From waste to cosmeceuticals: buccal in situ gelling formulation including a polyphenols-enriched secondary raw material from green recovery of grape processing waste

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GREEN EXTRACTION OF NATURAL PRODUCTS

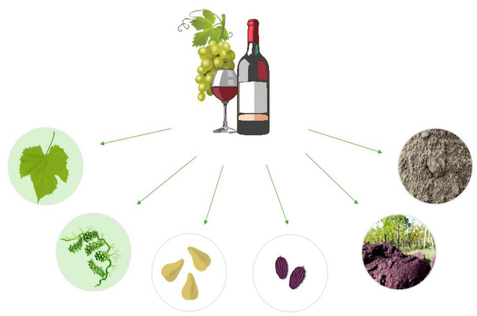
Background

Polyphenols have recently become useful bioactives to treat/prevent oral diseases[1]. These bioactives are widely synthetized in the plant kingdom, so a conscious way to maximize their recovery is to extract them from agri-food wastes and by-products. In a circular economy context, exploring further unconsidered wastes as additional sources of polyphenols can represent a sustainable and virtuous approach to meet the growing demand. Waste black bentonite (BB) (mixture of sodium activated bentonite and activated carbon, 1:1 w/w), used as a fining agent for white grape must (100 g/1 hL must), can be a rich source of polyphenols; the same can be treated to obtain a liquid excipient enriched with polyphenols, useful for various cosmetic and pharmaceutical purposes[2]

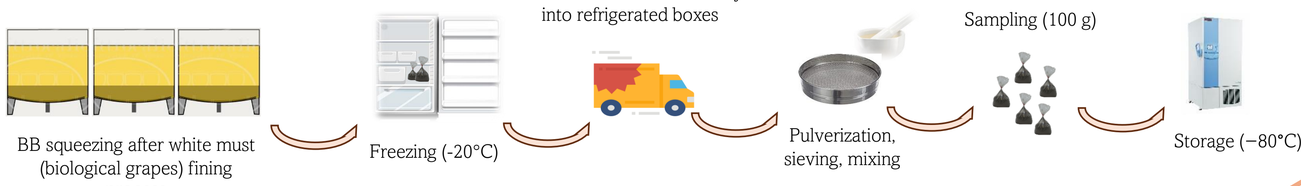
The black bentonite appears as a dark and moist mass after musts filtration and subsequent squeezing

Aim

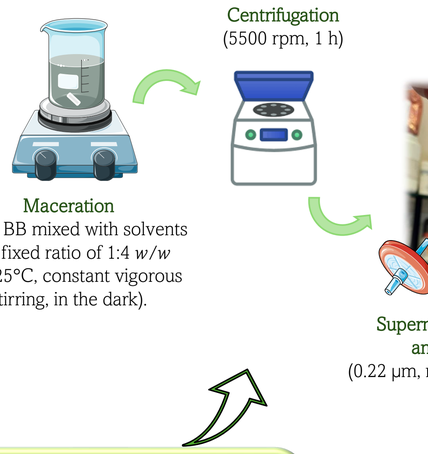
This study aims to cover the entire chain for valorization of the discarded waste BB: from the production of a new secondary raw material, by a green and scalable maceration process testing different unconventional solvents, to the development of an *ad hoc* designed topical formulation for preventing and treating oral disorders, using the enriched polyphenols' extract obtained. Indeed, in view of a waste-to-market approach the polyphenols-enriched extract is intended to be directly marketable as a liquid excipient for cosmetic and pharmaceutical products design



I. Waste recovery, pre-treatments and storage



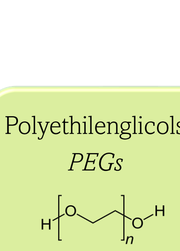
II. Green extraction



Freeze-drying

Evaluation of the water content: 50 ± 5 % (w/w)

Polyethilenglicols *PEGs*
- PEG200
- PEG400
- PEG600



Waste-to-market approach

Green extraction of polyphenols was achieved using **unconventional, eco-friendly solvents among the pharmaceutical/cosmetic excipients**. This approach yielded **high-value extracts directly suitable as liquid functional excipients**, with no need for further purification steps

Waste recovery

Green extraction, analysis and selection of the best extract

Design and characterization of *in situ* gelling system loaded with PEG200 extract

III. Characterization and choice of the best extract

Recovery %, density and pH after water dilution

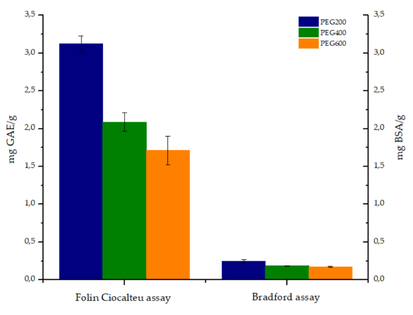
Means (n=6) ± standard error (SE)

Sample	Recovery %	Density (g/mL)	pH after water dilution
PEG 200	65.7 ± 2.4	1.140 ± 0.005	4.47 ± 0.14
PEG 400	63.3 ± 2.0	1.122 ± 0.005	3.54 ± 0.06
PEG 600	49.0 ± 10.0	1.111 ± 0.009	3.57 ± 0.02

$\rho_{PEG} = 1.115 \pm 0.005$ g/mL
$pH_{PEG} \sim 6.5$

Folin Ciocalteu and Bradford assay

Total phenolic (TPC) and protein (TPtC) contents (mg GAE/g and mg eq BSA/g of each extract). Means (n=18) ± SE



PEGs selectively extract polyphenols instead of proteins (TPC >> TPtC)

HPLC-MS analysis

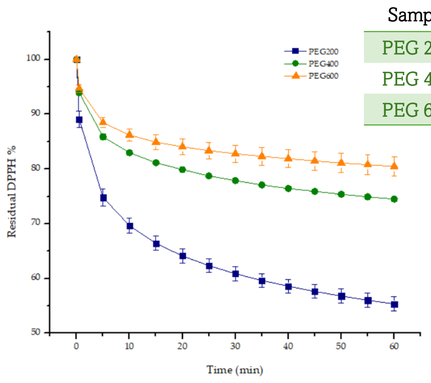
Analyte	Concentration (µg/mL)
Gallic acid	24.00
Gentisic acid	7.34
Catechin	50.37
Caffeic acid	18.96
Syringic acid	9.30
(-)-Epicatechin	25.00
Trans-cinnamic	39.90
Rutin	2.90
Resveratrol	1.50
Apigenin 7-glucoside	0.90
Kaempferol	31.00
Quercetin	330.30

Means (n=3). SD are always < 5%

DPPH assay

To evaluate the **antioxidant power** and kinetic of DPPH consumption each extract was kept reacting with a fixed amount of DPPH radical for 1 h and the percentage residual amount of DPPH radical was quantified every 5 min. Means (n=18) ± SE

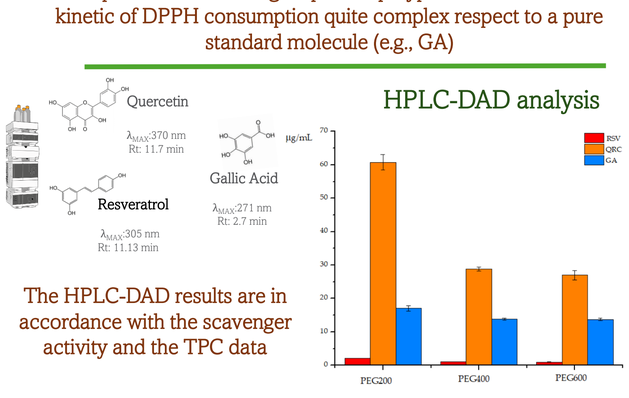
Sample	$GAE_{1\,h}$ (mg/g)
PEG 200	1.30 ± 0.05
PEG 400	0.60 ± 0.01
PEG 600	0.38 ± 0.07



GA standard curves

The presence of a variegate pool of polyphenols determine a kinetic of DPPH consumption quite complex respect to a pure standard molecule (e.g., GA)

HPLC-DAD analysis



The HPLC-DAD results are in accordance with the scavenger activity and the TPC data

Concentration of GA, RSV, QRC in the extracts (ug/mL). Means (n=18) ± SE

Best extract !

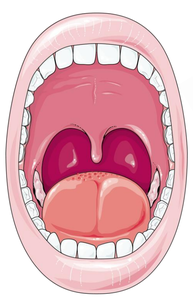
Complex pool of polyphenols

New secondary raw material

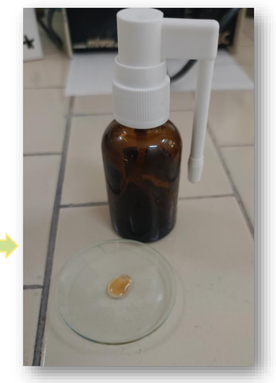
P200-based extract:
- Highest phenolic content (TPC, HPLC-DAD)
- Most powerful scavenger activity
- Rich and complex phenolic footprint (HPLC-MS)

IV. Formulation design

Optimised *in situ* gelling formulation



Components	*% w/w*
• **PEG200 extract**	**30**
• Pluronic F-127	10
• Polivynilpirrolydone K30	5
• Xylitol	3
• Benzyl alcohol	0.5
• Potassium sorbate	0.5
• Sodium metabisolfite	0.2
• Ascorbic acid	0.05
• Urea	0.05
• Sodium dehydrocholate	0.05
• Citrate buffer pH 5.5	50.65



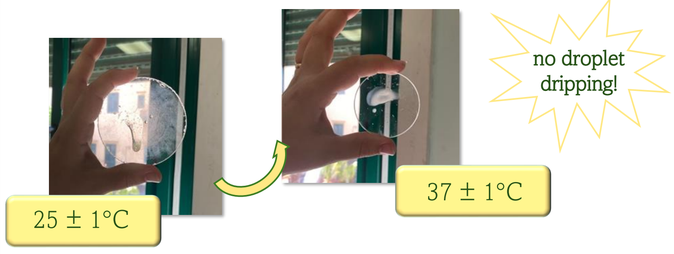
PEG200 extract was inserted into an *ad hoc* designed **liquid formulation intended to be administered as an oral spray for daily care of the whole oral cavity.**

To maximize the contact time between the formulation and the mucosae **thermoresponsive and mucoadhesive polymers** were used. Additionally, some **penetration enhancers** were included to promote polyphenols accumulation into the target tissues

V. *In situ* gelling system characterization

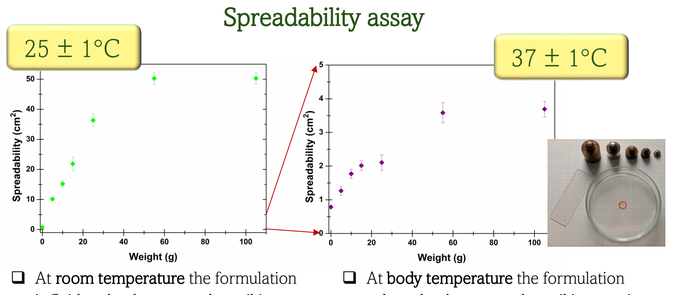
The resulting in situ-gelling buccal formulation was **fluid, colored, clear,** with a pH of 5.35±0.20

Temperature-dependent gelation behaviour (qualitative assay)



The buccal formulation (kept at room temperature) was sprayed on a watch glass kept at room temperature or pre-heated at the body temperature

Spreadability assay



- ❑ At **room temperature** the formulation is fluid and a drop spreads until it occupies a surface up to **64 times** the initial one
- ❑ At **body temperature** the formulation gels and a drop spreads until it occupies a surface up to less than **5 times** the initial one

The designed formulation let that polyphenols act locally:
- Polyphenols (GA, RSV, QRC) were not able to permeate the mucosae and reach the acceptor chamber and no other peaks were detected by HPLC-DAD until 3 h
- A rapid interaction between the actives and the target tissues occurred, resulting in their quick accumulation as no significant differences were highlighted for brief (15 min) or prolonged (3 h) contact times

Buccal/sublingual mucosa (250±25 µm *vs* 80±8 µm)

✓ No permeation
✓ Quick accumulation

	Buccal mucosa (ng/cm²)	Sublingual mucosa (ng/cm²)
GA	163.77 ± 5.23	55.00 ± 3.60
RSV	16.69 ± 1.02	5.72 ± 0.42
QRC	475.88 ± 16.16	139.62 ± 10.21

Amount of GA, RSV and QRC (ng) entrapped into the targeted buccal/sublingual mucosae per surface area (cm²) after 15 min of contact. Means (n=6) ± SE

Topical activity will be related to polyphenols' accumulation into the target tissues

Topical efficacy

DPPH assay

The antioxidant power of the whole formulation was evaluated to assess whereas the P200-based extract maintained its scavenging properties as well as to evaluate the contribution of the other antioxidants inserted into the formulation as preservatives

Means (n=3) ± SE

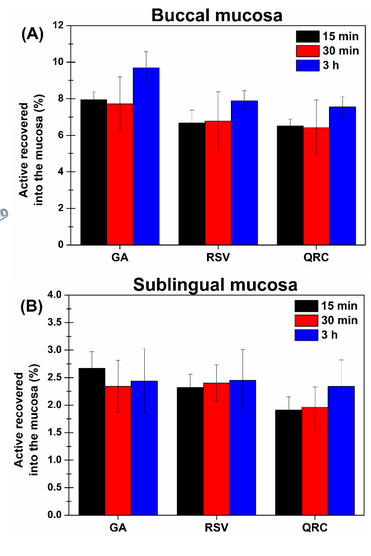
Sample	$GAE_{1\,h}$ (mg/g)
Complete formulation	0.574 ± 0.003
Preservatives-free formulation	0.373 ± 0.005

About the 60% of the GAE value is due to the PEG200 extract

Ex vivo tests: vertical Franz diffusion cells

As the **formulation** is **intended for the whole oral cavity daily care** the ability of polyphenols to permeate and/or penetrate through oral mucosae was studied using both **buccal** (A) and **sublingual** (B) **porcine tissues**

GA, RSV and QRC were quantified by HPLC-DAD as reference molecules



The higher amount of polyphenols entrapped into the buccal mucosa rater than the sublingual one could be attributed to the higher lipophilicity and thickness of the buccal tissue

Conclusions

Waste BB can be reused as a sustainable source of polyphenols. Green extraction by using unconventional solvents such as PEGs yielded novel polyphenols-enriched liquid excipients directly employable and marketable as such for cosmetics and pharmaceuticals. PEG200 emerged as the best extraction solvent, thus the resulting extract was incorporated into an *ad hoc* designed fluid sprayable buccal formulation intended for daily oral care. The latter was able to in situ-gelling thus prolonging the contact time with the target tissue and promoting polyphenols accumulation into the oral mucosae, ensuring the effectiveness of the cosmeceutical formulation.

References
1. Belfiore E et al., 2024, 10.3390/cancers16020260
2. Di Prima G et al., 2024, 10.1016/j.scp.2023.101414

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