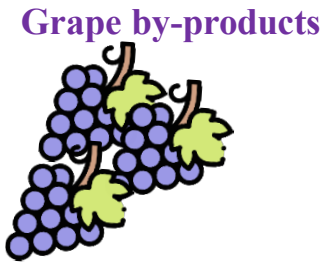
Chemical and nutritional characterization of by-products from the wine industry. Source of healthy ingredients for the formulation of nutraceuticals and functional foods



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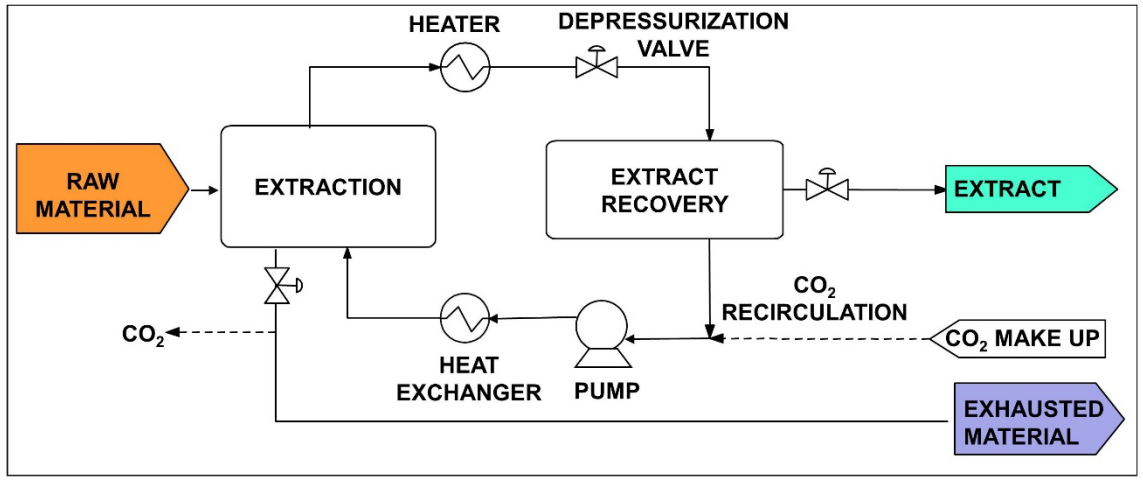
Feedstock supply strategy of grape by-products



Supercritical Fluid Extraction CO2 Optimization Process of fibers from Grape seeds

3 Products

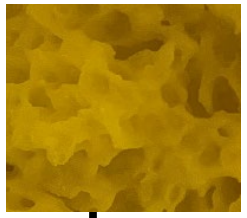
Grape seeds

Grinding and dehydration

Fiber (SPBF+) 1

CO2 Extaction

(20 MPa)



+

Extract (OSE) 2

Extracted Fiber (SPBF) 3

Objetive

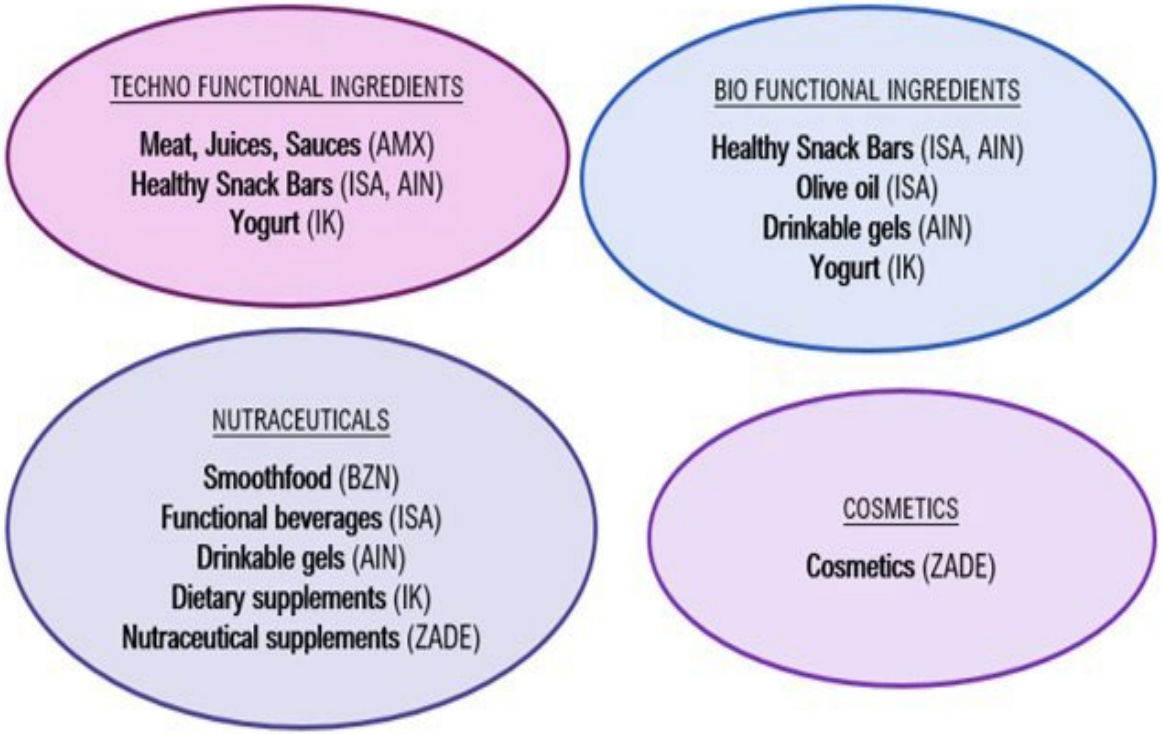
Evaluate 3 products from Grape Seeds

Fiber (SPBF+) | Extracted Fiber (SPBF) | Extract (OSE)

Chemical & nutritional characterisation of raw materials

Fortification of several food and cosmetic matrix

To comply with the requirements of each end users.



1. ***Analysis of Phenolic profile***
2. ***Analysis of Minerals***
3. ***Analysis of fatty acids (FA)***

1. Analysis of Phenolics by LC-MS/MS

C18 column (PHENOMENEX LUNA, 150 mm × 2 mm and 3 µm).

Column temperature:40°C

The gradient elution of:
A: 0.1% formic acid in water
B: 0.1% formic acid in acetonitrile

Phenolic compounds	
4-CBA	**4-chlorobenzoic acid**
VA	**Vanillic acid**
CA	**Cinnamic acid**
DA	**Dihydroxibenzoic acid**
FA	**Ferulic acid**
P-CA	**p-coumaric acid**
PA	**Phthalic acid**
SA	**Syringic acid**
M-TA	**m-toulic acid**
LU	**Luteolin**
SY	**Syringaldehyde**
PTA	**Protocatechuic acid**
QE	**Quercetin**
VN	**Vanillin**
SAA	Salycilic acid
RU	Rutin

Tyrosol and derivatives	
TYR	**Tyrosol**
HTYR	**Hydroxytyrosol**
LIG	**Ligstroside**
OLE	**Oleacein**
OLS	**Oleuroside**
OLP	**Oleuropein**

Flavonoids	
CAT	**Catechin**
EDPICAT	Epicatechin

Other phenolics	
RESV	**Resveratrol**

Comonly found in fruits

Grape

1. Analysis of Phenolics by LC-MS/MS

Grape by-products

Table 1. Quantification of phenolic compounds in grape seeds samples

Product	CODE	EXT.	PHENOLIC COMPOUNDS (mg/kg)														
		(MPa)	4-CBA	VA	CA	DA	FA	P-CA	PA	SA	M-TA	LU	SY	PTA	QE	VN	SAA	RU
Fiber	GPBF+	-	-	1.158	-	42.580	0.243	0.213	0.644	2.136	-	0.076	0.274	88.66	4.856	0.414	0.189	2.204
Extracted Fiber	GPBF	20	-	1.054	-	33.880	0.255	0.233	0.585	2.144	-	0.068	0.242	-	4.912	0.305	0.210	2.132
Extract	GSE*	20	-	9.638	-	0.884	0.278	0.164	0.196	0.208	-	0.004	3.320	-	0.024	9.326	0.047	-

Table 2. Quantification of tyrosol and their derivatives in grape seeds samples

Prodoct	CODE	EXT.	TYROSOL AND DERIVATIVES (mg/kg)					
		(MPa)	TYR	HTYR	LIG	OLE	OLS	OLP
Fiber	GPBF+	-	-	0.357	-	0.111	-	-
Extracted Fiber	GPBF	20	-	0.323	-	0.543	0.001	0.005
Extract	GSE*	20	8.644	10.226	-	156.942	0.001	0.004

Table 3. Resveratrol

Nº	PRODUCT	**Resveratrol** (mg/kg)
Fiber	GPBF+	1.00
Extracted Fiber	GPBF	1.15
Extract	GSE	0.04

Table 4. Quantification of catechin and epicatechin of selected grape seeds samples.

Nº	PRODUCT	CATECHIN (mg/kg)	EPICATECHIN (mg/kg)
Fiber	GPBF	81.05	28.52
Extracted Fiber	GPBF	83.35	26.65
Extract	GSE*	-	-

Main compounds: dihydroxibenzoic acid (DA), quercetin (QE), salicylic acid (SA), rutin (RU), catechin (CAT) and resveratrol in seed fibers (GPBF+ and GPBF).
Tyrosol (TYR), hydroxytyrosol (HTRY) and oleacein (OLE) in extracts (GSE)

Conclusions

1. Analysis of Phenolics by LC-MS/MS

Grape by-products 

***Targets*:**

1. Extracted Fiber (GPBF) →

- **Dihydroxibenzoic acid** (DA, 33.8 mg/kg)
- Quercetin (QE, 2.2 mg/kg)
- Salicylic acid (SA, 4.9 mg/kg)
- Rutin (RU, 2.1 mg/kg)
- **Catechin** (CAT, 83 mg/kg)
- Resveratrol (RESV, 1.1 mg/kg)

2. Fiber (GPBF+) →

- **Dihydroxibenzoic acid** (DA, 42.5 mg/kg)
- Quercetin (QE, 4.8 mg/kg)
- Salicylic acid (SA, 2.1 mg/kg)
- Rutin (RU, 2.2 mg/kg)
- **Catechin** (CAT, 81 mg/kg)
- Resveratrol (RESV, 1 mg/kg)

3. Extract (GSE) →

- **Tyrosol** (TYR, 8.64 mg/kg)
- **Hydroxytyrosol** (HTRY, 10.2 mg/kg
- **Oleacin** (OLE, 157 mg/kg)

2. Analysis of minerals by
INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY (ICP-OES)

Perkin–Elmer Optima 4300 DV spectrometer (Shelton, CT, USA), equipped with an AS-90 autosampler, axial system, a high dynamic range detector and a cross-flow type nebulizer for pneumatic nebulization.

Microelements:
[iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn)]
Macroelements:
[calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P), sodium (Na), sulfur (S), silicon (Si)]

WAVELENTS OF THE MINERALS														
Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn	S	Si	Al	B	Cr	Ni
317.933	327.393	238.204	766.490	285.213	257.610	589.592	213.617	206.200	181.975	251.611	167.079	249.773	267.716	231.604

2. Analysis of minerals by ICP-OES

Table 5. Quantification of minerals in grape seeds samples.

Nº	PRODUCT	EXT	MINERALS (mg/kg)													
			Ash (%)	**Ca**	Cu	**Fe**	**K**	**Mg**	Mn	Na	**P**	Zn	Al	B	Cr	Ni
Fiber	GPBF+	-	3,10	7805,8	38,2	113,4	3982,8	1461,7	23,6	580,2	3351,1	10,2	-	-	-	-
Extracted Fiber	GPBF	20	2,90	6530,5	33,0	27,7	3303,3	1188,3	14,5	505,6	2861,0	9,8	-	-	-	-
Extract	GSE	20	-	22,66	0,25	2,96	1,53	0,59	0,15	0,34	0,99	0,39	4,33	0,99	0,44	0,59

Grape by-products

***Targets*:**

1. Grape Seeds Fibers (GPBF+) →
- **Ca** (7.8 g/kg), **K** (3.9 g/kg), **Mg** (1.4 g/kg), **P** (3.3 g/kg).
- **Fe** (0.11 g)

2. Grape Seeds Extracted Fibers (GPBF) →
- **Ca** (6.5 g/kg), **K** (3.3 g/kg), **Mg** (1.1 g/kg), **P** (2.8 g/kg).
- **Fe** (0.027 g)

3. Grape Seeds Extract (GSEE) →
- **Low amounts of minerals**

Grape seed fibers are good sources of minerals

3. Analysis of Fatty acids by
GAS CHROMATOGRAPHY FLAME IONIZATION DETECTOR (GC-FID)

GC-FID operating conditions for fatty acids identification	
Injector Tª (ºC)	250
Injection volume (µL)	1
Injection	Split
Split ratio	1:50
Column	Agilent HP-88 30 m x 0,25 mm; 0,2 mm
Carrier	Helium
Flow (mL/min)	1

Temperature ramp (ºC/min)	T (ºC)	t hold (min)
-	120	1
10	175	10
3	220	5
25	260	10
Tª FID (ºC)	280	
Air flow (mL/min)	450	
H2 Flow (mL/min)	40	
Makeup Flow (mL/min)	30	
Analysis time (min)	45.1	

Short and long chain FA		Rt
Butyric acid	C4:0	1.675
Hexanoic acid	C6:0	1.873
Octanoic acid	C8:0	2.273
Decanoic acid	C10:0	3.004
Undecanoic acid	C11:0	3.507
Lauric acid	C12:0	4.089
Tridecanoic acid	C13:0	4.721
Myristic acid	C14:0	5.387
Myristoleic acid	C14:1	5.847
Pentadecanoic acid	C15:0	6.059
cis-10-Pentadecenoic acid	C15:1	6.526
Palmitic acid	C16:0	6.737
Palmitoleic acid	C16:1	7.152
Heptadecanoic acid	C17:0	7.489
cis-10-Heptadecanoic acid	C17:1	7.983
Stearic acid	C18:0	8.383
trans-9-Elaidic acid	C18:1 trans	8.723
Oleic acid	C18:1 cis	8.891
Linolelaidic acid	C18:2 trans	9.39
Linoleic acid	C18:2 cis	9.833
γ-Linolenic acid	C18:3 n6	10.596
Arachidic acid	C20:0	10.842
Linolenic acid	C18:3 n3	11.173
cis-11-Eicosenoic acid	C20:1	11.623
Heneicosanoic acid	C21:0	12.583
cis-11,14-Eicosadienoic acid	C20:2	13.147
cis-8,11,14-Eicosatrienoic acid	C20:3 n6	14.357
Behenic acid	C22:0	14.85
cis-11,14,17-Eicosatrienoic acid + Erucate acid	C20:3 n3+C22:1	15.333
cis-5,8,11,14-Eicosatetraenoic acid	C20:4	16.118
Tricosanoic acid	C23:0	17.679
cis-13,16-Docosadienoic acid	C22:2	17.991
cis-5,8,11,14,17-Eicosapentaenoic acid	C20:5	18.386
Lignoceric acid	C24:0	20.342
Nervonic acid	C24:1	21.459
cis-4,7,10,13,16,19-Docosahexaenoic acid	C22:6	23.991

4. Analysis of Fatty acids by GC-FID

Table 6. Short chain fatty acids in grape seeds samples.

	PRODUCT	EXT.	SHORT CHAIN FATTY ACID (mg/kg)														
		(MPa)	C4:0	C6:0	C8:0	C10:0	C11:0	C12:0	C13:0	C14:0	C14:1	C15:0	C15:1	C16:0	C16:1	C17:0	C17:1
Fiber	GPBF+		-	-	186	-	-	47	-	84	-	13.4	-	10100	117	101	-
Extracted Fiber	GPBF	20	-	-	152	45	-	30	-	65	-	-	-	10588	101	93	-
Extract	GSE*	20	-	-	205	67	-	209	-	721	16	156	-	64052	4962	671	875

Main fatty acids: oleic, linoleic and palmitic acids

Table 7. Long chain fatty acids in grape seeds samples.

	PRODUCT	EXT.	LONG CHAIN FATTY ACID (mg/kg)																		
		(MPa)	C18:0	C18:1 trans	C18:1 cis	C18:2 trans	C18:2 cis	C18:3 n6	C20:0	C18:3 n3	C20:1	C21:0	C20:2	C20:3 n6	C22:0	C20: n3 + C22:1	C20:4	C23:0	C22:2	C20:5	C24:0	C24:1	C22:6
Fiber	GPBF+		5216	57	16868	-	82606	-	261	612	178	-	-	-	158	-	-	-	-	31	64	-	-
Extracted Fiber	GPBF	20	5045	62	17335	-	84724	-	241	514	170	-	-	-	121	-	-	-	-	-	50	-	-
Extract	GSE*	20	17039	-	426519	-	161677	-	1567	3270	1010	166	-	-	1019	-	27	174	-	-	825	-	-

Table 8. Fatty acid proportion in grape samples.

Fatty acid (%)	GPBF+	GPBF (20MPa)	GSE (20MPa)
Total SFA	13.91	13.77	12.68
Total USFA	86.09	86.23	87.32
Total MUFA	14.76	14.81	63.25
Total PUFA	71.34	71.43	24.08

Good results for PUFA (Linoleic acid) in Fibers.

Conclusion

The by-products derived from the *Vitis vinifera* L processing industry contain different bioactive molecules such as polyphenols, flavonoids, minerals and fatty acids which can be recovered and re-used following circular economy policies.

Future work

A fortification plan is being created based on the functional ingredient requirements by the end users and matrix.

PRODUCTS	INCORPORATION STRATEGY	ENCAPSULATION NEED			BY-PRODUCT PROPOSAL
		Keep the organoleptic characteristics	Resistence to Temp.	Improve dispersion	
Meat	Antioxidants to increase the product shelf life. To replace butter/fat by vegetal extracts rich in antioxidants (and avoid fat oxidantion improve color, appearance).				**EXTRACT (OPE) FIBRE (PBF+ or PBF)**
Sauces					
Juice					
Nutraceutical Supplements	Minerals	x			**FIBRE (PBF+ or PBF)**
Cosmetics	Anti-aging, Antioxidants	x			
Smoothfood (Powder)	. Bioactive (neuroprotection, antiinflamatory)		80ºC		**FIBRE (PBF)**
Healthy Snack Bars	Antioxidants to increase the product shelf life.	x			**FIBRE (PBF+ or PBF)**
Drinkable gels		x	90ºC		
Yogurth	High Mineral Content, fibre and bioactive compounds	x		x	**FIBRE (PBF+ or PBF)**
Dietary supplements		x		x	

Addressing ingredient encapsulation to meet end users requirements

- To keep the organoleptic characteristics of the fortified product intact (avoiding undesirable odours, colour and flavours)
- To keep the phenolic compounds and FA intact after being thermally processed (90°C). Increase self life.
- To be able to work with water soluble or fat soluble matrix and to improve the dispersion

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