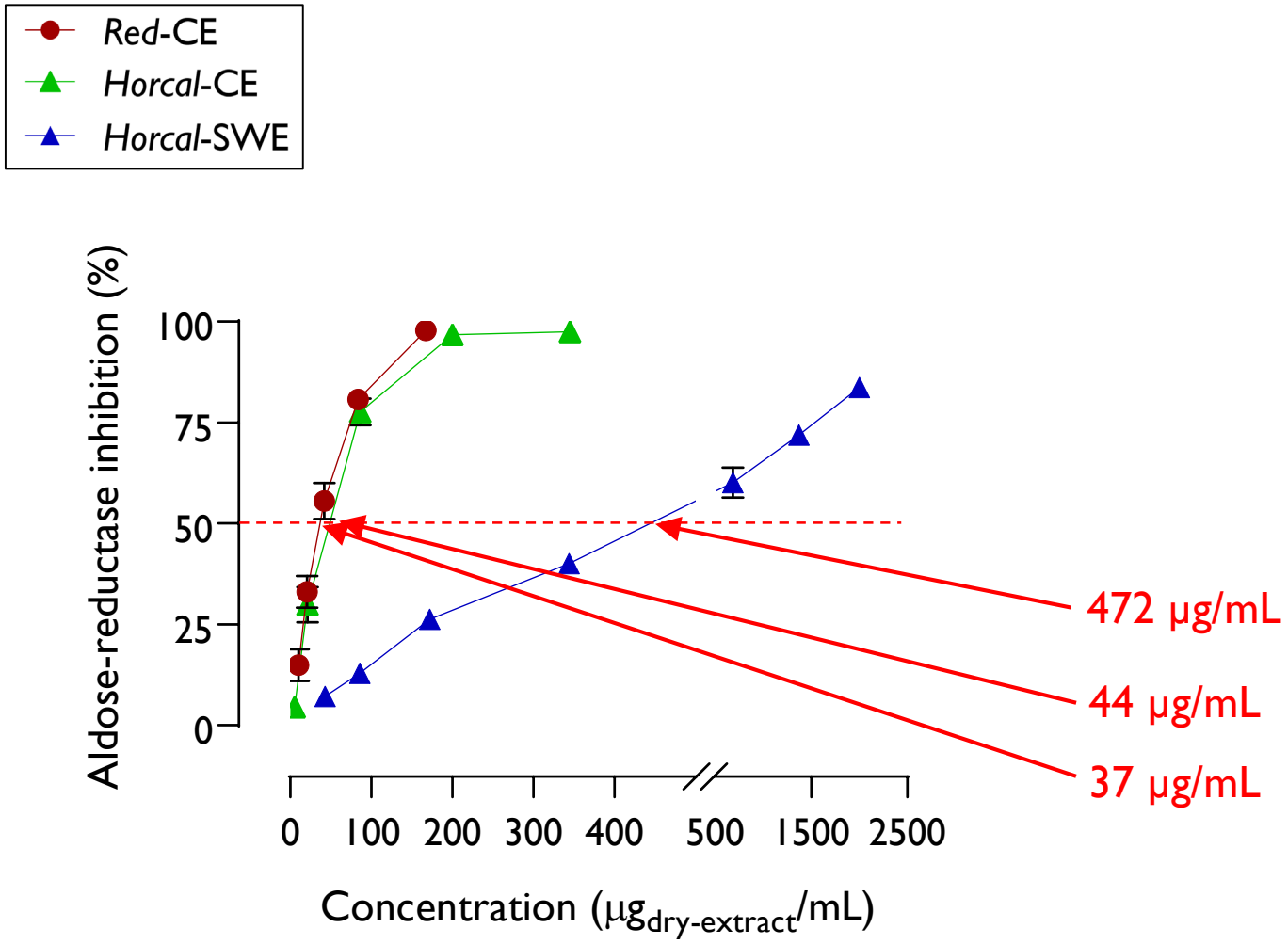
Enzyme inactivation → concentration dependent manner

Inactivation levels > 80%

CE extracts more active tan SWE extract.

Antidiabetic potential

Aldose-reductase

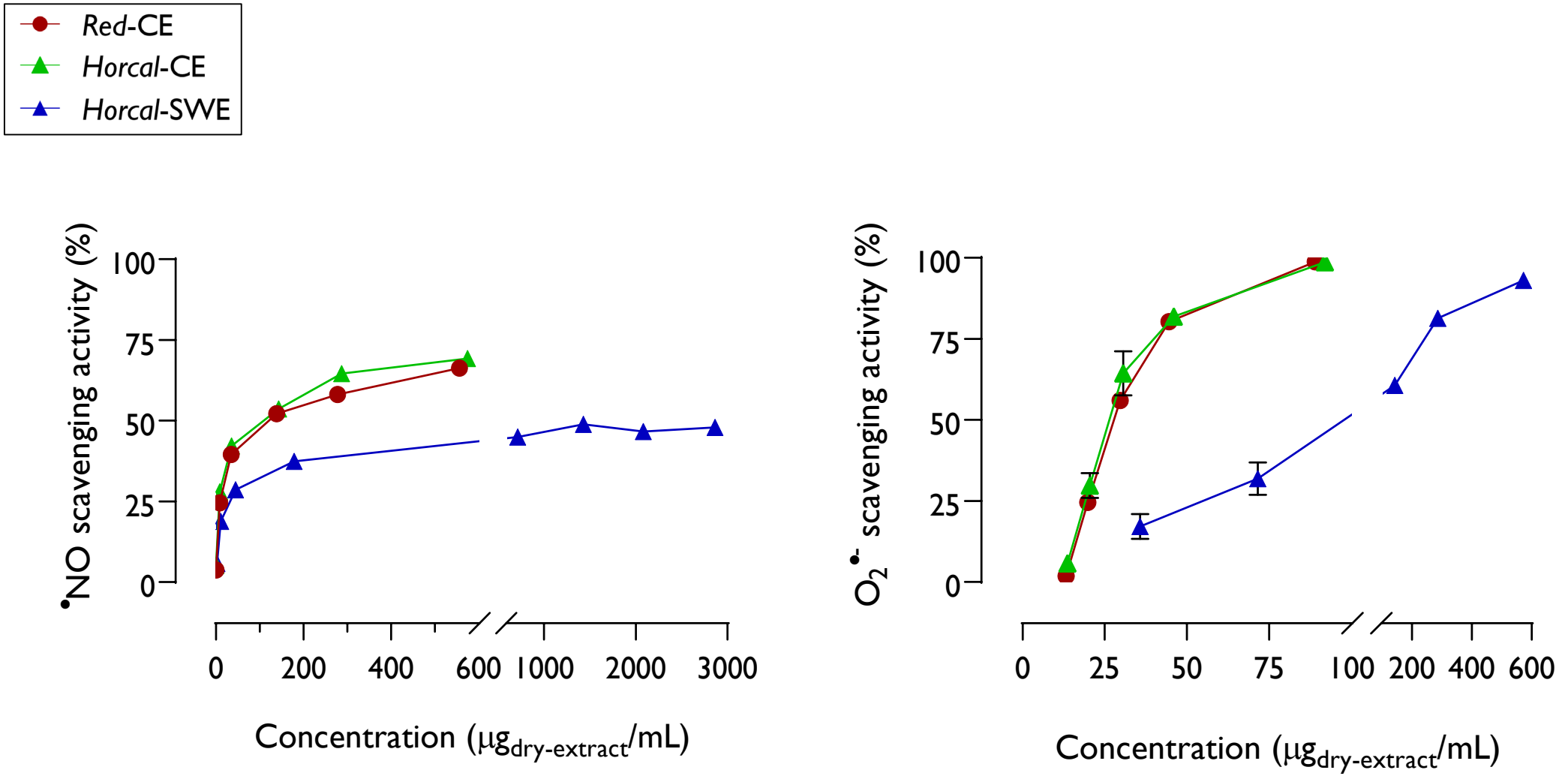


IC_{50}

Quercetin-3-*O*-glucopyranoside:
84 µg/mL

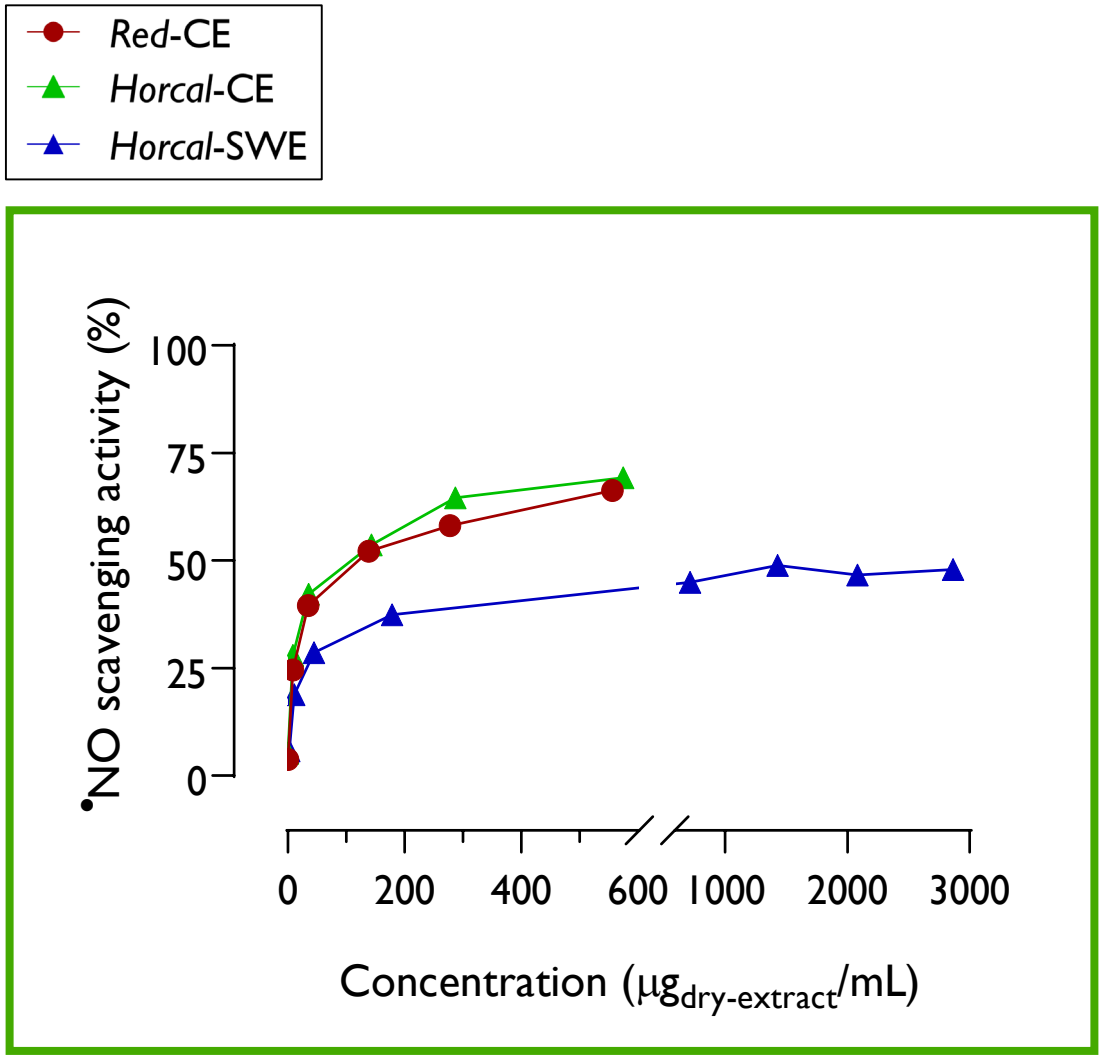
($p < 0.01$)

Antioxidant capacity



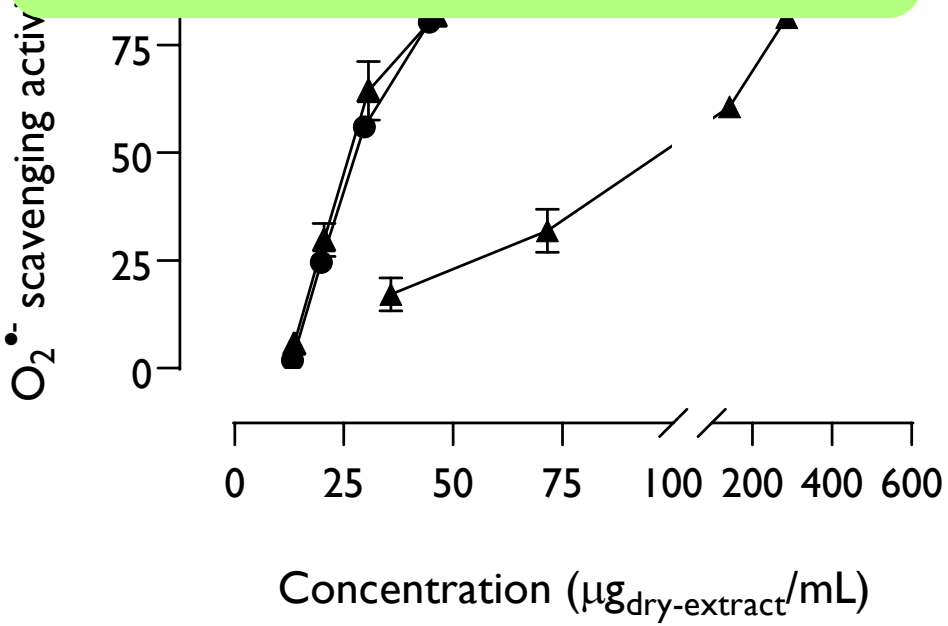
Antioxidant capacity

•NO



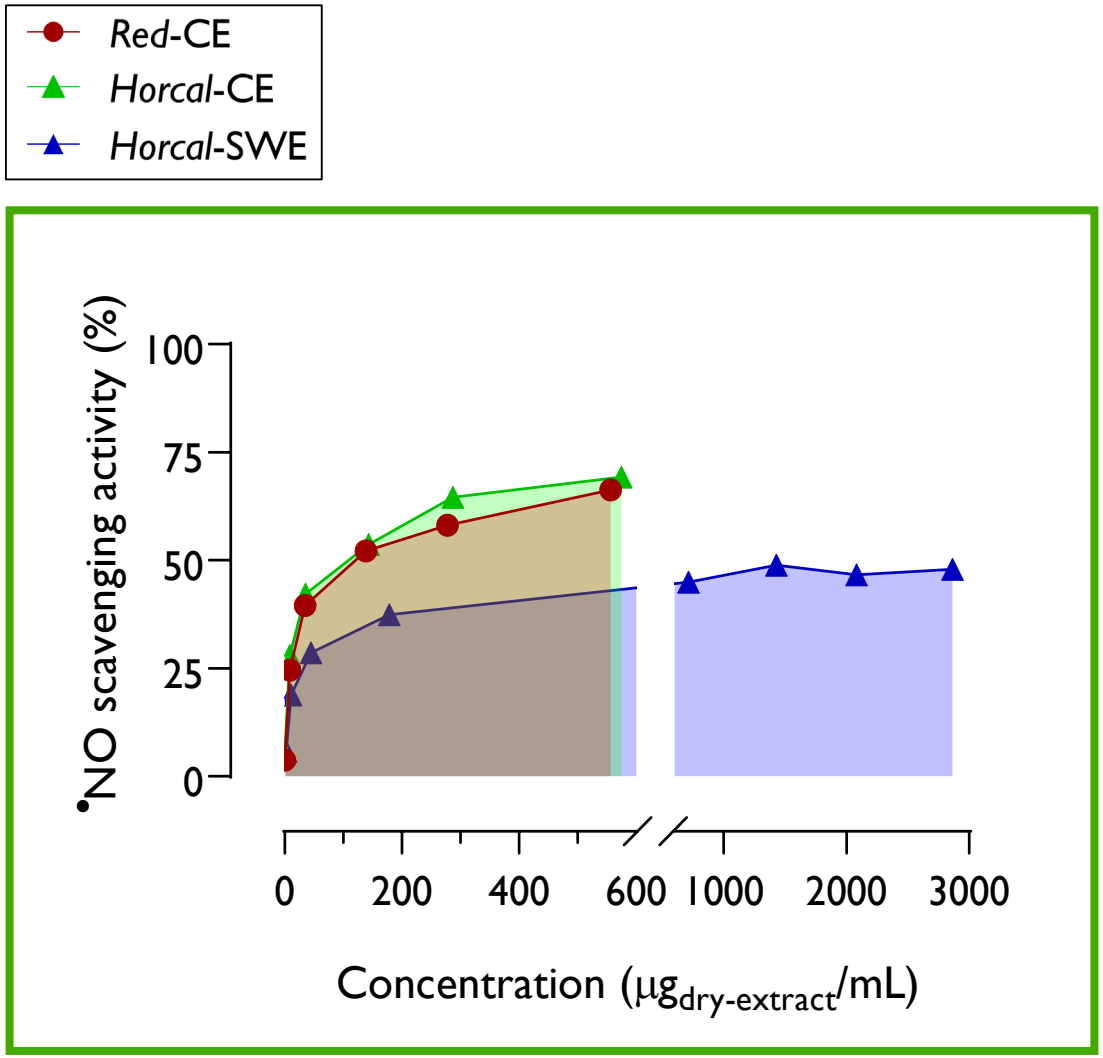
Scavenging activities < 75%

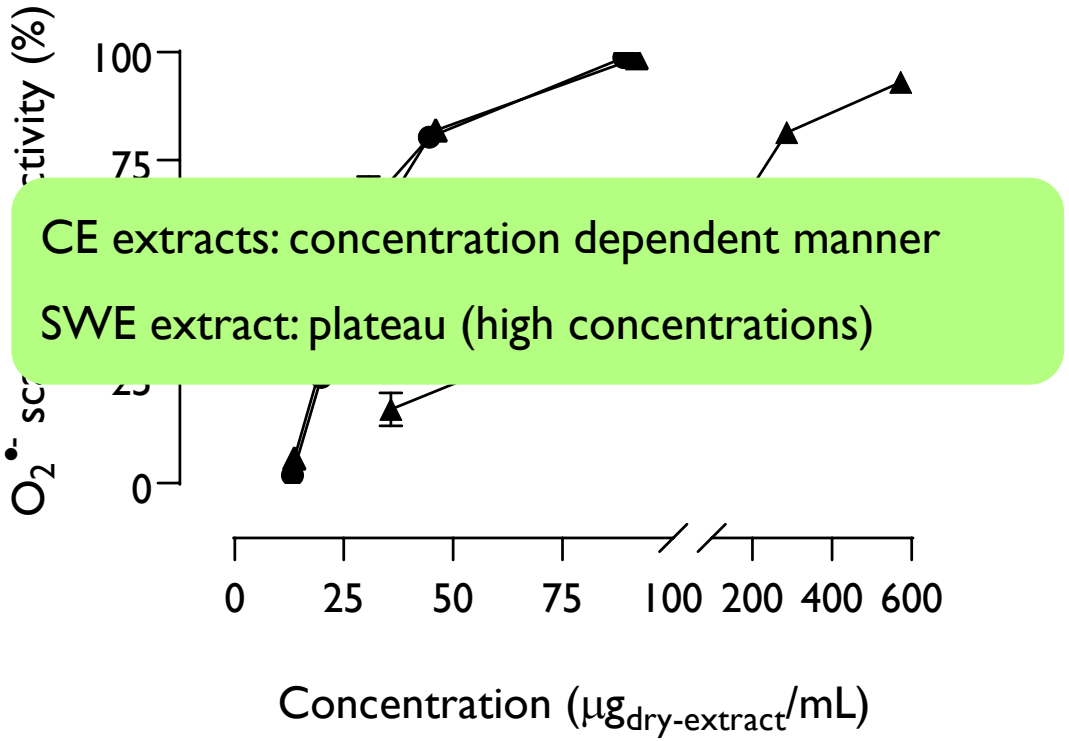
CE extracts > SWE extract



Antioxidant capacity

•NO



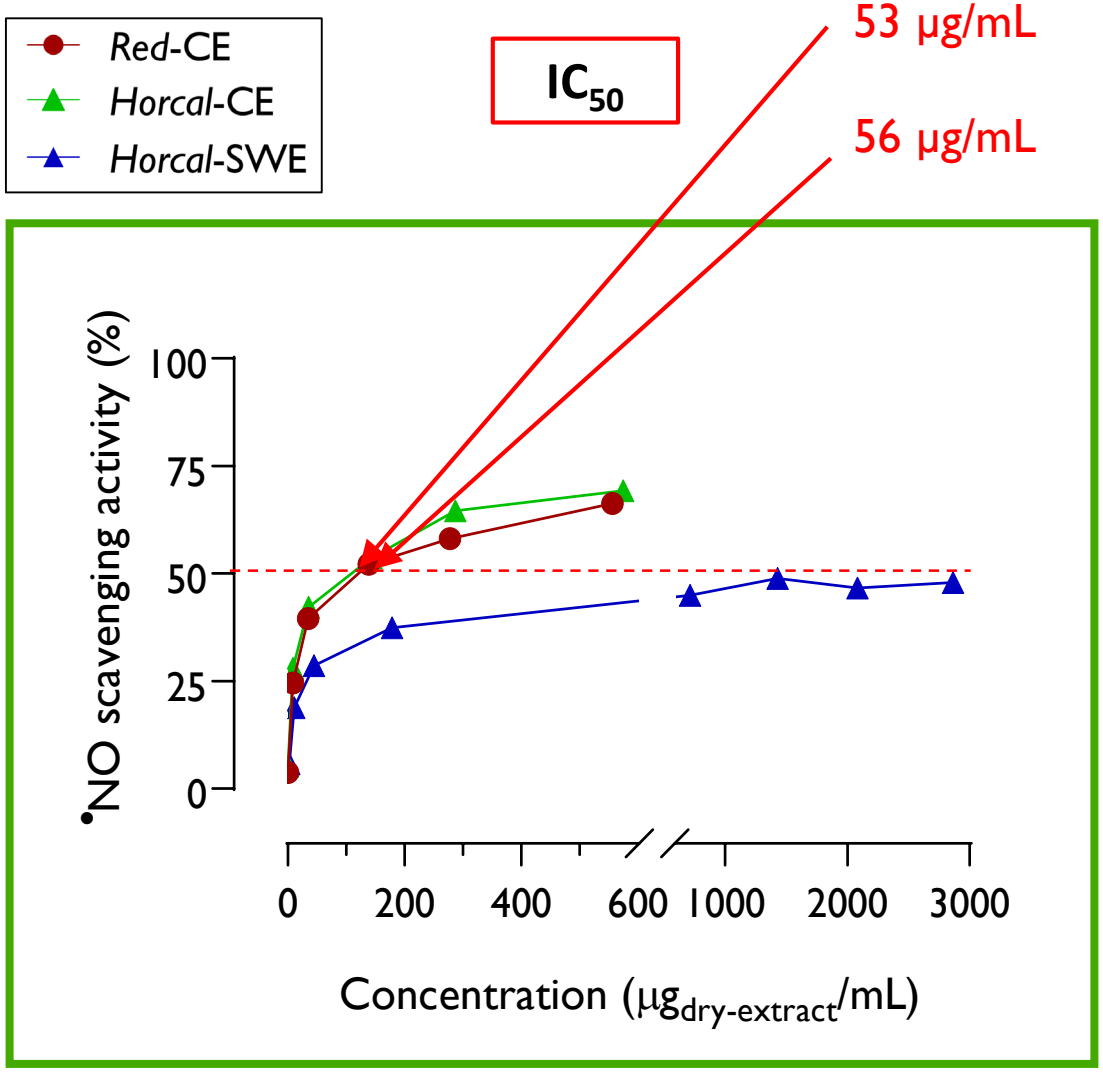


CE extracts: concentration dependent manner

SWE extract: plateau (high concentrations)

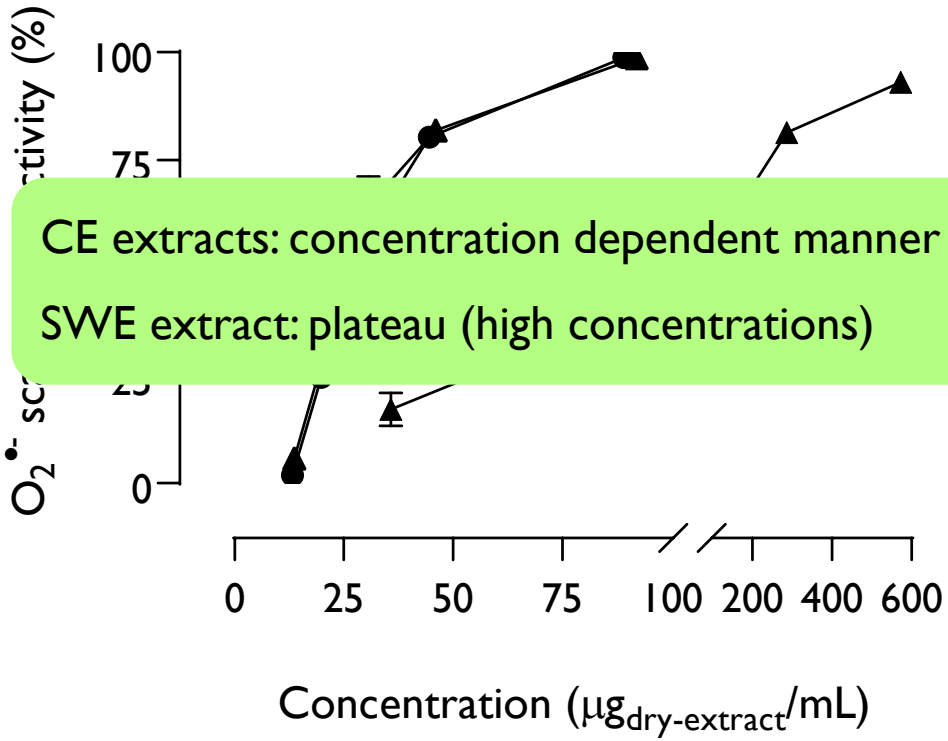
Antioxidant capacity

•NO



Quercetin: 63 µg/mL

($p < 0.01$)



CE extracts: concentration dependent manner

SWE extract: plateau (high concentrations)

Antioxidant capacity

$O_2^{\bullet-}$

Red-CE
Horcal-CE
Horcal-SWE

Quercetin: 24 µg/mL

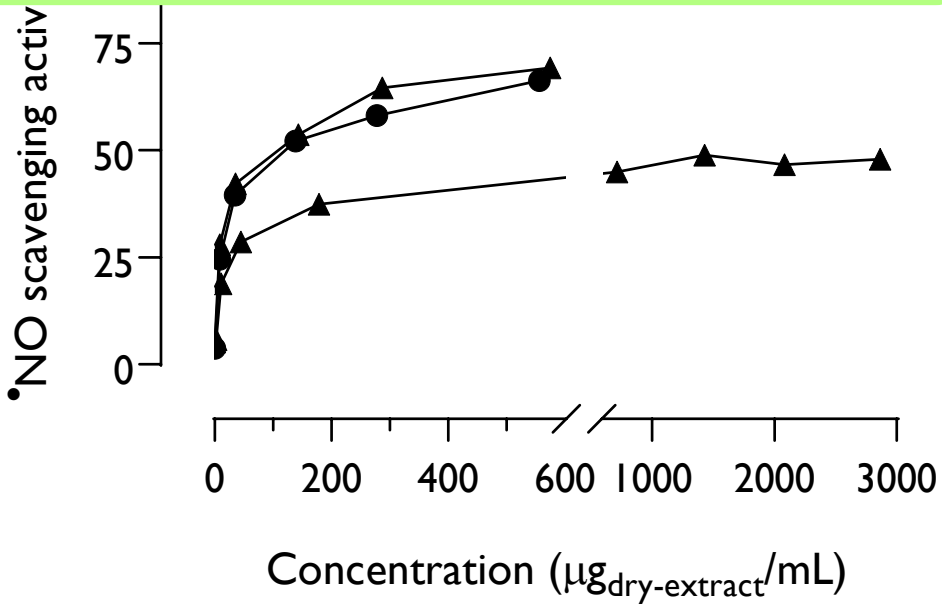
($p < 0.01$)

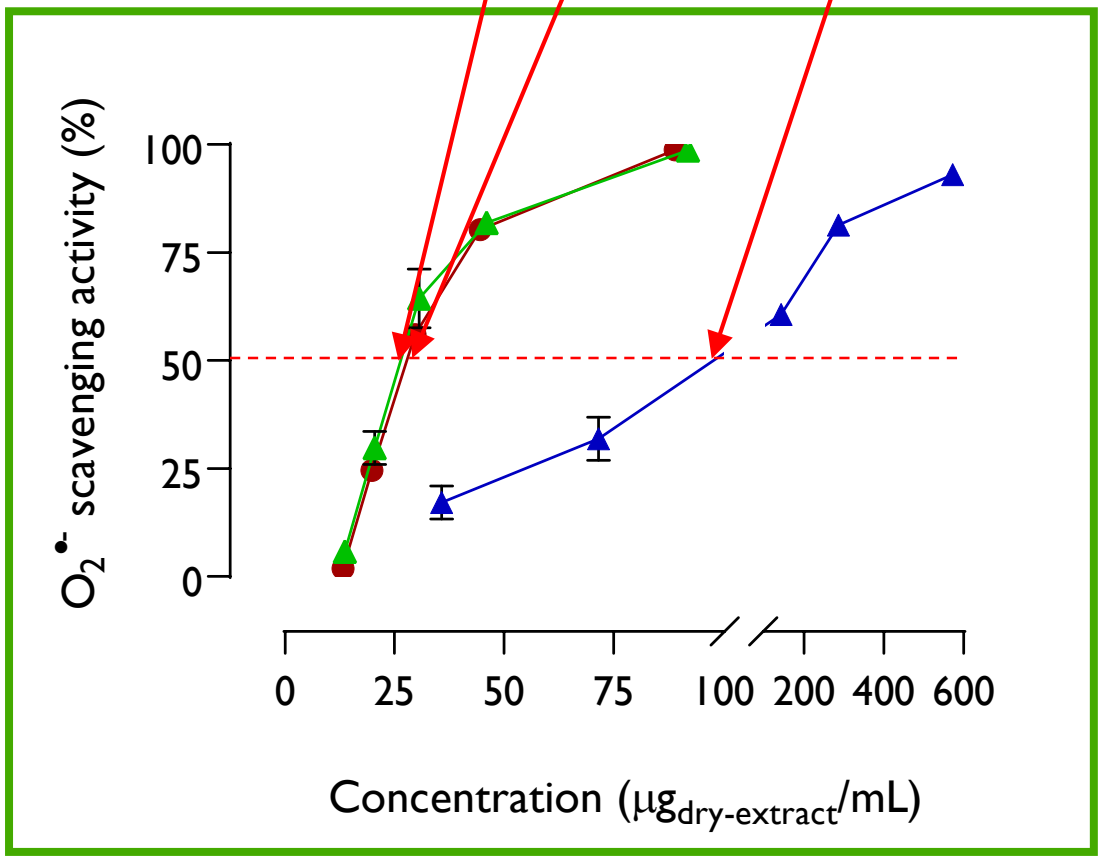
IC_{50}

26 µg/mL
28 µg/mL
112 µg/mL

Much more active for $O_2^{\bullet-}$ than for •NO

CE extracts more active tan SWE extract





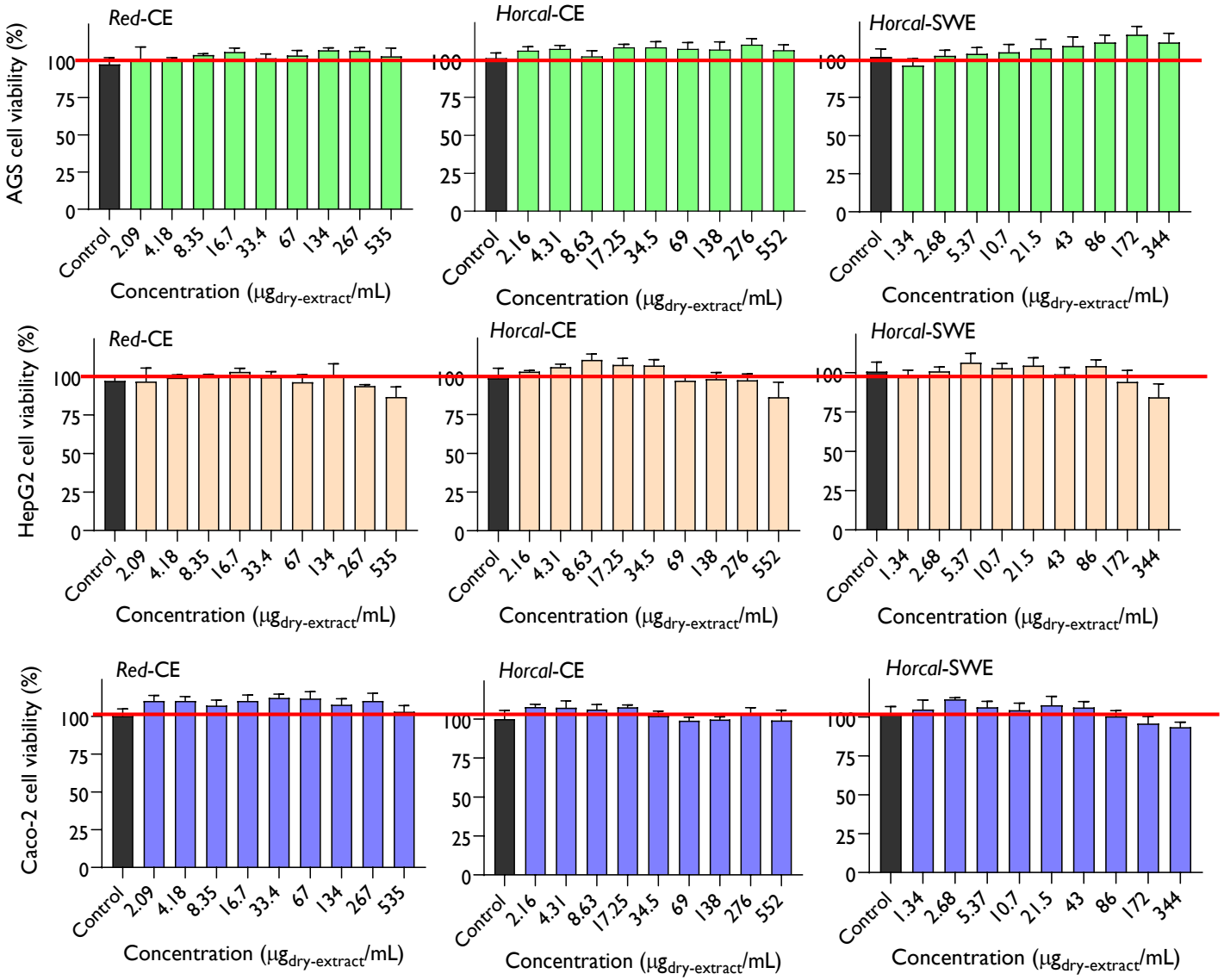
Cell viability

The OSW extracts did not cause significant changes on the mitocondrial activity of the evaluated cell lines

AGS cells: *Human gastric adenocarcinoma*

HepG2 cells: *Human liver hepatocelular carcinoma*

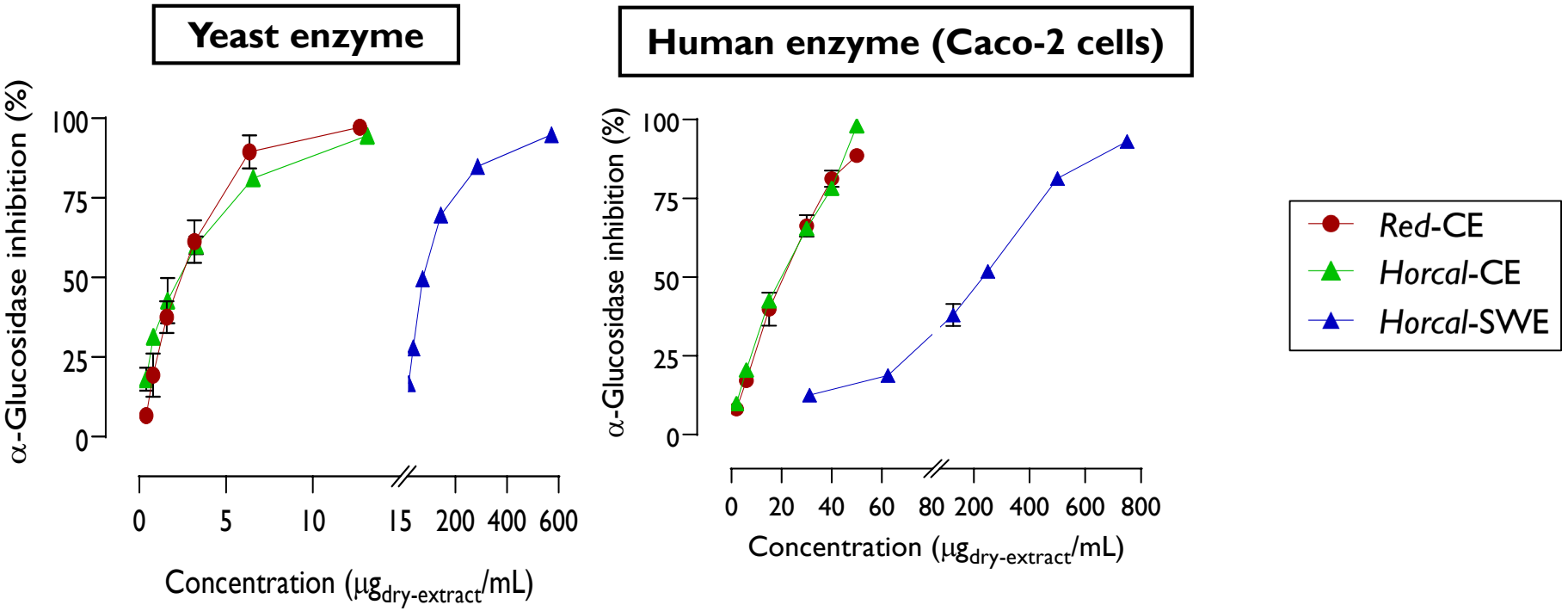
Caco-2 cells: *Human colorectal adenocarcinoma*



α-Glucosidase inhibition in Caco-2 cells

→ Sensitive of the enzyme/biological origin.

→ Enzyme-enriched cell supernatants from human Caco-2 cells (characteristics of human intestinal epithelium).

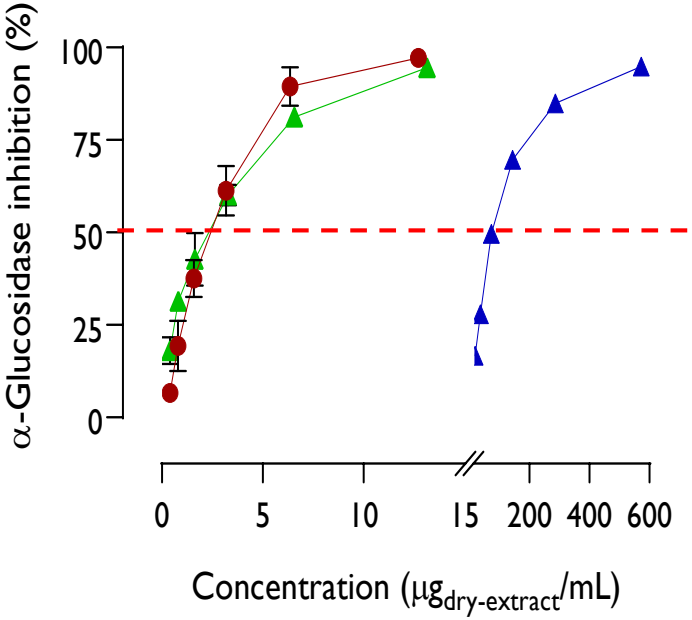


α-Glucosidase inhibition in Caco-2 cells

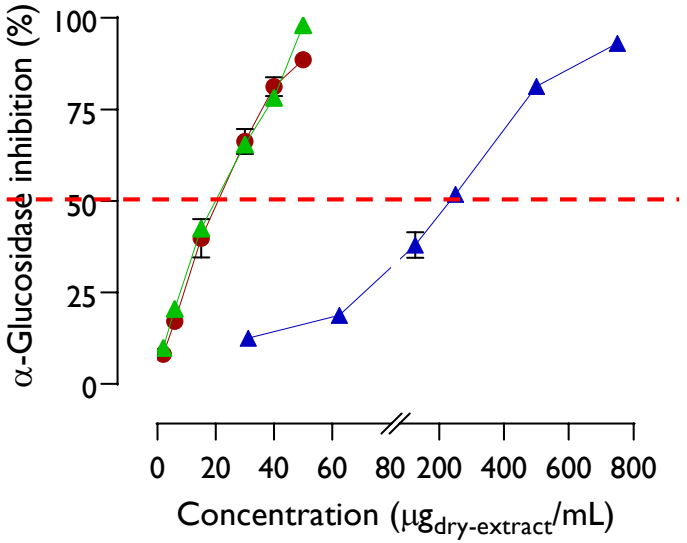
→ Sensitive of the enzyme/biological origin.

→ Enzyme-enriched cell supernatants from human Caco-2 cells (characteristics of human intestinal epithelium).

Yeast enzyme



Human enzyme (Caco-2 cells)



IC_{50}	Yeast α-Glucosidase	Human α-Glucosidase (Caco-2 cells)	
Horcal-CE	2.2 µg/mL	19 µg/mL	x 8,6
Red-CE	2.3 µg/mL	20 µg/mL	x 8,7
Horcal-SWE	76 µg/mL	215 µg/mL	x 2,8

Acarbose	136 µg/mL	(12,3 %)

Final Conclusions…

- The **extraction method resulted in varying phenolic compounds** profile.
(SWE = protocatechuic acid and quercetin-4'-*O*-glucoside)

- All the extracts **inhibited α-glucosidase,** but only those obtained from **CE inhibited α-amylase**.

- For **aldose-reductase**, **inactivation above 80%** was determined, and also managed to **neutralize •NO and anion $O_2^{\bullet -}$.**

- **None of the extracts caused cytotoxicity** of the tested cells, and all of them proved to effectively inactivate **α-glucosidase** from Caco-2 human cells.

SWE has proved to be a useful technology to obtain extracts from OSW with antidiabetic and antioxidant properties, besides avoiding the side effects resulted from strong α-amylase inhibition.

THANKS FOR YOUR ATTENTION

Funding

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CONTACT:

e-mail: eandres@ff.up.pt; etrigueros@ubu.es

ORCID: orcid.org/0000-0003-1701-0523

Biotechnology and Food Science Dept., Chemical Engineering Division, University of Burgos, Burgos, Spain

REQUIMTE/LAQV, Laboratório de Farmacognosia, Dept. Química, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal