Sustainability Impact for Extraction (SIX Score): from Concepts and Principles to Impact-based Metric for Green Extraction Assessment

Giorgio Capaldi, Clelia Aimone, Giorgio Mongardi, Emanuela Calcio Gaudino, Giorgio Grillo,* and Giancarlo Cravotto

Electronic Supporting Information (ESI)

Table of Abbreviations:

Abbreviation	Name
2-MeTHF	2-Methyltetrahydrofuran
AE	Atom Economy
AGREE	Analytical Greenness calculator
AGREEprep	Analytical Greenness metric for sample preparation
BAGI	Blue Applicability Grade Index
BOLD	Big Open Linked Data
CE	Carbon Economy
ComplexGAPI	Complementary Green Analytical Procedure Index
CPME	Cyclopentyl Methyl Ether
EATOS	Environmental Assessment Tool for Organic Syntheses
EFs	Elemental Factors
EMY	Effective Mass Yield
EQ	Environmental Quotient
Ex	Extraction
GAPI	Green Analytical Procedure Index
GE	Green Extraction
GHG	greenhouse gas
GHS	Globally Harmonized System
GRAS	Generally Recognized As Safe
h	severity of the hazard (Eq. 3)
iGAL	Innovation Green Aspiration Level
LCA	Life Cycle Assessment
MAE	Microwave-assisted Extraction
NaDES	Natural Deep Eutectic Solvent
NEMI	National Environmental Method Index

Org. Fr.	Organic Fraction
p	Prevention (Eq. 3)
P&E	Process and Equipment
PEF	Pulsed Electric Field
PME	Process Mass Efficiency
PMI	Process Mass Intensity
PP	Penalty Point
PP_{Add}	Penalty Point Additives
PP_{AddQnt}	Penalty Point Additive Quantity
PP_{CE}	Penalty Point Carbon Economy
PP_{CE}	Penalty Point Carbon Economy
PP_{Cont}	Penalty Point Contaminants
PP_{E}	Penalty Point Energy Consumption
PP_{MD}	Penalty Point Matrix Depletion
PP_{oh}	Penalty Point Occupational Hazard
PP_{p}	Penalty Point Pressure
PP_{pic}	Penalty Point Pictogram
PP_{PME}	Penalty Point Process Mass Efficiency
PP_{Sel}	Penalty Point Selectivity
PP_{SI}	Penalty Point Solvent Intensity
PP_{Sol}	Penalty Point Solvents
$PP_{SolvQnt}$	Penalty Point Solvent Quantity
PP_{sw}	Penalty Point Signal Words
PP_{TE}	Penalty Point Time Effectiveness
Pr	Product
Q	Unfriendliness Quotient
q	quantity factor (Eq. 3)
RM	Raw Material
RME	Reaction Mass Efficiency
S&A	Solvents and Additives
UAE	Ultrasound-assisted Extraction
UCD	User-Centered Design approach
W	Waste

Elemental Factors (EF): Details

1. Raw Material

Item	Produced ton in 2023	Notes
Hempseeds	417980	
Vanilla	769816	
Cranberries	919167	
Snails	1985763	
Hop Cones	2555716	
Locust Beans	2830824	
Gooseberries	8992507	Threshold for 10M tons
Hazelnut	16658433	
Blueberry	28195344	
Kapok Fruit	29028682	
Areca Nuts	52365482	
Currants	55830903	
Camel Meat	56019952	
Almonds	60612917	
Karite	64223479	
Safflawer Seed	64889913	
Artichoke	84659861	
Cherries	109988238	
Cashew apple	131892163	
Buckwheat	195950178	
Sunflower seeds	273961317	
Camels Raw Milk	281617612	
Okra	290557153	≈ Median
Ginger	319845089	
Chestnut	320324316	
Sesame Seeds	412637081	
Coffe	527322186	
Olive	566216341	
Barley	641177804	
Avocado	926808985	
Apple	1692969836	
Pineapple	2055963730	
Cattle meat	2117526104	
Orange	2900521724	≈ Average
Chicken meat	3660426289	
Mushroom	4795727024	
Soya	6283998992	
Banana	6339120285	
Pigs meat	6902727032	
Onion	8402661192	
Cattle Raw Milk	9863939733	
Wheat	13582666441	
Potato	16988996756	
Tomato	17911590299	
Cassava	18605185230	

Figure 1S. FAOSTAT data related to global production in ton of different food related biomass

A

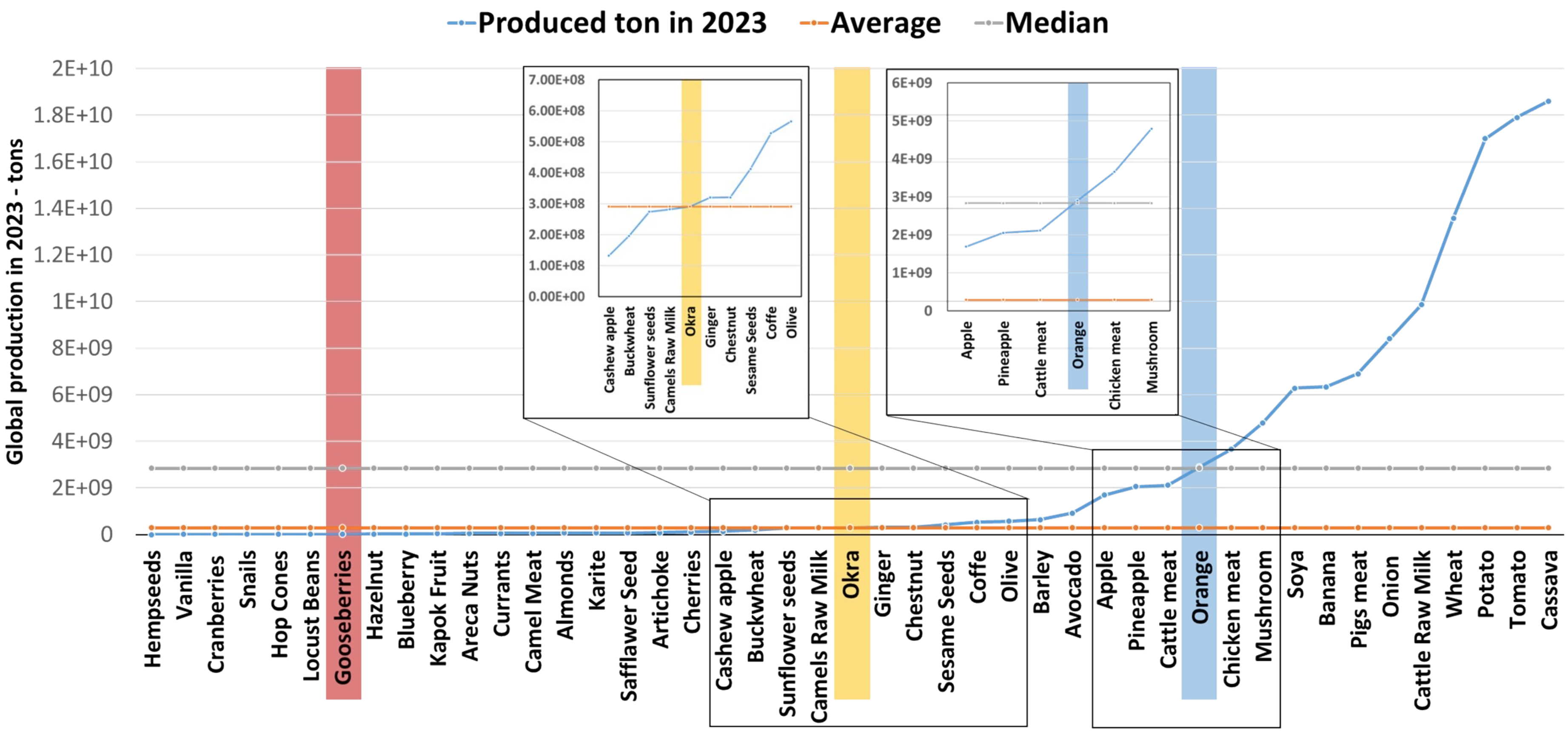


Figure 2S-A. Relative production quantity of food products in 2023 (FAOSTAT Database): General outlook and details on limit threshold, Average and Median values.

B

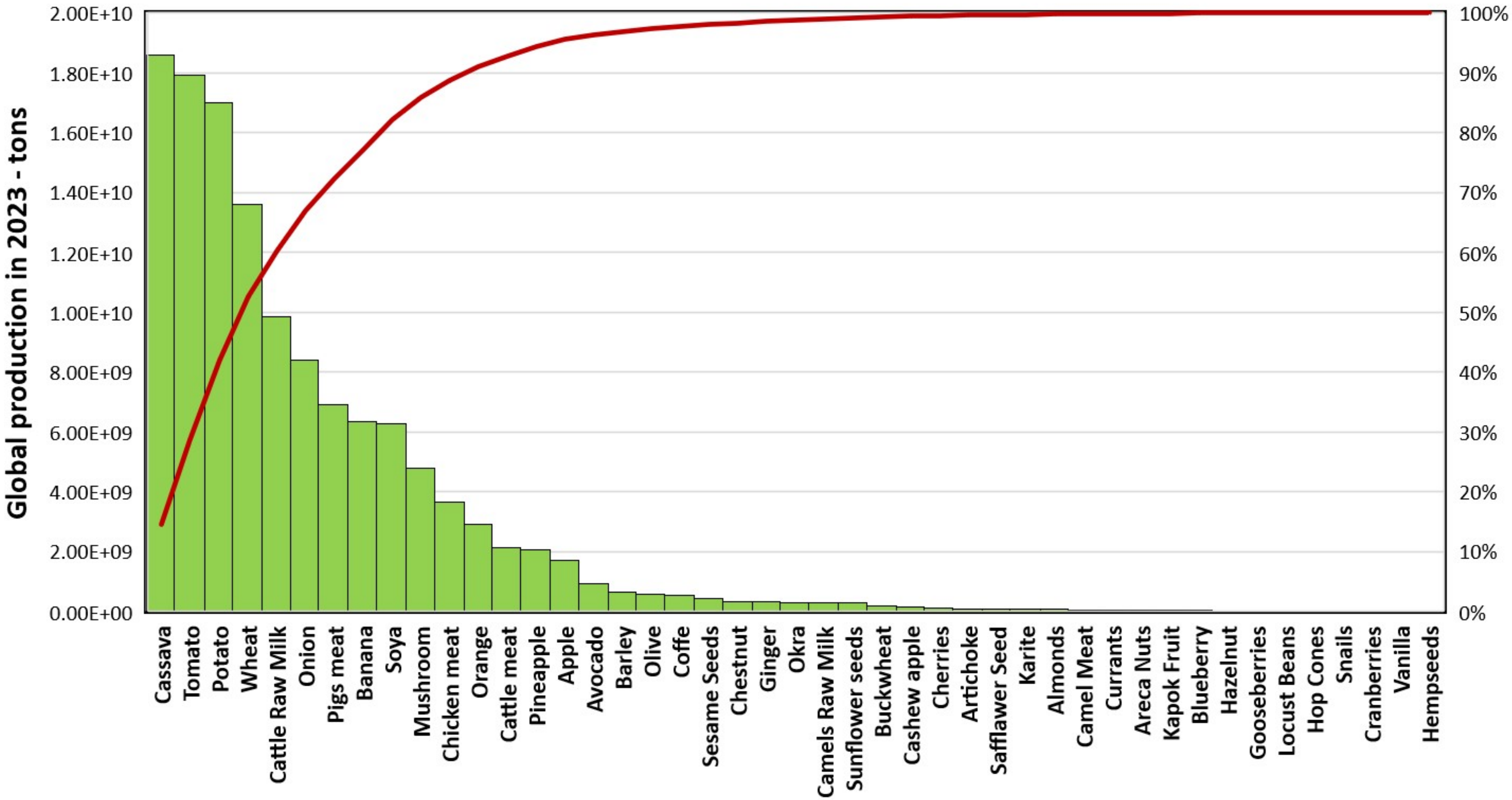


Figure 2S-B. Relative production quantity of food products in 2023 (FAOSTAT Database): Pareto Chart.

Table 1S: *Solvent & Additives EF, $PP_{Hazardousness}$ calculation for different classes of solvents.* **A:** *Alcohols/Polyols*; **B:** *Ethers*; **C:** *Esters*; **D:** *Acetals/Ketals*; **E:** *Ketones*; **F:** *Amides*; **G:** *Hydrocarbons (Linear)*; **H:** *Hydrocarbon (Aromatics)*; **I:** *Halogenated*; **J:** *Nitriles*; **K:** *Sulfoxides*; **L:** *Carbonates*.

		Penalty Points →	4	1	2	2	3	1	3	1	0	1	2	0	3	
		Hazard Group →	*Physical Hazards*				*Health Hazards*			*Environmental Hazards*						
		GHS Identifiers →	GHS01	GHS02	GHS03	GHS05	GHS06	GHS07	GHS08	GHS09	**Signal Words**			**Occupational Hazard**		
		Pictograms →														
		A. Alcohols/ Polyols	**Exploding Bomb**	**Flame**	**Flame Over Circle**	**Corrosion**	**Skull and Crossbones**	**Exclamation Mark**	**Health Hazard**	**Environment**	**None**	**Warning**	**Danger**	**Zero emission**	**Vapour/ Gas emission**	**$PP_{Hazardousness}$**
#	CAS	Name														
1	67-56-1	Methanol (MeOH)		X			X		X				X		X	**12**
2	64-17-5	Ethanol (EtOH)		X				X					X		X	**7**
3	71-23-8	1-Propanol (n-PrOH)		X		X		X					X		X	**9**
4	67-63-0	2-Propanol (iPrOH)		X				X					X		X	**7**
5	71-36-3	1-Butanol (n-BuOH)		X		X		X					X		X	**9**
6	78-92-2	2-Butanol (sec-BuOH)		X				X				X			X	**6**
7	75-65-0	tert-Butanol (t-BuOH)		X				X					X		X	**7**
8	100-51-6	Benzyl alcohol						X				X		X		**2**
9	107-21-1	Ethylene glycol						X	X			X		X		**5**
10	57-55-6	Propylene glycol (1,2-propanediol)									X			X		**0**
11	56-81-5	Glycerol									X			X		**0**
12	504-63-2	1,3-propanediol									X			X		**0**
13	25322-68-3	PEG-200									X			X		**0**
14	25322-68-3	PEG-400									X			X		**0**

	GHS01	GHS02	GHS03	GHS05	GHS06	GHS07	GHS08	GHS09						
Penalty Points →	4	1	2	2	3	1	3	1	0	1	2	0	3	
Hazard Group →	*Physical Hazards*			*Health Hazards*				*Environmental Hazards*	**Signal Words**			**Occupational Hazard**		
GHS Identifiers →	GHS01	GHS02	GHS03	GHS05	GHS06	GHS07	GHS08	GHS09						
Pictograms →	**Exploding Bomb**	**Flame**	**Flame Over Circle**	**Corrosion**	**Skull and Crossbones**	**Exclamation Mark**	**Health Hazard**	**Environment**	**None**	**Warning**	**Danger**	**Zero emission**	**Vapour/ Gas emission**	**$PP_{Hazardousness}$**

B. Ethers

#	CAS	Name	Exploding Bomb	Flame	Flame Over Circle	Corrosion	Skull and Crossbones	Exclamation Mark	Health Hazard	Environment	None	Warning	Danger	Zero emission	Vapour/ Gas emission	$PP_{Hazardousness}$
15	5614-37-9	Cyclopentyl methyl ether (CPME)			X				X						X	**7**
16	96-47-9	2-Methyltetrahydrofuran (2-MeTHF)			X		X		X						X	**9**
17	5306-85-4	Isosorbide dimethyl ether										X		X		**0**
18	109-99-9	Tetrahydrofuran (THF)			X				X	X					X	**10**
19	2679-89-2	Diethyl ether (Et_2O)			X				X						X	**7**
20	1634-04-4	tert-Butyl methyl ether (MTBE)			X				X						X	**7**
21	108-20-3	Diisopropyl ether (DIPE)			X				X						X	**7**
22	100-66-3	Anisole (methoxybenzene)			X				X				X	X		**3**

C. Esthers

#	CAS	Name	Exploding Bomb	Flame	Flame Over Circle	Corrosion	Skull and Crossbones	Exclamation Mark	Health Hazard	Environment	None	Warning	Danger	Zero emission	Vapour/ Gas emission	$PP_{Hazardousness}$
23	687-47-8	Ethyl lactate			X		X		X					X		**6**
24	539-88-8	Ethyl levulinate							X				X	X		**2**
25	141-78-6	Ethyl acetate (EtOAc)			X				X						X	**7**
26	108-21-4	Isopropyl acetate (iPrOAc)			X				X						X	**7**
27	123-86-4	n-Butyl acetate (n-BuOAc)			X				X				X		X	**6**
28	79-20-9	Methyl acetate (MeOAc)			X				X						X	**7**
29	123-92-2	Isoamyl acetate			X								X		X	**5**
30	102-76-1	Triacetin (glycerol triacetate)										X		X		**0**

	Exploding Bomb	Flame	Flame Over Circle	Corrosion	Skull and Crossbones	Exclamation Mark	Health Hazard	Environment	None	Warning	Danger	Zero emission	Vapour/ Gas emission	$PP_{Hazardousness}$
Penalty Points →	4	1	2	2	3	1	3	1	0	1	2	0	3	
Hazard Group →	*Physical Hazards*				*Health Hazards*			*Environmental Hazards*	**Signal Words**			**Occupational Hazard**		
GHS Identifiers →	GHS01	GHS02	GHS03	GHS05	GHS06	GHS07	GHS08	GHS09						
Pictograms →														

D. Acetals/ Ketals

#	CAS	Name	Exploding Bomb	Flame	Flame Over Circle	Corrosion	Skull and Crossbones	Exclamation Mark	Health Hazard	Environment	None	Warning	Danger	Zero emission	Vapour/ Gas emission	$PP_{Hazardousness}$
31	100-79-8	Solketal						X			X			X		**1**
32	646-06-0	1,3-Dioxolane		X				X	X				X		X	**10**
33	646-06-0	1,3-Dioxane		X				X	X				X		X	**10**
34	77-76-9	2,2-Dimethoxypropane (DMP)		X				X					X		X	**7**

E. Ketones

#	CAS	Name	Exploding Bomb	Flame	Flame Over Circle	Corrosion	Skull and Crossbones	Exclamation Mark	Health Hazard	Environment	None	Warning	Danger	Zero emission	Vapour/ Gas emission	$PP_{Hazardousness}$
35	53716-82-8	Dihydrolevoglucosenone						X				X		X		**2**
36	67-64-1	Acetone		X				X					X		X	**7**
37	78-93-3	2-Butanone (MEK)		X				X					X		X	**7**
38	108-10-1	Methyl isobutyl ketone (MIBK)		X				X	X				X		X	**10**
39	108-94-1	Cyclohexanone		X		X		X					X	X		**6**
40	107-87-9	2-Pentanone (methyl propyl ketone)		X				X					X		X	**7**

F. Amides

#	CAS	Name	Exploding Bomb	Flame	Flame Over Circle	Corrosion	Skull and Crossbones	Exclamation Mark	Health Hazard	Environment	None	Warning	Danger	Zero emission	Vapour/ Gas emission	$PP_{Hazardousness}$
41	68-12-2	dimethylformamide (DMF)		X				X	X				X	X		**7**
42	872-50-4	N-Methyl-2-pyrrolidone (NMP)						X	X				X	X		**6**
43	127-19-5	Dimethylacetamide (DMA)						X	X				X	X		**6**

		Penalty Points →	4	1	2	2	3	1	3	1	0	1	2	0	3	
		Hazard Group →	*Physical Hazards*			*Health Hazards*				*Environmental Hazards*						
		GHS Identifiers →	GHS01	GHS02	GHS03	GHS05	GHS06	GHS07	GHS08	GHS09	**Signal Words**			**Occupational Hazard**		
		Pictograms →	**Exploding Bomb**	**Flame**	**Flame Over Circle**	**Corrosion**	**Skull and Crossbones**	**Exclamation Mark**	**Health Hazard**	**Environment**	**None**	**Warning**	**Danger**	**Zero emission**	**Vapour/ Gas emission**	**$PP_{Hazardousness}$**

G. <u>Hydrocarbons (Linear)</u>

#	CAS	Name	Exploding Bomb	Flame	Flame Over Circle	Corrosion	Skull and Crossbones	Exclamation Mark	Health Hazard	Environment	None	Warning	Danger	Zero emission	Vapour/ Gas emission	$PP_{Hazardousness}$
44	473-55-2	Dihydropinene		X						X		X		X		**3**
45	5989-27-5	d-Limonene		X				X	X	X			X	X		**8**
46	80-56-8	α-Pinene		X				X	X	X			X		X	**11**
47	127-91-3	β-Pinene		X				X	X	X			X	X		**8**
48	99-87-6	p-Cymene		X				X				X		X		**3**
49	110-82-7	Cyclohexane		X				X	X	X			X		X	**11**
50	142-82-5	n-Heptane		X				X	X	X			X		X	**11**
51	110-54-3	hexane		X				X	X	X			X		X	**11**

H. <u>Hydrocarbon (Aromatics)</u>

#	CAS	Name	Exploding Bomb	Flame	Flame Over Circle	Corrosion	Skull and Crossbones	Exclamation Mark	Health Hazard	Environment	None	Warning	Danger	Zero emission	Vapour/ Gas emission	$PP_{Hazardousness}$
52	108-88-3	Toluene		X				X	X				X		X	**10**
53	1330-20-7	Xylene		X				X	X				X		X	**10**
54	100-41-4	Ethylbenzene		X				X	X				X		X	**10**
55	71-43-2	Benzene		X				X	X				X		X	**10**

	GHS01	GHS02	GHS03	GHS05	GHS06	GHS07	GHS08	GHS09						
Penalty Points →	4	1	2	2	3	1	3	1	0	1	2	0	3	
Hazard Group →	*Physical Hazards*			*Health Hazards*				*Environmental Hazards*	**Signal Words**			**Occupational Hazard**		
GHS Identifiers →	GHS01	GHS02	GHS03	GHS05	GHS06	GHS07	GHS08	GHS09						
Pictograms →	**Exploding Bomb**	**Flame**	**Flame Over Circle**	**Corrosion**	**Skull and Crossbones**	**Exclamation Mark**	**Health Hazard**	**Environment**	**None**	**Warning**	**Danger**	**Zero emission**	**Vapour/ Gas emission**	**$PP_{Hazardousness}$**

I. Halogenated

#	CAS	Name	Exploding Bomb	Flame	Flame Over Circle	Corrosion	Skull and Crossbones	Exclamation Mark	Health Hazard	Environment	None	Warning	Danger	Zero emission	Vapour/ Gas emission	$PP_{Hazardousness}$
56	75-09-2	Dichloromethane (DCM)						X	X			X			X	**8**
57	67-66-3	chloroform					X		X				X		X	**11**
58	107-06-2	1,2-dichloroethane		X			X		X				X		X	**12**

J. Nitriles

#	CAS	Name	Exploding Bomb	Flame	Flame Over Circle	Corrosion	Skull and Crossbones	Exclamation Mark	Health Hazard	Environment	None	Warning	Danger	Zero emission	Vapour/ Gas emission	$PP_{Hazardousness}$
59	75-05-8	Acetonitrile		X				X					X	X		**4**

K. Sulfoxides

#	CAS	Name	Exploding Bomb	Flame	Flame Over Circle	Corrosion	Skull and Crossbones	Exclamation Mark	Health Hazard	Environment	None	Warning	Danger	Zero emission	Vapour/ Gas emission	$PP_{Hazardousness}$
60	67-68-5	dimethyl sulfoxide (DMSO)									X			X		**0**

L. Carbonates

#	CAS	Name	Exploding Bomb	Flame	Flame Over Circle	Corrosion	Skull and Crossbones	Exclamation Mark	Health Hazard	Environment	None	Warning	Danger	Zero emission	Vapour/ Gas emission	$PP_{Hazardousness}$
61	616-38-6	Dimethyl carbonate (DMC)		X									X		X	**6**
62	105-58-8	Diethyl carbonate (DEC)		X								X			X	**5**
63	623-53-0	Ethyl methyl carbonate		X									X		X	**6**
64	108-32-7	Propylene carbonate						X				X		X		**2**
65	931-40-8	Glycerol carbonate									X			X		**0**
66	931-40-8	CO_2													X	**3**

Table 2S: Solvent & Additives EF, $PP_{Hazardousness}$ calculation for different classes of NaDES components. **A:** *Nitrogen Compounds*; **B:** *Carboxylic/Hydroxy Acids*; **C:** *Aromatic Acids*; **D:** *Fatty Acids*; **E:** *Polyols/Sugar alcohols*; **F:** *Hydrophobic/Natural Alcohols*; **G:** *Aliphatic alcohols*.

		Penalty Points →	4	1	2	2	3	1	3	1	0	1	2	0	3	
		Hazard Group →	*Physical Hazards*			*Health Hazards*				*Environmental Hazards*	**Signal Words**			**Occupational Hazard**		$PP_{Hazardousness}$
		GHS Identifiers →	GHS01	GHS02	GHS03	GHS05	GHS06	GHS07	GHS08	GHS09						
		Pictograms →	**Exploding Bomb**	**Flame**	**Flame Over Circle**	**Corrosion**	**Skull and Crossbones**	**Exclamation Mark**	**Health Hazard**	**Environment**	**None**	**Warning**	**Danger**	**Zero emission**	**Vapour/ Gas emission**	

A. Nitrogen Compounds

#	CAS	Name	GHS01	GHS02	GHS03	GHS05	GHS06	GHS07	GHS08	GHS09	None	Warning	Danger	Zero emission	Vapour/ Gas emission	$PP_{Hazardousness}$
											X			X		**0**
65	67-48-1	Choline chloride									X			X		**0**
66	107-43-7	Betaine									X			X		**0**
67	57-13-6	Urea									X			X		**0**

B. Carboxylic/ Hydroxy Acids

#	CAS	Name	GHS01	GHS02	GHS03	GHS05	GHS06	GHS07	GHS08	GHS09	None	Warning	Danger	Zero emission	Vapour/ Gas emission	$PP_{Hazardousness}$
68	64-19-7	Acetic acid		X		X							X		X	**8**
69	79-09-4	Propionic acid		X		X		X					X	X		**6**
70	79-33-4	Lactic Acid				X							X	X		**4**
71	123-76-2	Levulinic acid				X		X					X	X		**5**
72	77-92-9	Citric acid						X				X		X		**2**
73	6915-15-7	Malic acid						X				X		X		**2**
74	141-82-2	Malonic acid				X		X					X	X		**5**
75	144-62-7	Oxalic acid				X		X					X	X		**5**

		Penalty Points →	4	1	2	2	3	1	3	1	0	1	2	0	3
		Hazard Group →	*Physical Hazards*			*Health Hazards*				*Environmental Hazards*	**Signal Words**			**Occupational Hazard**	
		GHS Identifiers →	GHS01	GHS02	GHS03	GHS05	GHS06	GHS07	GHS08	GHS09					
		Pictograms →	**Exploding Bomb**	**Flame**	**Flame Over Circle**	**Corrosion**	**Skull and Crossbones**	**Exclamation Mark**	**Health Hazard**	**Environment**	**None**	**Warning**	**Danger**	**Zero emission**	**Vapour/ Gas emission**	**$PP_{Hazardousness}$**

C. Aromatic Acids

#	CAS	Name	Exploding Bomb	Flame	Flame Over Circle	Corrosion	Skull and Crossbones	Exclamation Mark	Health Hazard	Environment	None	Warning	Danger	Zero emission	Vapour/ Gas emission	$PP_{Hazardousness}$
76	69-72-7	Salicylic acid				X		X	X				X	X		**8**
77	121-34-6	Vanillic acid												X		**0**
78	140-10-3	trans-cinnamic acid						X				X		X		**2**
79	501-98-4	p-coumaric acid						X				X		X		**2**
80	537-98-4	trans-ferulic acid						X				X		X		**2**
81	99-96-7	p-hydroxybenzoic acid						X				X		X		**2**

D. Fatty Acids

#	CAS	Name	Exploding Bomb	Flame	Flame Over Circle	Corrosion	Skull and Crossbones	Exclamation Mark	Health Hazard	Environment	None	Warning	Danger	Zero emission	Vapour/ Gas emission	$PP_{Hazardousness}$
82	107-92-6	Butyric acid				X		X					X	X		**5**
83	142-62-1	Hexanoic acid				X							X	X		**4**
84	124-07-2	Octanoic acid				X							X	X		**4**
85	334-48-5	Decanoic acid				X						X		X		**3**
86	143-07-7	Lauric acid				X							X	X		**4**

			Exploding Bomb	Flame	Flame Over Circle	Corrosion	Skull and Crossbones	Exclamation Mark	Health Hazard	Environment	None	Warning	Danger	Zero emission	Vapour/ Gas emission	$PP_{Hazardousness}$
		Penalty Points →	4	1	2	2	3	1	3	1	0	1	2	0	3	
		Hazard Group →	*Physical Hazards*			*Health Hazards*				*Environmental Hazards*	**Signal Words**			**Occupational Hazard**		
		GHS Identifiers →	GHS01	GHS02	GHS03	GHS05	GHS06	GHS07	GHS08	GHS09						
		Pictograms →														

E. Polyols/ Sugar alcohols

#	CAS	Name	Exploding Bomb	Flame	Flame Over Circle	Corrosion	Skull and Crossbones	Exclamation Mark	Health Hazard	Environment	None	Warning	Danger	Zero emission	Vapour/ Gas emission	$PP_{Hazardousness}$
87	107-21-1	Ethylene glycol						X	X			X		X		**5**
88	56-81-5	Glycerol									X			X		**0**
89	50-70-4	Sorbitol									X			X		**0**
90	87-99-0	Xylitol									X			X		**0**
91	69-65-8	Mannitol									X			X		**0**

F. Hydrophobic/ Natural Alcohols

#	CAS	Name	Exploding Bomb	Flame	Flame Over Circle	Corrosion	Skull and Crossbones	Exclamation Mark	Health Hazard	Environment	None	Warning	Danger	Zero emission	Vapour/ Gas emission	$PP_{Hazardousness}$
92	104-54-1	Cinnamyl alcohol						X		X		X		X		**3**
93	2217-02-9	Fenchyl alcohol						X				X		X		**2**
94	89-78-1	Menthol						X				X		X		**2**
95	89-83-8	Thymol				X		X		X			X	X		**6**
96	76-22-2	Camphor		X		X		X	X	X			X	X		**10**

G. Aliphatic alcohols

		Penalty Points →	4	1	2	2	3	1	3	1	0	1	2	0	3	
		Hazard Group →	*Physical Hazards*			*Health Hazards*				*Environmental Hazards*	**Signal Words**			**Occupational Hazard**		
		GHS Identifiers →	GHS01	GHS02	GHS03	GHS05	GHS06	GHS07	GHS08	GHS09						
		Pictograms →	**Exploding Bomb**	**Flame**	**Flame Over Circle**	**Corrosion**	**Skull and Crossbones**	**Exclamation Mark**	**Health Hazard**	**Environment**	**None**	**Warning**	**Danger**	**Zero emission**	**Vapour/ Gas emission**	**$PP_{Hazardousness}$**
#	CAS	Name														
97	111-27-3	Hexanol		X				X				X		X		**3**
98	111-87-5	Octanol						X				X		X		**2**
99	112-30-1	Decanol						X				X		X		**2**
100	112-53-8	Dodecanol						X				X		X		**2**

Table 3S: Solvent & Additives EF, PP_{Solv} case studies calculation for different classes of NaDES. **A. Binary componets**: Choline chloride, glycerol, lactic acid and ethylene glycol, thymol, decanoic acid; **B. Ternary components:** thymol, menthol, decanoic acid, lauric acid, decanol.

A. Binary

	Components	Molar ratio	Matrix/Solvent	Weight	PP_{Qnt}	$PP_{Hazardousness}$	PP_{Solv} Partial	PP_{Solv} TOT
ChCl:Gly	Choline chloride	1 : 2	1:30	18.08	2	0	0	**0**
	Glycerol			11.92	3	0	0	
ChCl:LactA	Choline chloride	1 : 2		13.10	2	0	0	**8**
	Lactic Acid			16.90	2	4	8	
ChCl:EtGly	Choline chloride	1 : 2		15.88	2	0	0	**10**
	Ethylene Glycol			14.12	2	5	10	
Ty:DecA	Thymol	1 : 2	1:20	6.85	2	6	12	**18**
	Decanoic acid			13.15	2	3	6	
	Thymol	1 : 3		5.15	2	6	12	**18**
	Decanoic acid			14.85	2	3	6	
	Thymol	1 : 4		4.13	1	6	6	**12**
	Decanoic acid			15.87	2	3	6	

B. Ternary

	Components	Molar ratio	Matrix/Solvent	Weight	PP_{Qnt}	$PP_{Hazardousness}$	PP_{Solv} Partial	PP_{Solv} TOT
Ment:DecA:DecOH	Menthol	1 : 2 : 3	1:20	3.16	1	2	2	**12**
	Decanoic acid			6.40	2	3	6	
	Decanol			10.44	2	2	4	
Ment:LauA:DecOH	Menthol	1 : 2 : 3		2.91	1	2	2	**14**
	Lauric acid			7.46	2	4	8	
	Decanol			9.63	2	2	4	
Ty:DecA:DecOH	Thymol	1 : 2 : 3		3.05	1	6	6	**16**
	Decanoic acid			6.44	2	3	6	
	Decanol			10.51	2	2	4	

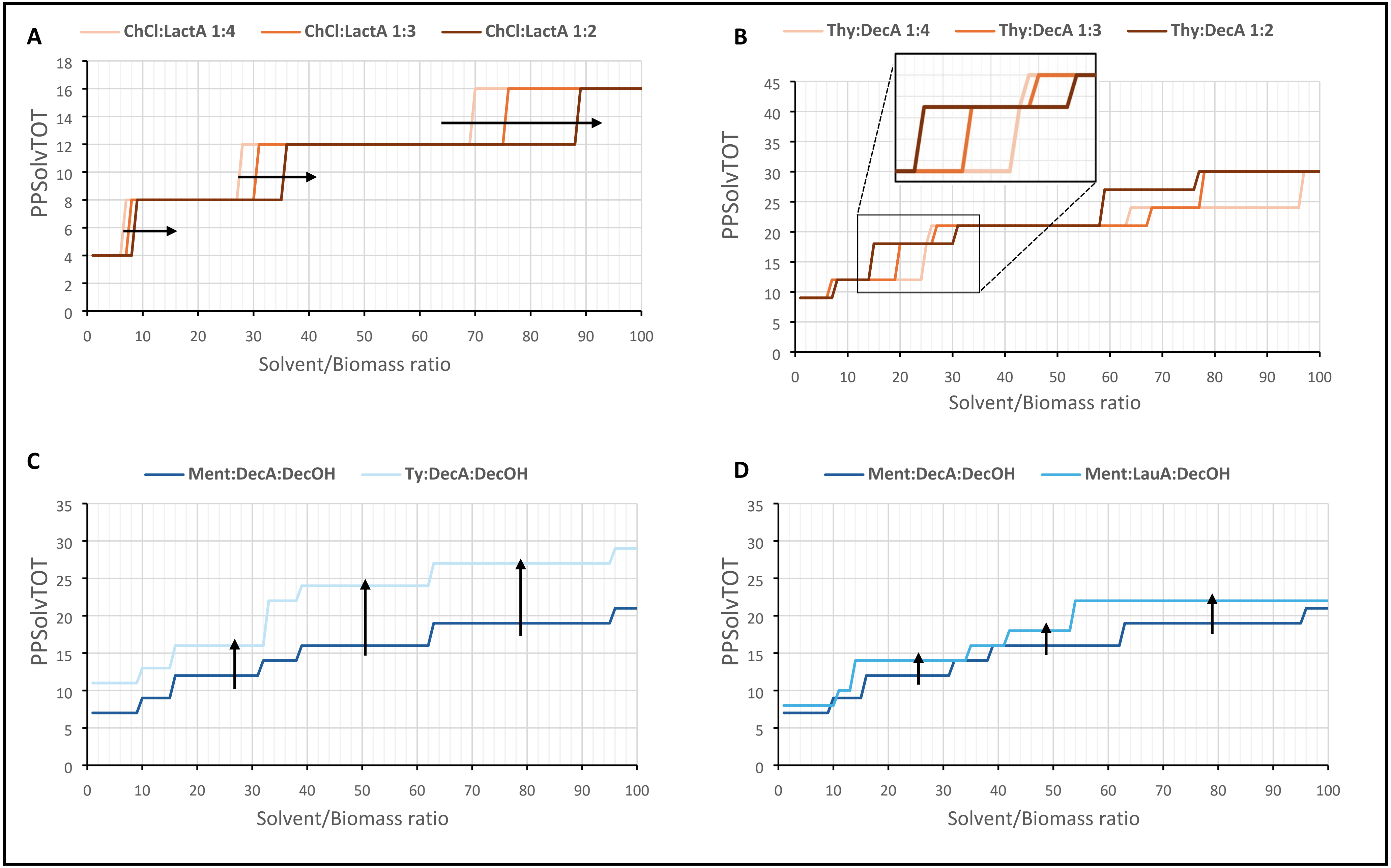


Figure 3S. Solvent & Additives EF, *case studies* calculation for different classes of *NaDES*. Graphical representation of PP_{Solv} in relation to Solvent/Biomass ratio.

3. Extraction

- Subcategory: *Matrix Depletion (MD)*

%	PP_{MD}
≤ 10	25.0
27	20.0
44	15.0
61	10.0
71	7.0
81	4.0
88	2.0
92	1.0
93	0.5
≥ 95	0.0

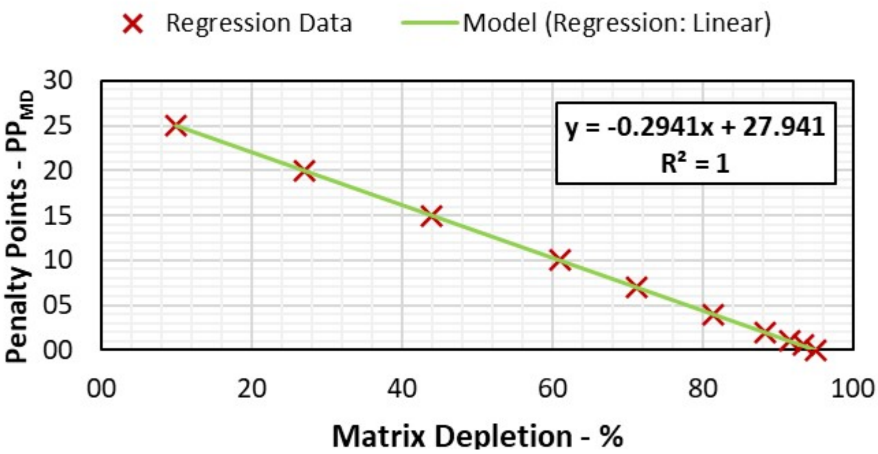


Figure 4S. Relation between the matrix depletion % and the attributed PPs.

- Subcategory: *Solvent Impact (SI)*

Score	PP_{SI}
0.0	0
1.0	2.5
2.0	5
3.0	7.5
4.0	10
5.0	12.5
6.0	15
7.0	17.5
8.0	20
9.0	22.5
≥ 10.0	25

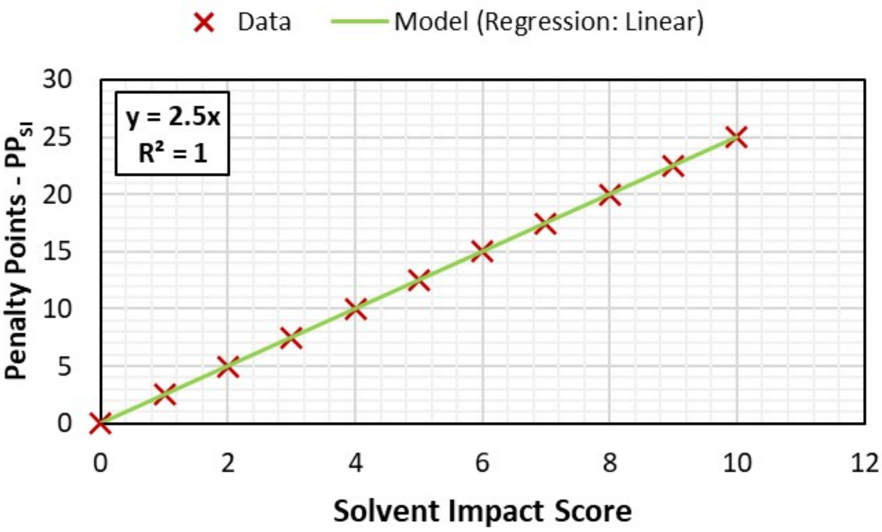
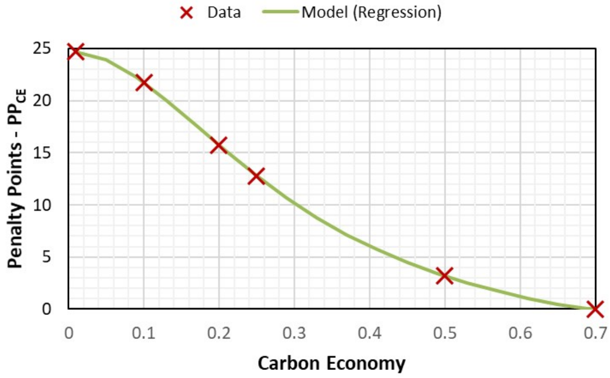


Figure 5S. Relation between the Solvent Score and the attributed PPs.

- Subcategory: *Carbon Economy (CE)*

	CE	PP_{CE}
≤	0.01	25
	0.10	22
	0.20	16
	0.25	12
	0.50	3
≥	0.70	0

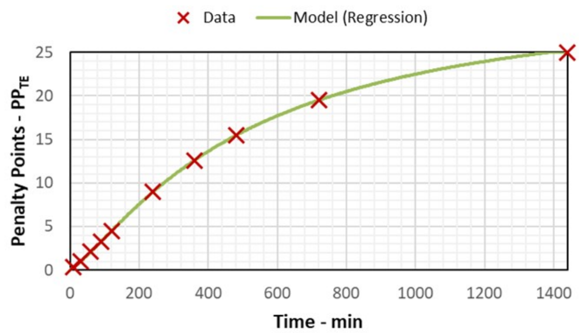


Goodness Measures What's this?		
R^2		0.9941
aR^2		0.9823
P		0.01178
SE		1.208
SSE		2.918
F		84.14
AIC		20.7
BIC		19.87
DoF		2
AICc		60.7
Coefficients		
a	24.74817	± 1.203
b	1.962412	± 0.5883
c	0.306275	± 0.07001
d	−5.071388	± 5.321
Equation		
$y = -5.071388 + (24.74817 - -5.071388)/(1 + (x/0.306275)^{1.962412})$		

Figure 6S. Relation between the CE value and the attributed PPs

- Subcategory: *Time Effectiveness (TE)*

	Min	PP_{TE}
≤	10	0
	30	1
	60	2
	90	3.5
	120	5.5
	240	8
	360	12
	480	16
	720	20
≥	1440	25



Goodness Measures What's this?		
R^2		0.9954
aR^2		0.9924
P		$3.845*10^{-7}$
SE		0.7165
SSE		3.08
F		325.6
AIC		24.6
BIC		25.81
DoF		6
AICc		32.6
Coefficients		
a	0.1271015	± 0.6654
b	1.273563	± 0.186
c	507.1445	± 87.89
d	31.90947	± 3.257
Equation		
$y = 31.90947 + (0.1271015 - 31.90947)/(1 + (x/507.1445)^{1.273563})$		

Figure 7S. Relation between the time used for the extraction and the attributed PPs

4. Process & Equipment

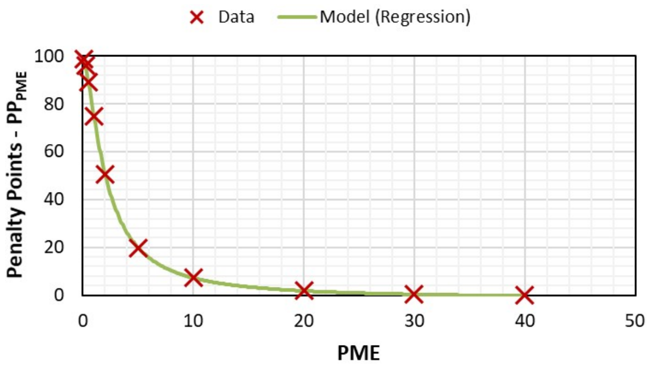
Equipment	Producer	**Energy** Power Input kWh	**Biomass** Load kg	**kWh/kg**
Blender - Scale-up	Ceccato	1.5	10	0.15
Hydrodynamic Cavitation (Rotor-stator System) Model 1	APV Cavitator - SPX Flow	45	200	0.23
Membrane Filtration - Scale-up	HydroAir	4	12	0.33
High Shear Homogenizator	KONMIX	4	10	0.40
Acoustic Cavitation (US) - Flow	Weber Ultrasonic	6.4	10	0.64
Ball mill - Scale-up	Chang	0.75	1	0.75
Supercritical CO_2	Carredi	18	15	1.20
Membrane Filtration - Lab-scale	HydroAir	0.55	0.4	1.38
Heating Plate - Model 1	Stuart	0.05	0.03	1.67
MW (Ethos X) - Lab-scale	Milestone	3.8	2.25	1.69
Ohmic heating	CFT Food Machinery	1.9	1	1.89
Autoclave	De-Lama	215.9	100	2.16
MW (Ethos XL) - Scale-up	Milestone	13.3	5	2.66
Hydrodynamic Cavitation (Rotor-stator System) Model 2	EPIC srl	4	1.4	2.86
Rotovapor - Scale-up	ZZKD	9.25	2.5	3.70
Freeze-dryer - Plates	MCGS	1.3	0.25	5.20
Supercritical CO_2 - Model 1	SepareCo 40 L	30	4	7.50
Subcritical Water Extr. 15 L	TFM	12.62	1.5	8.41
Freeze-dryer - Flasks - Model 1	LyoQuest	3.9	0.4	9.75
Acoustic Cavitation (US) - Immersion horn	Hairnertec	0.5	0.05	10.00
Acoustic Cavitation (US) - Cup-horn	SARL REUS	0.15	0.01	15.00
Centrifuging Separators	Alfalaval C10	15	1	15.00
Freeze-dryer - Flasks - Model 2	LabTech	5	0.3	16.67
Heating Plate - Model 2	LLG Labware	0.515	0.03	17.17
Blender - Lab-scale - Model 1	Waring Commercial	88	5	17.60
Acoustic Cavitation (US) - Bath	Weber Ultrasonic	3.68	0.2	18.40
Spray-drier - Model 1	ICF-Welco	200	10	20.00
Heating Plate - Model 3	IDL	0.65	0.03	21.67
MW (SynthWAVE)	Milestone	2.73	0.1	27.30
Ball mill - Lab-scale	Retch	2.76	0.1	27.60
Blender - Lab-scale - Model 2	Waring Commercial	141.9	5	28.38
Rotovapor	LAB Tech	1.54	0.05	30.80
PEF	Energy Pulse System	3.5	0.1	35.00
Spray-drier - Model 2	MCGS	8.8	0.2	44.00
Supercritical CO_2 - Model 2	SepareCo 1.7 L	8	0.17	47.06
MW (MonoWave300)	Sairem	0.69	0.011	62.73
Supercritical CO_2 - Model 3	ExtractLab	19.75	0.25	78.98
Autoclave (Parr)	Parr	2.76	0.025	110.40

Figure 8S. Collected data on the specific energy consumption (expressed in kWh/kg) for different extraction instruments. The mass values were derived from the maximum operating volume of each device, applying a standard solid-to-liquid ratio of 1:10, which represents the most used extraction condition.

5. Waste

- Subcategory: *PME*

	PME	PP_{PME}
≤	0.10	99
	0.25	95
	0.50	90
	1	75
	2	50
	5	20
	10	7
	20	2
	30	0.3
≥	40	0



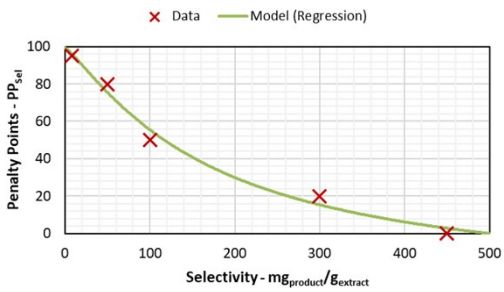
Goodness Measures What's this?		
R²		0.9998
aR²		0.9997
P		$3.384*10^{-11}$
SE		0.7447
SSE		3.328
F		7360
AIC		25.38
BIC		26.59
DoF		6
AICc		33.38
Coefficients		
a	99.82682	± 0.667
b	1.510155	± 0.04183
c	2.080652	± 0.0402
d	−1.473811	± 0.5574
Equation		
$y = -1.473811 + (99.82682 - -1.473811)/(1 + (x/2.080652)^{1.510155})$		

Figure 9S. Plot showing the relationship between the Process Mass Efficiency (PME) of the evaluated extraction protocols and their assigned Penalty Points.

6. Product

- Subcategory: *Selectivity*

	$mg_{product}/g_{extract}$	PP_{Sel}
≤	10	95
	50	75
	100	55
	300	15
≥	500	0



Goodness Measures What's this?		
R²		0.9872
aR²		0.9359
P		0.1692
SE		9.004
SSE		81.07
F		19.24
AIC		36.12
BIC		34.56
DoF		1
Coefficients		
a	99.3915	± 14.76
b	1.185792	± 1.041
c	171.945	± 211.1
d	−28.17362	± 83.85
Equation		
$y = -28.17362 + (99.3915 - -28.17362)/(1 + (x/171.945)^{1.185792})$		

Figure 10S. Relation between extraction selectivity in terms of $mg_{product}/g_{extract}$ and the attributed PPS.

Web Platform Development:

The SIX Score web platform is implemented as a client-side single-page application developed in JavaScript using the React framework. Data persistence and the public dataset are handled through Google Firebase, with records organized in dedicated collections (e.g., metadata and full submissions) and accessed directly from the front-end through Firestore API/SDK calls for read–write operations. To improve security against automated or non-authorized traffic, the application integrates Firebase App Check with reCAPTCHA v3, enabling Firestore to validate App Check tokens and reject requests not originating from the deployed application context. The website is hosted on GitHub Pages as a fully static deployment, i.e., without a dedicated custom back-end layer, resulting in a lightweight, free-to-host, and easy-to-maintain architecture while delegating database services to Firebase.

SIX Score Application to Case Studies

In this paragraph are described the results obtained using the SIX Score methodologies applied to three case studies previously published by our research group, focused on the recovery of secondary metabolites from pomegranate peels and blueberry pomace, where each extraction is developed across multiple configurations, covering processing technology, residue management, and solvent/additives options. The outcome wants to evidence how the developed metrics effectively integrates multiple dimensions, such as material hazard, process efficiency and environmental impact, into a unified framework.

First Case Study: Pomegranate Peels Extraction [44]

Pomegranate peels extraction protocols account for: i) conventional hydroalcoholic extraction, ii) optimized subcritical water extraction (SWE) and iii) optimized SWE extraction combined with nanomembrane filtration (**Figure 11S, A, B** and **C**, respectively). The evaluation in SIX Score across different pomegranate peel extraction protocols revealed slight differences**:** 66 for the conventional ethanol-based method (Conv. 70% EtOH), 71 for microwave-assisted extraction (MAE), and a maximum value of 80 when MAE is combined with nanofiltration (MAE+NF). It is immediately evident that the MAE–NF protocol constitutes the greenest approach among those evaluated. Analyzing the individual EFs of the score allows for a deeper understanding of where improvements can be implemented and how.

The *Raw Material* factor scored only 40% because, although pomegranate peel is a second-generation feedstock, its global production is limited to approximately 3.8 million tons per year and is strictly seasonal. Accordingly, this distinctive value is inherent to the selected valorization protocol, underscoring that, irrespective of the protocols employed, a substantive component of process sustainability inevitably derives from the intrinsic nature of the selected matrix. Under *Solvents&Additives*, the first major distinction between the protocols emerges. The use of ethanol penalized the conventional method in comparison to the two water-based alternatives. *Extraction* performance was similar across all methods; however, analysis of the subcategories within this EF showed that the conventional method achieved a higher polyphenol yield, whilst MAE protocols reached approximately 70% of that value. This difference, however, came at the cost of a longer extraction time (60 min *vs*. 10 min) and the use of two solvents instead of one. When all factors are considered (extraction time, solvent use and selectivity), the three methodologies are balanced resulting in similar scores. Regarding *Process&Equipment*, MAE+NF scored highest. The ethanol-based extraction ranked lower due to the need for solvent removal by rotary evaporation, which increases process complexity. Although NF is an additional step operating at 10 bars, it concentrates the aqueous extract and reduces lyophilization time by a factor of four. Because lyophilization is highly energy-intensive, this reduction outweighs the added NF step, allowing MAE+NF to outperform MAE alone by 1 PP. In the *Waste* category, solvent recovery significantly influenced the output. In the conventional method, ethanol was recovered using rotary evaporation with an efficiency of 80%. Ethanol waste, estimated at around 20% due to rotavapor losses, adds further PPs for the solvent's hazardousness. Moreover, the rotary evaporator's refrigeration system continuously consumes non-recirculated water, leading to significant waste. A more sustainable alternative is a closed-loop chiller, which greatly reduces water consumption but introduces additional equipment with high energy demand, a factor to be considered under *Process & Equipment*.

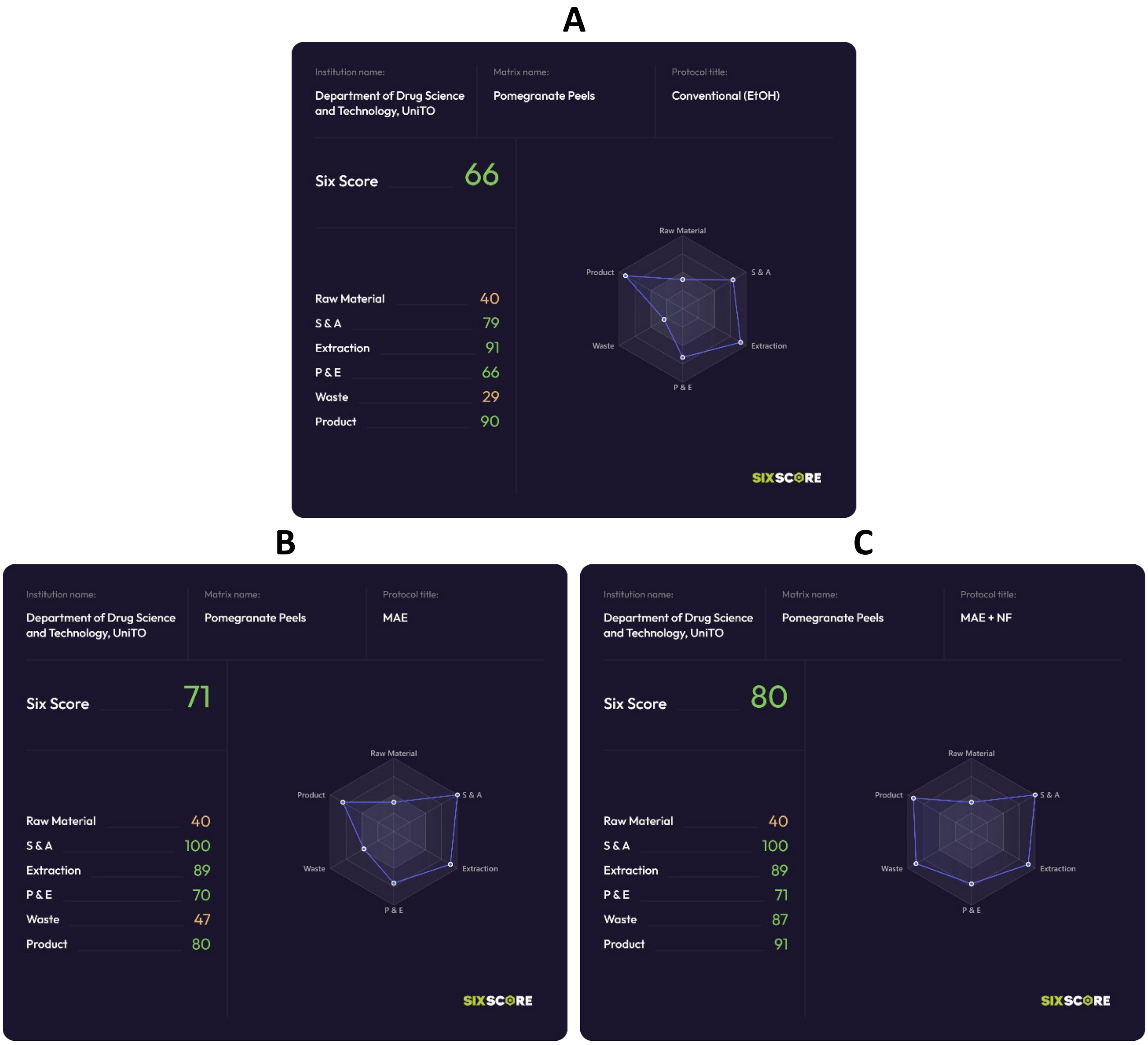


Figure 11S. Six Score and EFs values determined for the different extraction protocols applied on pomegranate peels. A. conventional hydroalcoholic extraction; B. optimized subcritical water extraction (SWE); C. optimized SWE extraction combined with nanomembrane filtration.

The MAE+NF process achieved the highest score in this category by enabling the recovery of approximately 75% of process water and a relative score for the *Waste* EF of 87 against the 47 obtained by only MAE, in which all the water was discarded consequently to the lyophilization process. Finally, the NF step also improved the selectivity of the extract, achieving performance comparable to the ethanol protocol in the *Product* EF. This contributed to the overall superiority of the MAE- NF approach, which ultimately received the highest final green score.

This case study demonstrated the utility of the SIX Score in assessing the sustainability of extraction protocols by considering a comprehensive set of factors, including material hazardousness, process efficiency, waste minimization strategies, and the quality of the final extract. However, this evaluation did not allow for comparisons involving the use of additives or sequential extraction protocols. Therefore, an additional case study will be presented to address these aspects deep in detail.

Second Case Study: Blueberry Pomace Extraction [45]

The second case study analyzed with SIX Score revolves around the optimized blueberry extraction protocols: i) the conventional method, ii) MAE 1° (first step), iii) MW-assisted subcritical water extraction (MASWE) 2° (second step), and iv) the sequential MAE+MASWE process**.** In this case the target compound were both polyphenols and anthocyanin which, consequently, have been accounted together in the voice Matrix Depletion (in EF 3) and Selectivity (in EF 6) (**Figure 12S A, B, C** and **D**, respectively).

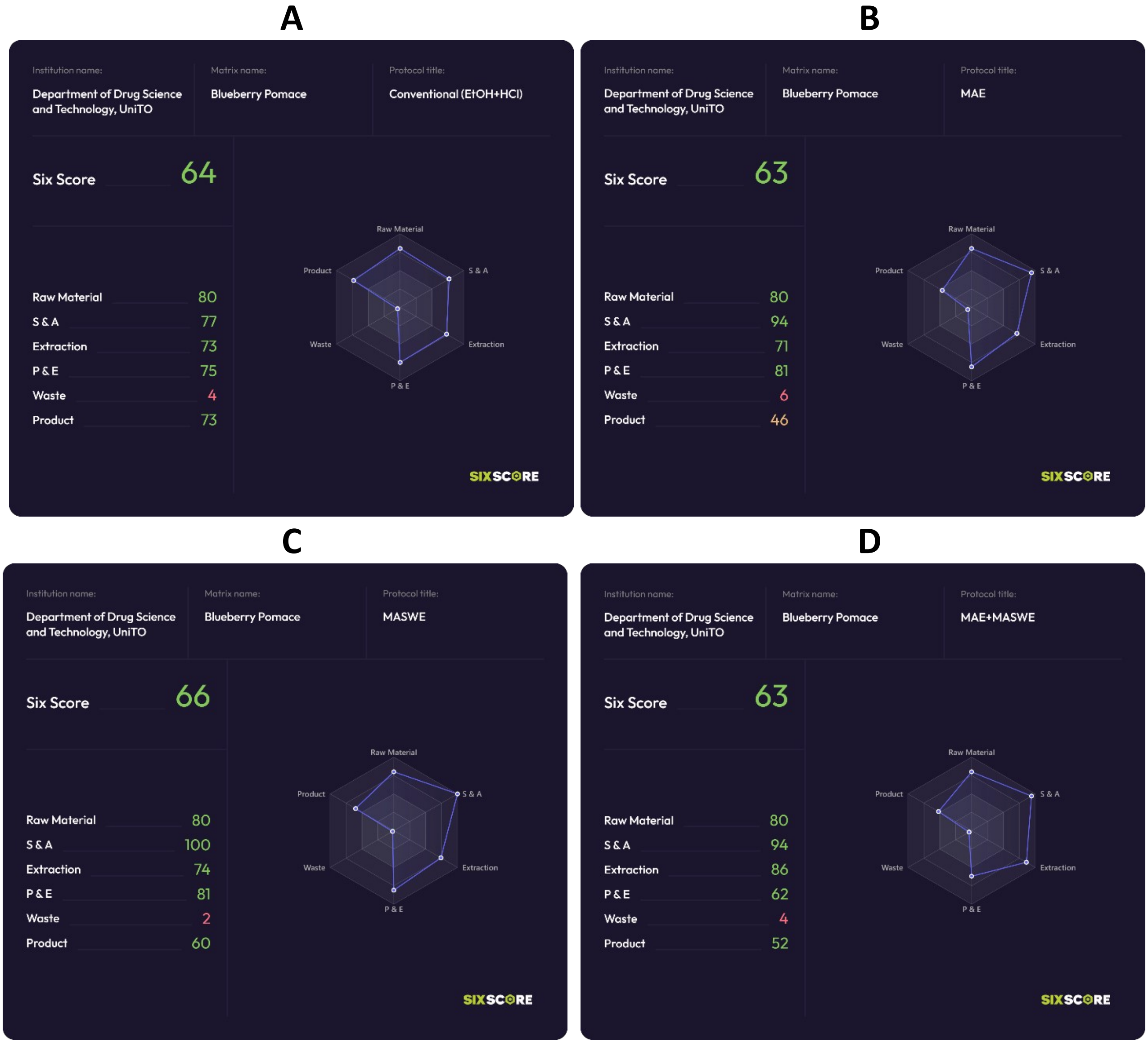


Figure 12S. Six Score and EFs values, together with subcategories, determined for the different extraction protocols applied on blueberry pomace. A) conventional method, B) MAE 1° (first step), C) MW-assisted subcritical water extraction (MASWE) 2° (second step); D) sequential MAE+MASWE process.

In this evaluation, all protocols yielded final SIX Scores within a narrow range, indicating comparable overall sustainability performance. The MAE protocol achieved a score of 63, followed by MASWE 2° with 65, while both the sequential MAE+MASWE approach and the conventional protocol scored 63 and 64, respectively. Given the limited dispersion of the scores, the final SIX value alone does not allow for an immediate or clear interpretation of sustainability improvements. Instead, analysis of the individual EF contributions provides more meaningful insight, enabling identification of protocol-specific strengths and weaknesses and highlighting potential areas for optimization in future experimental designs. Broadly, the following analysis gave an additional evidence on how the SIX Score is a valuable comparative tool for extraction strategies, intrinsically tied to the EF that shape it, such that any interpretation necessarily requires consideration of those generating factors.

All protocols shared a *Raw Material* score of 80%, as they employed blueberry pomace, a second-generation feedstock that, according to FAOSTAT, had a global production exceeding 28 million tons in 2023 (**Figure S1-S2**). However, this voice did not reach 100% due to the seasonal nature of blueberry harvesting, which typically occurs between June and August. The *Solvents&Additives* EF penalized the conventional protocol due to its use of a 60:40 ethanol-water mixture with an additional 0.8% on total volume of HCl. This solution received a score of 77, which was significantly lower than the scores of MAE 1° (94), which used citric acid, and MASWE 2° (100), which used only subcritical water. In terms of extraction performance, MAE exhibited the lowest score among all protocols (71), primarily due to its comparatively low degree of matrix depauperation. In contrast, the sequential MAE+MASWE protocol achieved the highest score (86), reflecting enhanced biomass exhaustion, with improvements observed in both Matrix Depletion and Carbon Economy subcategories. Conversely, despite achieving high matrix depletion of target compounds, the conventional protocol scored only 73, as the use of ethanol and HCl, together with poor carbon economy, negatively affected its overall extraction score. In the *Process&Equipment* component, MAE and MASWE exhibited comparable performance, achieving identical scores (81) due to their use of the same extraction equipment. The conventional protocol scored slightly lower (75), as it requires an additional rotary evaporation step for ethanol removal. In contrast, the sequential extraction protocol showed a markedly lower score (62), reflecting the cumulative time and energy demands associated with performing two consecutive extraction steps. Following, *Waste* emerged as a crucial aspect. Despite their eco-friendly intent, the green protocols showed surprisingly low scores, 6 for MAE, 2 for MASWE, and 4 for MAE+MASWE, primarily due to high solid-to-liquid ratios (1:30 for single-step methods and 1:60 for the sequential process). On other hand, the conventional protocol, score 4, which employed a lower solid-to-liquid ratio (1:5), was penalized for ethanol and HCl losses, as well for the significant water waste deriving from the rotary evaporator's refrigeration system. As a result, *Waste* emerged as the lowest-scoring factor across all metrics and presents a key challenge to be addressed to enhance the sustainability of the protocols. The last EF, *Product*, showed how the product deriving from conventional protocols have a higher selectivity toward anthocyanin and polyphenols with the highest score of 73.

Overall, the SIX Score values show only marginal differences among the protocols, thereby tempering its use as standalone indicators of sustainability gains. However, examination of the individual EFs provides clearer insight. This analysis reveals that the proposed protocols perform better than the conventional approach in terms of chemical selection and overall extraction efficiency, although they still exhibit limited selectivity toward the target compounds. In addition, waste management emerged as a critical weakness across all protocols, with consistently low scores highlighting the need for improved strategies in this area. To point out with an example, in MAE protocol an hypothetic water recovery by means of NF as previously reported for pomegranate (*ca.* 75%) would raise the *Waste* EF from 6 to 31. The development of the SIX Score framework, which followed the completion of the blueberry extraction work, proved particularly valuable in identifying an important limitation that had not been fully recognized during experimental design: excessive water consumption and the absence of purification steps to enhance extract selectivity. If applied earlier, this framework could have more effectively guided protocol optimization, for example by encouraging lower solid-to-liquid ratios, water recovery or recycling approaches, and the integration of downstream purification techniques to improve selectivity toward bioactive compounds.

Third Case Study: Blueberry Pomace Extraction – NaDES [46]

Blueberry pomace extraction with Natural Deep Eutectic Solvents (NaDES) can be exploited for a comparison with previous extraction set-ups. The resulting SIX Score highlight a slight reduction in the *S&A* Score due to the use of NADES instead of water (**Figure 13S**).

Figure 13S. Six Score and EFs values, together with subcategories, determined for the NaDES extraction protocol applied on blueberry pomace.

Nevertheless, this value remains substantially more sustainable than the conventional extraction, which relies on ethanol and HCl. An improvement is also observed in the *P&E* component, as the solvent is not removed after extraction. Consequently, energy- and time-intensive steps such as solvent evaporation and separation are avoided, positively influencing the score. A similar effect is observed for the waste management factor, which reaches a value of 100, since no solvent removal is applied and the solvent, together with the extracted fraction, are considered product. However, these advantages are offset by a major limitation: retaining the solvent in the final extract results in very low selectivity toward the target compound. As a consequence, the *Product* elemental factor scored 0, due to the combined effect of poor selectivity and the presence of lactic acid, which carries a corrosive hazard pictogram and therefore introduces additional penalty points. Moreover, the current scoring framework does not capture additional limitations associated with storing the extract in a solubilized form, including reduced chemical and physical stability, as molecules in solution are generally more susceptible to degradation. More critically, this condition entails an increased microbiological risk due to the high-water activity of the extract. This provides a paradigmatic example that the use of the SIX Score (and of the individual EFs that constitute it) for assessment purposes should nevertheless always be guided by a criterion that realistically accounts for the practical applicability of the extraction protocol (potentially industrial or market-oriented setting)

The presented studies proved a balanced methodology able to identify principal weakness in the extraction protocols and evaluating proportionally different aspects. As example, the addition of a filtration step in the MASWE+NF protocol for pomegranate peels have resulted in lowest score in the *P&E* EF but highly improving the score for the *Waste* EF, demonstrating that the advantage of the additional step surpasses the disadvantages. On other hand, the double extraction protocol for blueberry pomace showed to increase some aspect such as the Matrix Depletion in the *Extraction* EF but weighting too much on the *P&E* and on the *Waste* resulting in a not proportional increase of the final score.

In conclusion, the collected insights propose to shape future research, where alternative methodologies will be implemented from the outset, helping to avoid earlier shortcomings and supporting more sustainable design choices for green extraction protocols. The idea is to offer a flexible tool that propose a structure (updated with the latest literature), on which the scientific community could rely on to evaluate the greenness of extraction procedures.

Fourth Case Study: Green extraction of hemp seeds cake (*Cannabis sativa* L.) with 2-methyloxolane: A response surface optimisation study [47]

This case study presents a sustainability evaluation of lipophilic compounds extracted from hemp seed cake, comparing the conventional solvent n-hexane with the greener lipophilic solvent 2-MeTHF. Two different evaluation approaches are considered. The first focuses on the total oil yield (**Figure 14S A** and **B**), while the second reports the bioactive compounds co-extracted with the oil fraction.

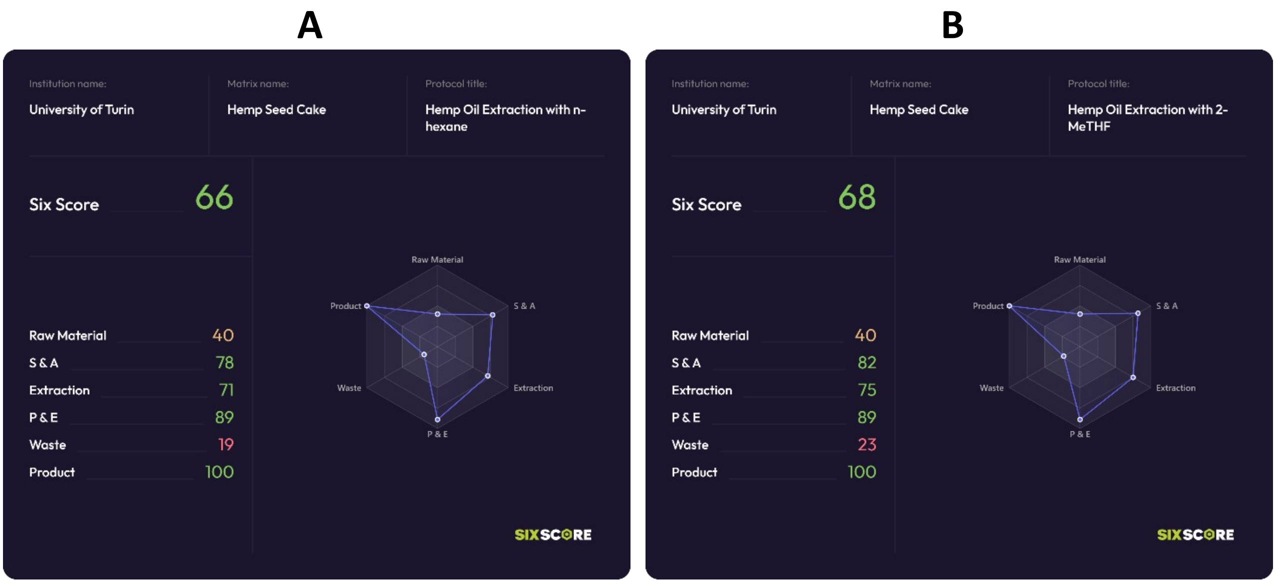


Figure 14S: Six Score and EFs values, together with subcategories, determined for the different extraction protocols targeting oil applied on hemp seed cake. A) n-hexane, B) 2-MeTHF

The aim of this comparison is to illustrate how the SIX Score can be adjusted according to the specific objective of process optimization, by considering different target compounds within the extraction process.

The comprehensive SIX Score for total oil extraction shows a slightly more favorable outcome for 2-MeTHF, with a final score of 68 compared to 66 for n-hexane. Analyzing each factor, the main differences were observed in the Solvents & Additives, Extraction, and Waste elemental factors. Regarding Solvents & Additives, 2-MeTHF presents fewer hazardous pictograms than n-hexane, which explains the higher score obtained for this elemental factor. For the Extraction factor, the subcategory *overall target content* was set at 110 mg oil/g matrix, based on the reference method protocol "ISO 659, 2009 – Oilseeds - Determination of Oil Content (https://www.iso.org/standard/43169.html)." Under the tested conditions, 2-MeTHF showed greater depletion of the matrix compared with n-hexane, resulting in a higher process yield. Consequently, the subcategories *Depletion Efficiency* and *Carbon Economy* both scored more favourably for 2-MeTHF. Additionally, since 2-MeTHF is a bio-derivable solvent, it was less penalized in the *solvent impact* subcategory compared with n-hexane.

The higher extraction yields also influenced the Waste factor, specifically the Process Mass Intensity (PMI) subcategory. As 2-MeTHF exhibited better extraction performance, the resulting PMI value was more favourable. The Product elemental factor reached the maximum value of 100 for both solvents. This is because the extracted oil was evaluated in terms of general selectivity, without targeting specific classes of compounds, resulting in an optimal score.

Conversely, if the objective is to evaluate specific compounds present in the oil, the SIX Score can be recalculated by targeting those compounds in the assessment (**Figure 15S A** and **B)**. In this second assessment, polyphenols and carotenoids present in the final oil were selected as the target compounds.

A B

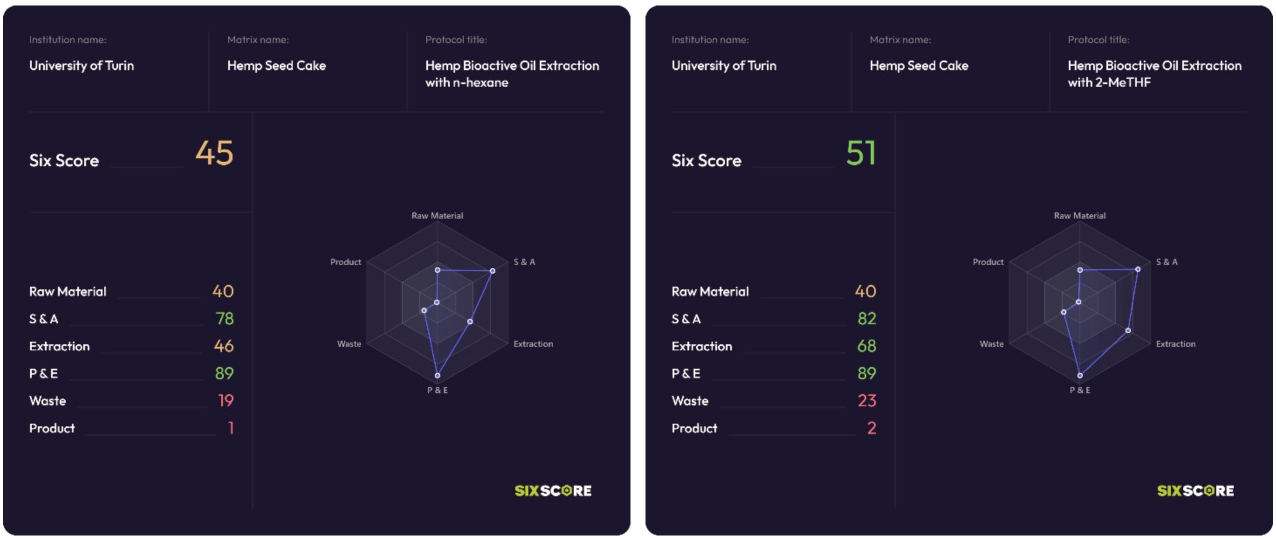


Figure 15S: Six Score and EFs values, together with subcategories, determined for the different extraction protocols targeting the bioactives in oil applied on hemp seed cake. A) n-hexane, B) 2-MeTHF

In this case, the scores decrease significantly, dropping from 66 to 45 for the n-hexane extraction and from 68 to 51 for the 2-MeTHF extraction. Compared with the previous evaluation, the most notable differences are observed in the Extraction and Product elemental factors. Within the Extraction factor, the *depletion efficiency* subcategory shows different values, with 2-MeTHF demonstrating a greater ability to extract polyphenols and carotenoids than n-hexane. The Product factor shows the largest decrease, dropping from 100 in the previous evaluation to 1 for n-hexane and 2 for 2-MeTHF. This is because the concentration of these metabolites in the final oil is very low. Although their presence is beneficial, the main product of the process is the oil itself, and the extraction is not specifically optimized to target these minor compounds.

Overall, both evaluation approaches indicate that 2-MeTHF represents a more favourable alternative to n-hexane in terms of process efficiency and sustainability. The final scores are relatively close, and only minor differences, such as the lower hazard profile of the 2-MeTHF and its slightly higher extraction efficiency, contribute to its improved performance. In this case, the initial solvent selection was already effective, which explains the limited differences observed in process efficiency parameters between the two tested solvents. Nevertheless, the final SIX Score values allowed for an effective comparison, confirming the sustainability advantages associated with the use of 2-MeTHF.

Fifth Case Study: Enhanced and Selective Lipid Extraction from the Microalga *P. tricornutum* by Dimethyl Carbonate and Supercritical CO_2 Using Deep Eutectic Solvents and Microwaves as Pretreatment [48]

This case study evaluates different lipid extraction protocols from the microalga *P. tricornutum*, comparing the conventional Bligh and Dyer (B&D) method, which uses methanol and chloroform, with alternative solvent protocols such as dimethyl carbonate (DMC) and supercritical CO_2 ($ScCO_2$), applied either alone or in combination with a biomass pretreatment using a natural deep eutectic solvent (NADES) composed of choline chloride and oxalic acid (ChCl/OA, 1:2). In this case study, the evaluation was performed using total lipid yield and targeting the total fatty acid (TFA) fraction as the class of compounds of interest. The conventional B&D method resulted in an overall SIX Score of 59 (**Figure 16S**).

Figure 16S. Six Score and EFs values, together with subcategories, determined for the B&D extraction protocol applied on *P. tricornutum*.

This protocol was used as the benchmark methodology to improve upon and to provide reference data regarding the overall target content (mg lipids / g matrix). Analysis of the elemental factors shows that the lowest scores are associated with the *Solvent & Additives* and *Waste* categories. The low score for *Solvent & Additives* is mainly due to the use of highly hazardous solvents such as methanol and chloroform in large quantities relative to the treated biomass. While this method is exhaustive in terms of extraction efficiency, it raises significant sustainability concerns. This issue is also reflected in the *Waste* category, since no solvent recovery protocol was applied and toxic chemicals were discarded after the extraction process.

The two alternative approaches showed modest improvements in the sustainability score, reaching 60 for DMC and 62 for $ScCO_2$ (**Figure 17S A and B**). The *Solvent & Additives* category was improved by selecting solvents with lower hazardousness, resulting in scores of 82 for DMC and 88 for $ScCO_2$, which represents the least hazardous solvent among the tested systems. However, the *Extraction* elemental factor showed a reduction compared to the conventional method for both alternatives. This was expected, as the conventional methodology is exhaustive, while the alternative protocols did not achieve complete depletion of lipids. In particular, the B&D method yielded 110 mg lipids/g matrix, compared to 45 mg/g and 3 mg/g obtained with DMC and $ScCO_2$, respectively.

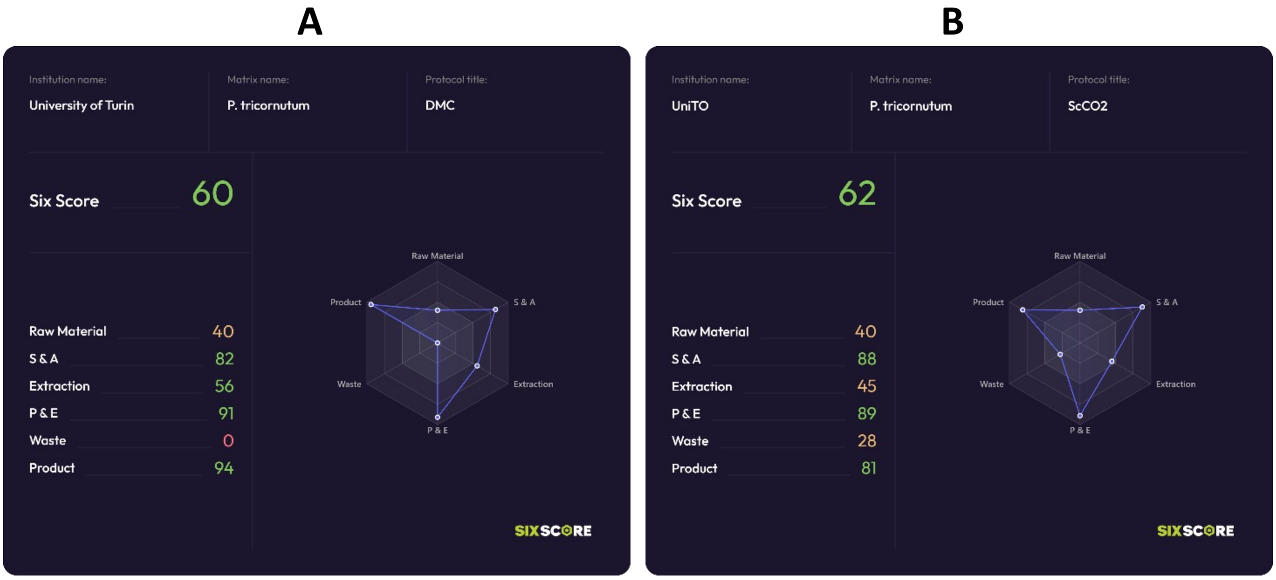


Figure 17S: Six Score and EFs values, together with subcategories, determined for the different extraction protocols applied on *P. tricornutum*. A) DMC, B) $ScCO_2$

Regarding *Process and Equipment*, DMC is favored because $ScCO_2$ extraction requires high-pressure instrumentation. On the other hand, the *Waste* elemental factor rewards the $ScCO_2$ extraction due to the implementation of a solvent recycling system, unlike the DMC extraction. Finally, the higher selectivity observed with DMC represents an additional advantage. Overall, although $ScCO_2$ achieved the highest comprehensive sustainability score, it showed low efficacy in terms of extraction performance, particularly due to its low yield and selectivity. Therefore, despite its high potential as a green technology, $ScCO_2$ alone is not suitable for direct extraction from untreated biomass.

To combine the sustainability advantages of $ScCO_2$ with improved extraction efficiency, a pretreatment strategy was introduced. The relatively low depletion and selectivity observed in the initial experiments were likely related to the thick cell wall of the algae. For this reason, a pretreatment step using a DES system (ChCl/OA, 1:2) was applied to both DMC and $ScCO_2$ protocols to enhance solvent accessibility through the algal cell wall (**Figure 18S A and B**).

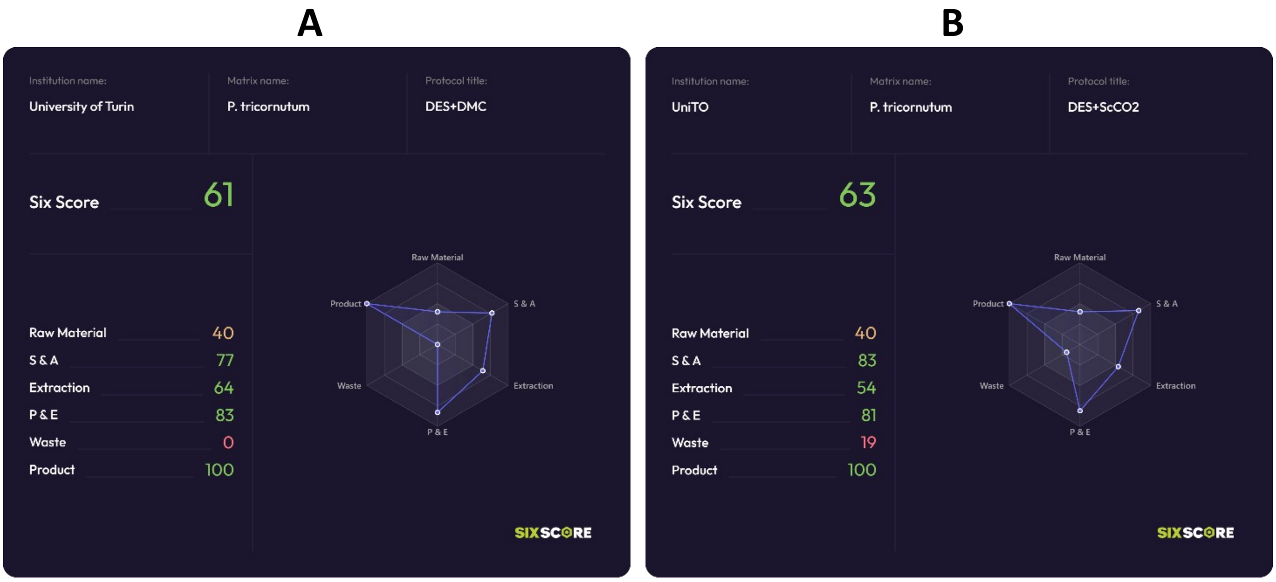


Figure 18S: Six Score and EFs values, together with subcategories, determined for the different extraction protocols applied on *P. tricornutum*. A) DES+DMC, B) DES+ScCO2

The two protocols including NADES pretreatment showed an overall increase of 1 percentage point in the final SIX Score. The addition of the pretreatment step slightly reduced the scores of some elemental factors (*Solvent & Additives*, *Process & Equipment*, and *Waste*), due to the introduction of an additional processing step and chemicals. However, it significantly improved the *Extraction* and *Selectivity* factors, demonstrating a balanced trade-off among the evaluated parameters.

In particular, $ScCO_2$ combined with NADES pretreatment achieved a depletion efficiency of 64% for total fatty acids, corresponding to a target yield of 71 mg TFA/g matrix, compared to only 3 mg/g in the untreated sample. Similarly, the DMC protocol improved from 45 to 110 mg TFA/g matrix, reaching a depletion efficiency of 99% relative to the conventional B&D method. Consequently, the *Extraction* score reached 64 for DES+DMC and 54 for DES+$ScCO_2$. Nevertheless, improvements in other elemental factors, such as *Solvent & Additives* and *Waste*, compensate for this difference, resulting in a slightly higher final overall score for the DES+$ScCO_2$ protocol.

This case study illustrates how the SIX Score can be used for the iterative optimization of extraction protocols, starting from a technology, such as ScCO2, that is promising in terms of solvent sustainability but shows significant limitations in terms of mass yield. The improvements obtained through the introduction of a DES pretreatment resulted in an increase in the SIX Score, justifying the additional processing step and providing a more balanced overall assessment of the extraction process. Based on these results, further experiments could be performed to optimize process yields, with the aim of improving the performance of the protocol that demonstrated the promising sustainability potential.