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DEEP EUTECTIC SOLVENTS AS EXTRACTION MEDIA FOR POLAR BIOACTIVES FROM WHEAT BRAN

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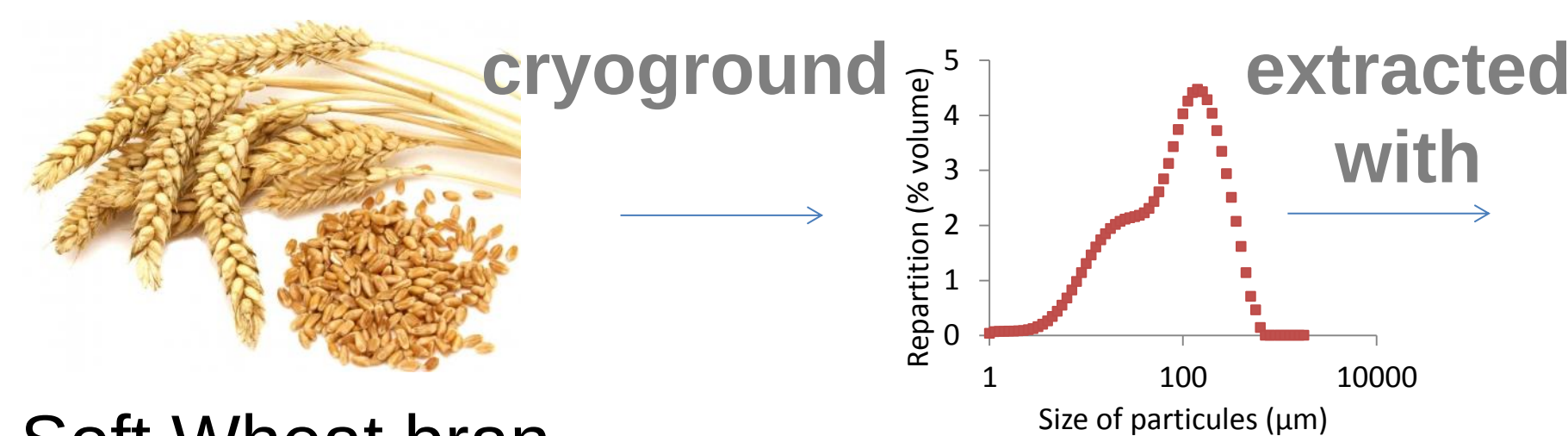
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Context & Objectives

Deep eutectic solvents (DES) based on a Hydrogen Bond Acceptor (HBA) generally a quaternary ammonium and a Hydrogen Bond Donor (HBD) of very diverse chemical natures, have been presented since 2003 as cheap, easy to prepare and biodegradable solvents. They are interesting alternative to ionic liquids or organic solvents and were employed in this project to extract bioactive polar compounds from wheat bran in comparison to classic organic solvents.

to determine the potential of DES to extract antioxidants from wheat bran and characterize DES extracts' composition and reducing power

Materials & Methods



Soft Wheat bran
(Bermude variety)



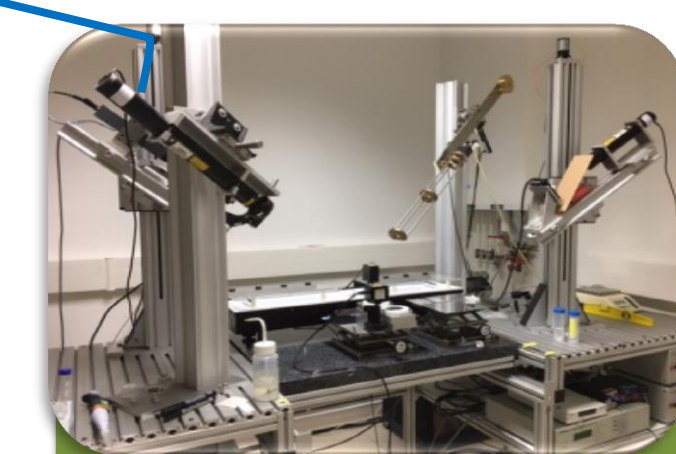
contents in
phenolic acids
(gallic, vanillic,
ferulic, sinapic,
coumaric) and
hexyl resorcinol
=> by UV-HPLC

Putative identity of other
compounds => mass
spectrometry (Esi-MS/MS)

Characterization of extracts



Reducing power =>
DPPH radical scavenging
capacity assay



Surface
interactions

Tensiometry/Ellipsometry
Ellipsometry Δ (°)
Wilhelmy
Balance
 Π (mN/m)
 π (surface pressure) is indicative
of protein insertion,
 Δ (ellipsometric angle) is related
to the amount of matter at the
interface

7 DES

Name	Composition	Molar ratio	A_w	VC	KF	VC	pH
DES 1	ChCl* 1,2-propanediol	W 1:1:1	0,51	0,05	7,2	0,07	5,42
DES 2	ChCl Glycerol	x 1:2	0,12	0,12	0,2	0,03	3,90
DES 2b	ChCl Glycerol	W 1:2:2	0,13	0,17	0,4	0,01	3,95
DES 3	ChCl Lactic Acid	x 1:10	0,29	0,01	6,3	0,04	-0,77
DES 3b	ChCl Lactic Acid	W 1:10:6	0,41	0,03	13,6	0,02	-0,34
DES 4	ChCl Citric Acid	x 1:2			Eutectic not reached		
DES 4b	ChCl Citric Acid	W 1:2:3	0,36	0,01	13,3	0,03	-0,90
DES 5	Betaine Citric Acid	x 1:1			Eutectic not reached		
DES 5b	Betaine Citric Acid	W 1:1:2	0,38	0,09	10,1	0,12	2,36

*ChCl, cholinium chloride; W, water; A_w , water activity; KF, Karl Fisher; VC= variation coefficient

3 three reference organic solvents:
acetone/water 50:50 v/v, n-propanol/water
70:30 v/v, ethanol/water 60:40 v/v

RESULTS

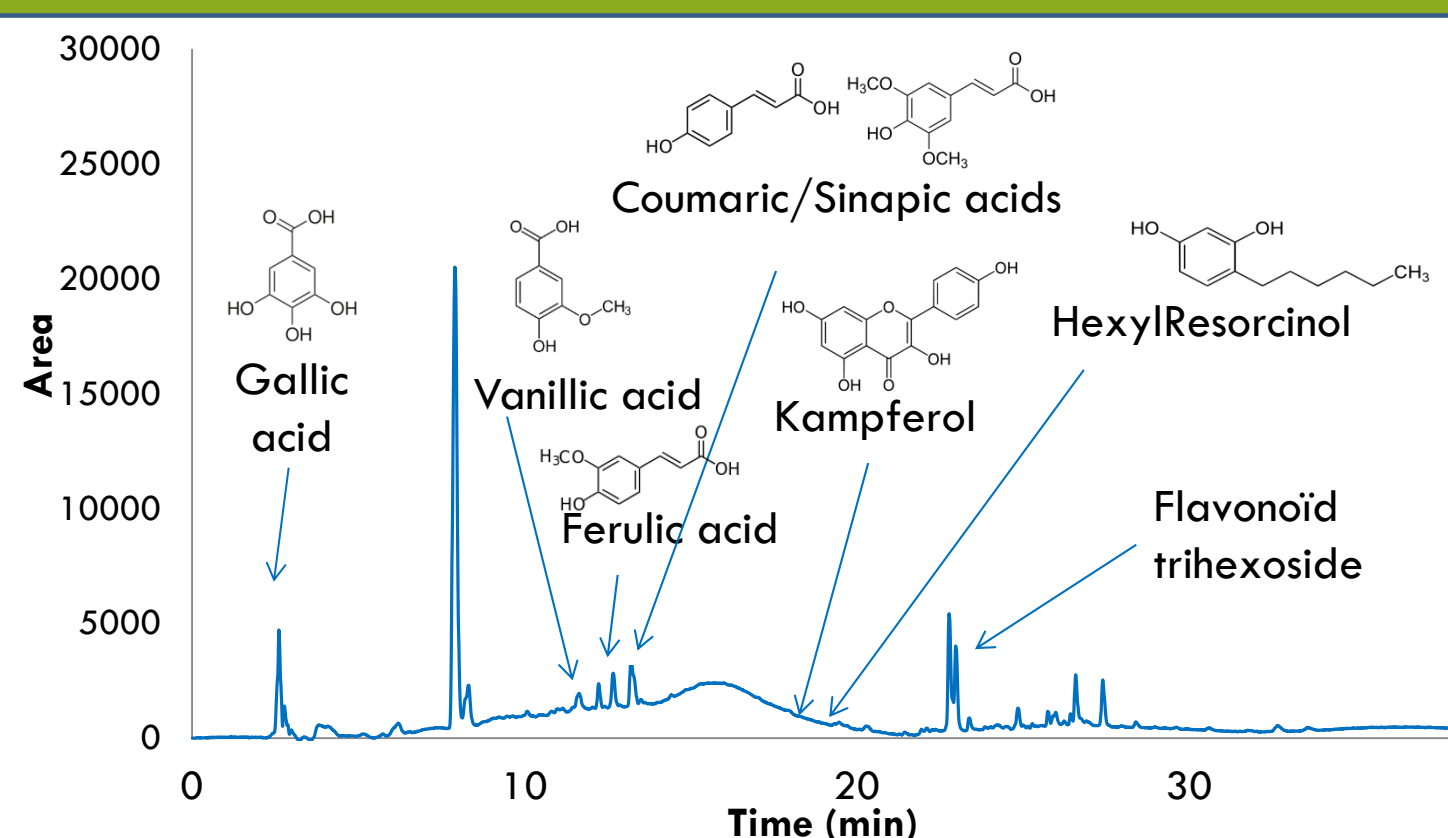


Figure 1: Identification of compounds on HPLC spectra (UV=280 nm, ethanol/water 60:40 v/v, 40°C, 25 min).

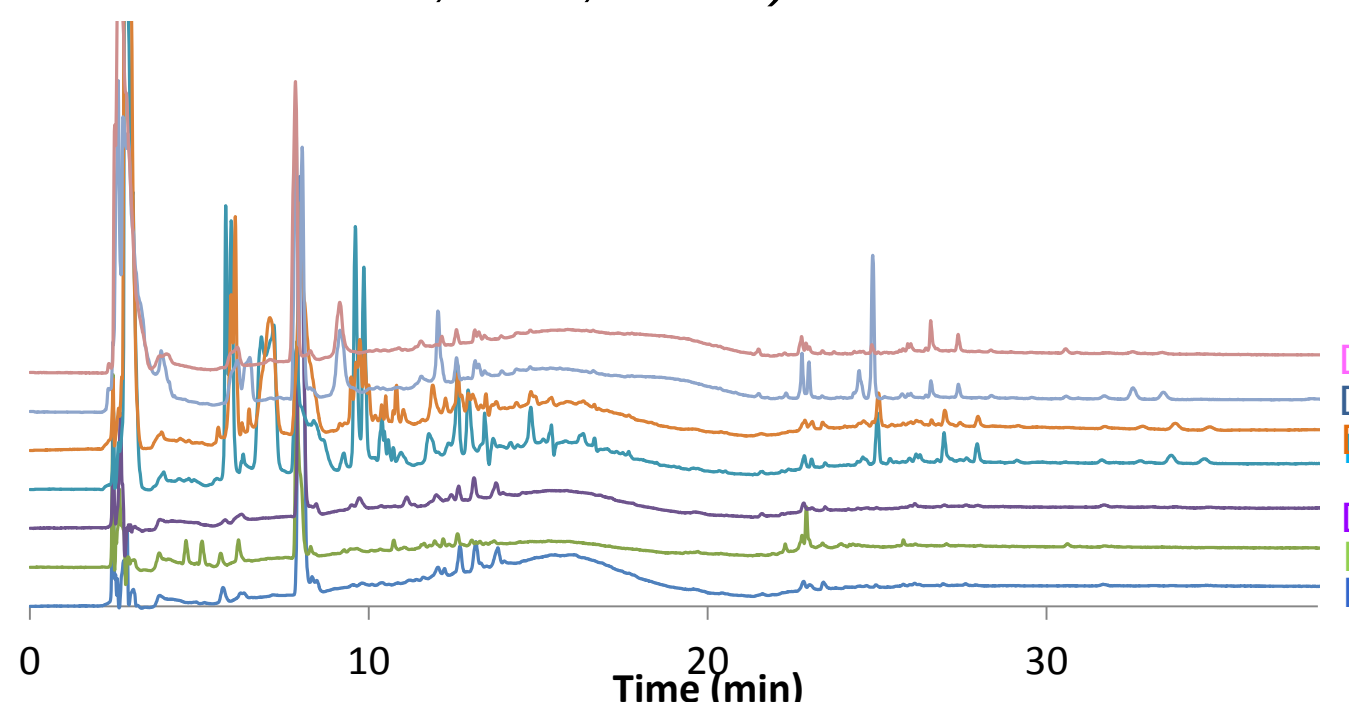


Figure 2: Compared chromatographic profiles of wheat bran DES extracts (UV=280 nm, 40°C, 25 min).

DES 1, 2 et 2b present quite similar profiles to reference solvents. Other DES allowed the extraction of other compounds. DES 3 and 3 b extracted the highest number of compounds (diverse and numerous peaks) among which flavonoids, quinones, resorcinols and small MW compounds were identified by Ms/Ms.

Conclusions

DES are good alternative to conventional organic solvents and could constitute good media to concentrate wheat bran antioxidants and add value to this abundant low-cost by-product of the milling industry.

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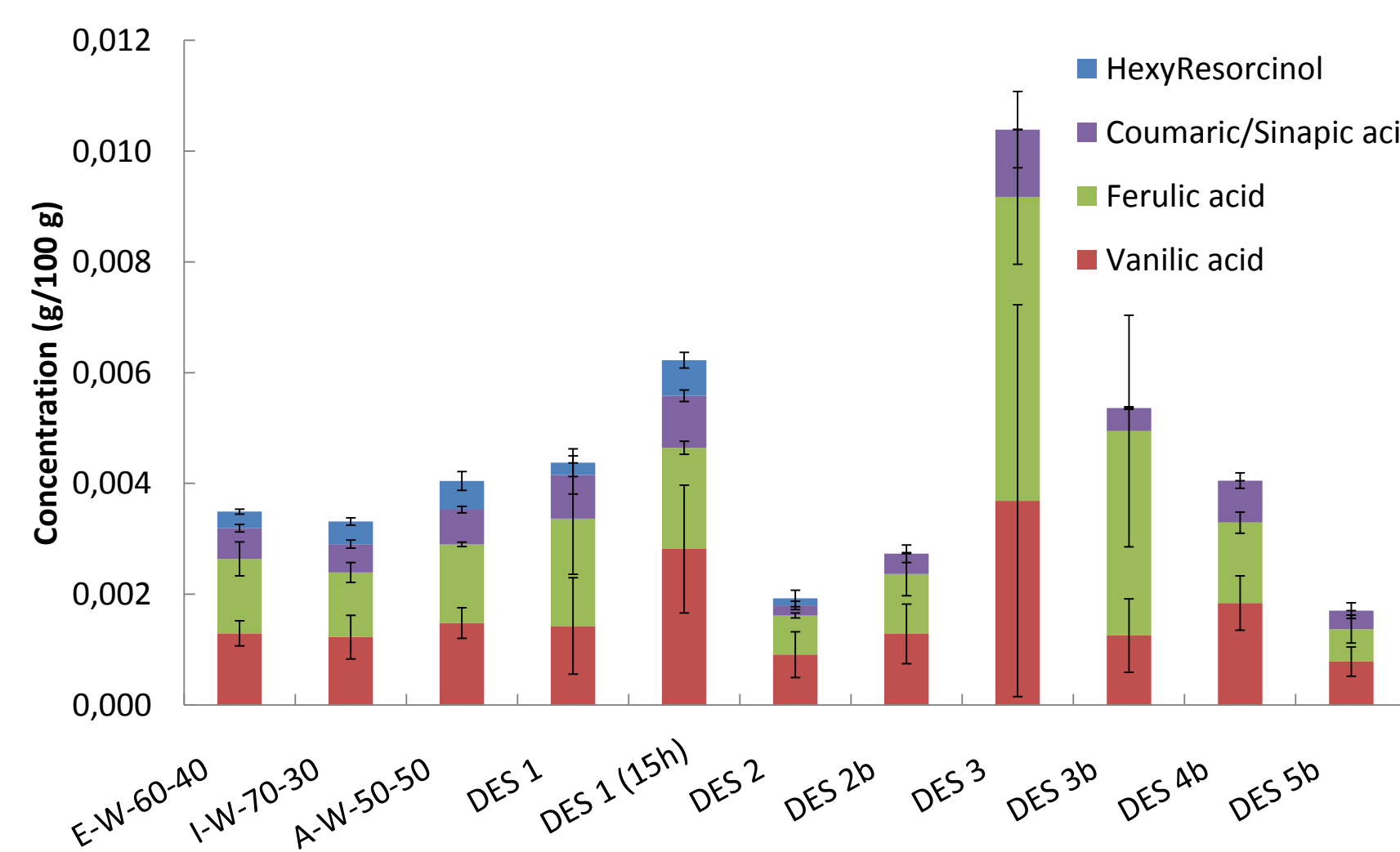


Figure 3: Comparison of contents in phenolic acids obtained by reference solvent extraction or using DES. Abbreviations : E-W-60-40 ethanol/water 60 :40 v/v, I-W-70-30 isopropanol/water 70:30 v/v, A-W-50-50 acetone/water 50:50 v/v.

The best organic solvent was acetone/water 50:50 v/v allowed extracting ~4 mg/100 g phenolic acids among which 1.4 mg/100 g ferulic acid. This quantity was 2.6 fold enhanced using DES 3 (cholinium chloride/lactic acid 1:10). Glycosylated flavonoids were also detected by mass spectrometry in this extract.

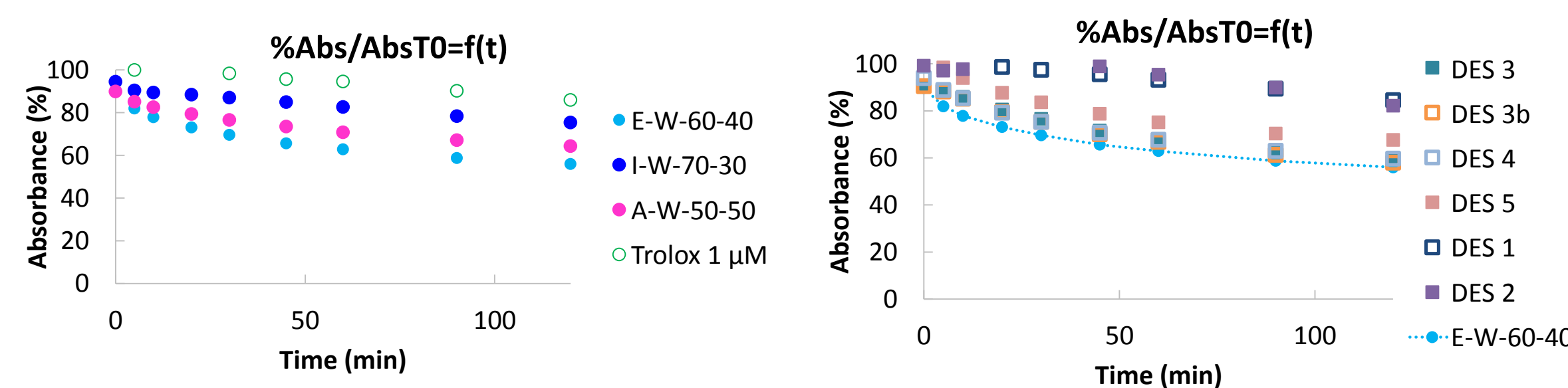


Figure 4: DPPH assay absorbance of wheat bran DES extracts in comparison with reference solvents extraction. Abbreviations : E-W-60-40 ethanol/water 60 :40 v/v, I-W-70-30 isopropanol/water 70:30 v/v, A-W-50-50 acetone/water 50:50 v/v.

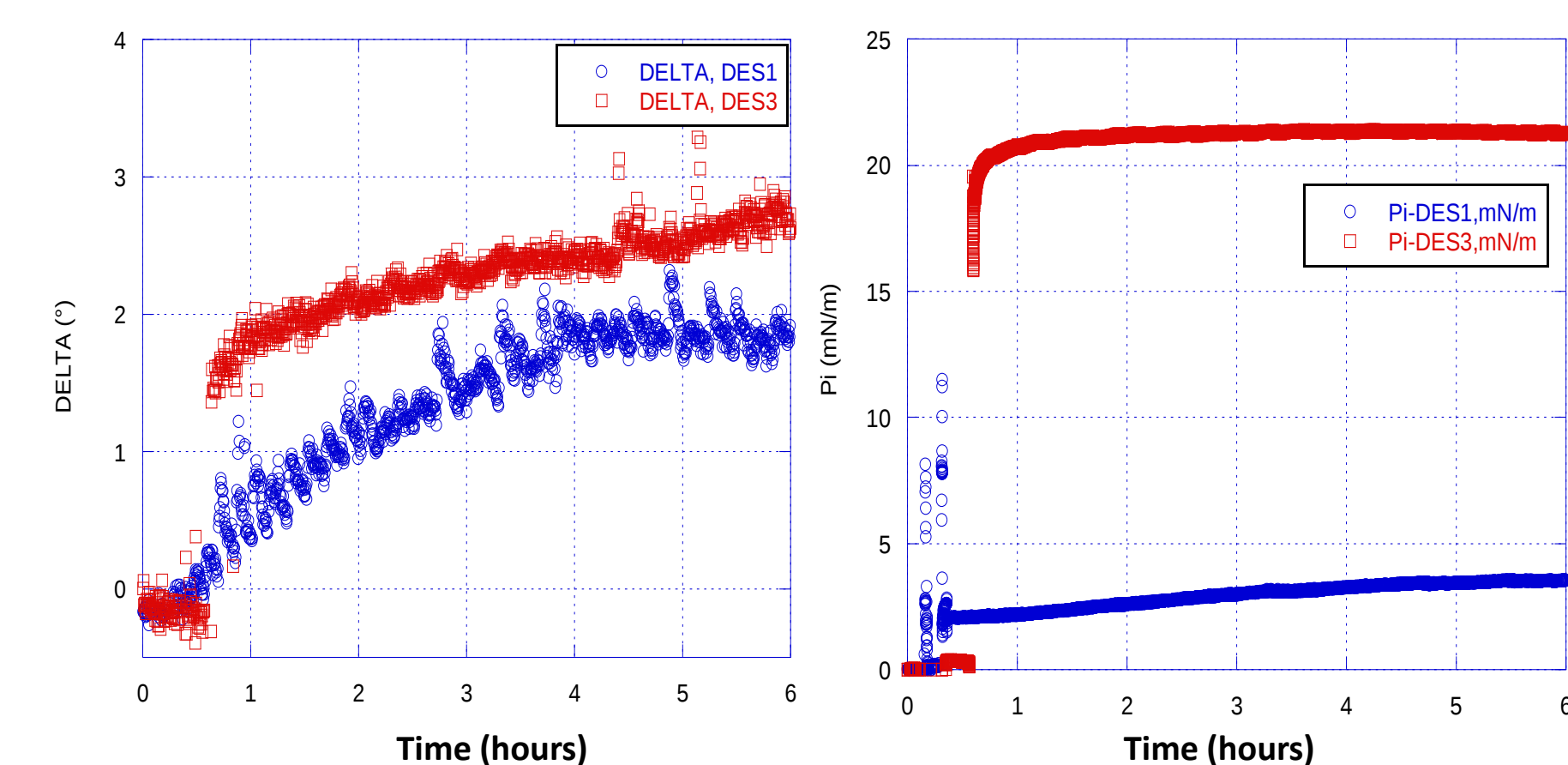


Figure 5: Comparison of ellipsometric angle (Delta, °) and surface tension (Pi, N/m) increases induced by the presence of DES 1 and DES 3 in Langmuir cuve. (Buffer Tris/HCl pH 7, DES at 0.5 % w/v).

An insight into the tensioactive properties of DES 3 indicated that it was strongly tensioactive (22 mN/m at 0.5 % in phosphate buffer), property which could contribute to its high extractive power.

The reductive power of DES 3 extract was also the strongest among DES and more important than Trolox at 1 μM although DES extracts were 5 times more diluted.