Eco-friendly extraction of bioactive metabolites from Ecuadorian quinoa (*Chenopodium quinoa* Willd.) by natural deep eutectic solvents (NADES)

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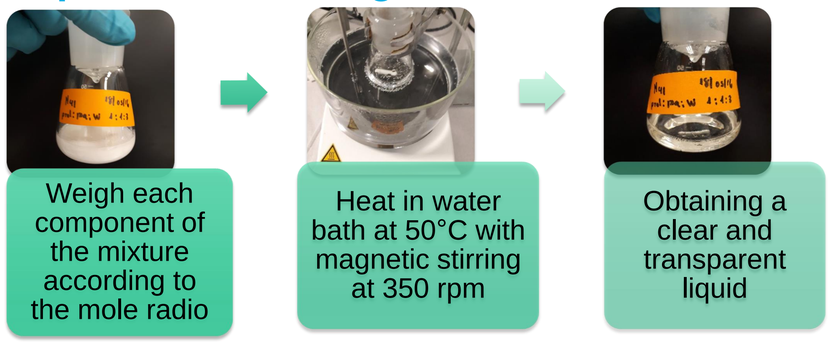
Introduction

Natural deep eutectic solvents (NADES), prepared with abundant and low-cost primary metabolites, are eco-friendly, non-toxic, biodegradable, easy to prepare and possess a remarkable solubilizing potential. Quinoa is an Andean pseudocereal whose production and consumption have increased worldwide as it was reported to contain bioactive compounds with beneficial effects on health. As other parts of the plant could yield to interesting and health-promoting new by-products, different types of NADE (viscous and less viscous) solvents were investigated, using simple and rapid HPTLC-bioautographic methods, for their capacity to extract antioxidant compounds from quinoa leaves. The most abundant sapogenins were identified in the husk of bitter quinoa seeds and in bitter quinoa seeds by LC-MS.

Natural Deep Eutectic Solvents

Preparation: Heating method



Weigh each component of the mixture according to the mole radio

Heat in water bath at 50°C with magnetic stirring at 350 rpm

Obtaining a clear and transparent liquid

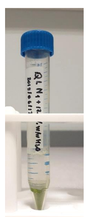
NADES composition	
Viscous	**Less viscous**
N_1: Malic acid : choline chloride : water (1:1:2)	N_5: 1,2-propanediol : choline chloride : water (1:1:1)
N_2: Glucose : choline chloride : water (2:5:5)	N_6: Lactic acid : glucose : water (5:1:3)
N_3: Proline : malic acid : water (1:1:3)	N_7: Glycerol : choline chloride : water (2:1:1)
N_4: Fructose : glucose : sucrose : water (1:1:1:11)	N_8: Xilitol : choline chloride : water (1:2:3)

Extraction

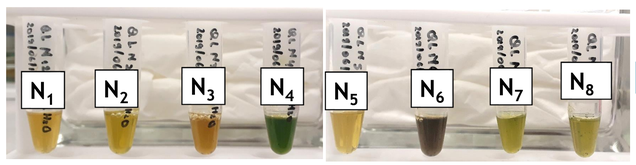
Water was added to improve the extraction process by reducing the viscosity of NADES: N_1+17.5%w/w H_2O, N_2+20%w/w H_2O, N_3+10%w/w H_2O and N_4+10%w/w H_2O.
The thermograms do not present melting points, characterising these diluted NADES as eutectic solvents.

N_1, 17.5 % w/w H_2O +

Lyophilized and milled quinoa leaves INIAP Tunkahuan Ecuadorian variety



Vortex 5 min
Heat 40°C, 60 min
Ultrasonicate RT, 30 min
Centrifugate 25°C, 40 min, 4000 g



NADES extracts

Recover the bioactive compounds using solid-phase extraction (SPE) with Strata X Phenomenex cartridges. The cartridge was placed in a vacuum manifold, equilibrated with 5 mL of EtOH and 5 mL of water, loaded with 1 mL of sample, rinsed twice with 6 mL of water and then eluted with 6 mL of EtOH. The EtOH was dried and the sample was dissolved in 1 mL of MeOH

Phenolic compounds extracted with eight different NADES (N_1 to N_8): (1) HPTLC CHEMICAL PROFILING AND (2) HPTLC-BIOAUTOGRAPHY

METHODOLOGY AND RESULTS

(1) HPTLC CHEMICAL PROFILING OF PHENOLIC COMPOUNDS

HPTLC conditions:

Mobile phase: formic acid - water – methyl ethyl ketone - ethyl acetate (10:10:30:50 v/v)

Derivatization

a) 10 g/L solution of diphenylboric acid aminoethyl ester in MeOH
b) 50 g/L solution of PEG400 in MeOH

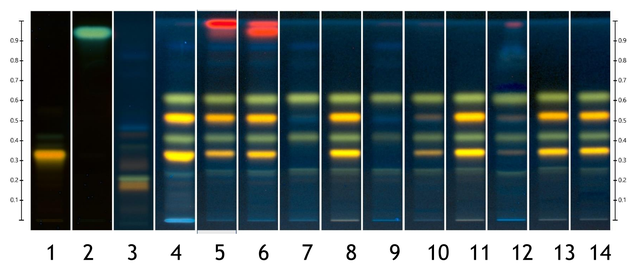


Fig. 1 High performance thin-layer chromatograms of standard solutions: Rutin (1), Kaempferol (2). Samples: quinoa seed methanolic extract (3) and quinoa leaf extract in water (4), methanol (5) methanol +20 % H_2O (6); quinoa leaf NADES N_1 (7), N_2 (8) N_3 (9) N_4 (10), N_5 (11), N_6 (12), N_7 (13) and N_8 (14) extracts

The green and yellow zones in quinoa leave samples correspond to kaempferol and quercetin derivatives respectively, which are flavonoids that have shown *in vitro* anticancer effect in different cell lines.

(2) DPPH ASSAY

Yellowish zones against the purple background show antioxidant activity

HPTLC conditions:

Mobile phase: formic acid - water – methyl ethyl ketone - ethyl acetate (10:10:30:50 v/v)

Derivatization

0.5 mg/mL solution of 2,2-di-(4-tert-octylphenol)-1-picrylhydrazyl in MeOH. Leave the plate in dark for 60 min

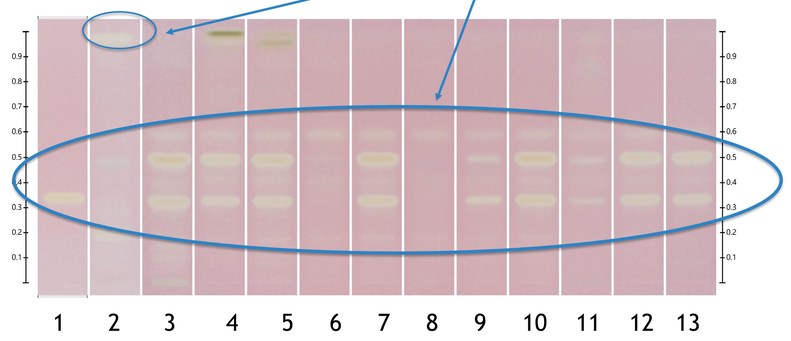


Fig. 2 DPPH assay of standard solution of Rutin (1). Samples: quinoa seed methanolic extract (2) and quinoa leaf extract in water (3), methanol (4) methanol +20 % H_2O (5); quinoa leaf NADES N_1 (6), N_2 (7) N_3 (8) N_4 (9), N_5 (10), N_6 (11), N_7 (12) and N_8 (13) extracts

Saponins extracted with NADES N_7: LC-MS identification

METHODOLOGY AND RESULTS

LC-MS conditions:

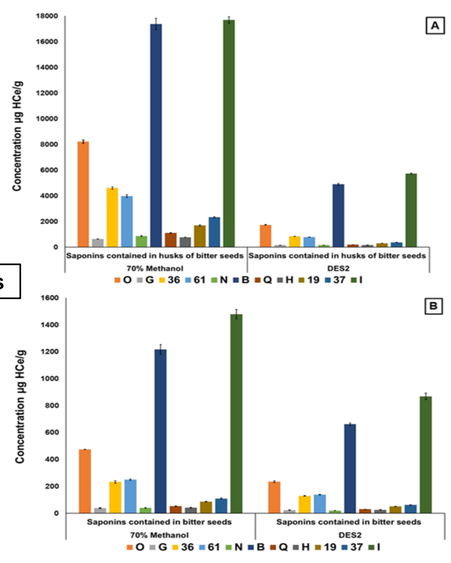
Non polar columna (Acquity UPLC BEH C18)
Mobile phase: 0.1 % (v/v) formic acid in water and acetonitrile in gradient elution

Electrospray ionization in the positive ionization mode

Ion monitoring mode was based on full scan recording in the *m/z* 50 to 2000 range

Husk of bitter seeds

Bitter seeds



Relative amounts of saponins identified in the samples: (A) husks of bitter seeds; and (B) bitter seeds, extracted either with 70 % methanol or NADES (choline chloride - glycerol - water at a molar ratio 1:2:1)

n = 3 technical replicates.

ID	Composition	m/z $[M+H]^+$	Δ (ppm) (a)	R_1	R_2	Aglycone (b)	R_3 (c)	Retention time (min)	Samples of bitter quinoa	
									Convent. extract	NADES extract
O	$C_{54}H_{86}O_{25}$	1135.5536	0.3	- $COOCH_3$	- CH_2OH	PA	Glc-Glc-Ara -	5.31	√	√
G	$C_{54}H_{86}O_{24}$	1119.5587	1.5	- $COOCH_3$	- CH_2OH	SA	Glc-Glc-Ara -	6.31	√	√
36	$C_{53}H_{84}O_{24}$	1105.5431	1.1	- C_2H_5	- CH_2OH	AG487	Hex-Hex-Pent-	4.84	√	√
61	$C_{53}H_{84}O_{23}$	1091.5638	3.6	- CH_3	- CH_2OH	Hed	Glc-Glc-Ara -	6.51	√	√
N	$C_{49}H_{78}O_{21}$	1003.5114	0.9	- $COOCH_3$	- CH_2OH	PA	Glc-Gal -	4.73	√	√
B	$C_{48}H_{76}O_{20}$	973.5008	0.1	- $COOCH_3$	- CH_2OH	PA	Glc-Ara -	5.36	√	√
Q	$C_{48}H_{78}O_{19}$	959.5215	2.2	- $COOCH_3$	- CH_2OH	Hed	Glc-Gal -	5.90	√	√
H	$C_{48}H_{76}O_{19}$	957.5059	4.1	- $COOCH_3$	- CH_3	SA	Glc-Ara -	6.32	√	√
19	$C_{47}H_{76}O_{19}$	945.5059	1.4	- CH_2OH	- CH_2OH	AG489	Glc-Xyl -	3.69	√	√
37	$C_{47}H_{74}O_{19}$	943.4902	4.6	- C_2H_5	- CH_2OH	AG487	Hex-Pent-	4.87	√	√
I	$C_{47}H_{76}O_{18}$	929.5110	0.3	- CH_3	- CH_2OH	Hed	Glc-Ara -	6.58	√	√

Conclusions and perspectives

Extraction of quinoa leaves bioactive compounds is possible using NADES; given their non-toxicity, these solvents allow direct addition of extracts to food formulations and pharmaceutical products. NADES quinoa leaves extracts are antioxidants, qualities for possible new functional ingredients. Regardless the sample or extraction method, the major detected sapogenins were hederagenin and phytolaccagenic acid. Interestingly, the extracts of quinoa metabolites in NADES show high stability (they retain initial organoleptic characteristics) in storage at 5°C for several weeks, which represents an advantage for the industry avoiding separation and purification processes. The elaboration of products based on quinoa NADES-extracts such as edible film coating or multifunctional wound dressing hydrogel is in process.

Acknowledgment
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