Delivery Technologies for Diversified Products I

Maria Rosa Gigliobianco, PhD

Post-doc
Department of Pharmacy
University “ G. d’Annunzio” of Chieti and Pescara
Chieti, Italy
mariarosa.gigliobianco@gmail.com

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INTEGRATING
Delivery Science
ACROSS DISCIPLINES

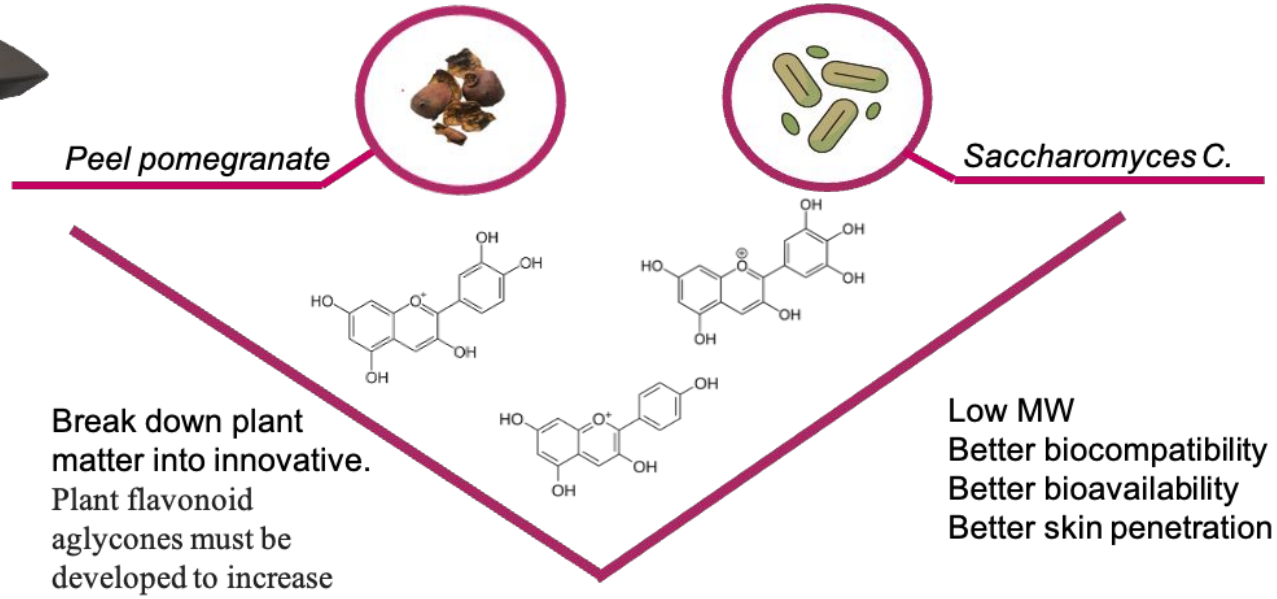
Green approach for the activation of polyphenolic compounds: *in vivo* evaluation of novel cosmetic formulations

Purpose of the study

We developed a novel and eco-friendly method to prepare fermented extracts from fruit peel, exploiting the potential of enzymatic sources to promote the bioconversion of glycosylated molecules.

Recovery

Punica Granatum



Valorization

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Experimental section

Ultrasonication and enzymatic fermentation were combined to obtain the final extract, which contained bioactive molecules. High-performance liquid Chromatography (HPLC-DAD) assessed the fermented extracts' aglyconic bioactive chemical production (Figure 2). The fermented extract was tested for in vitro evaluation and cell line compatibility.
Twenty volunteers were chosen to quantify skin redness, hydration, and trans-epidermal water loss before and after skin care product application.

Experimental section

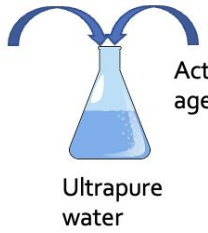


Table 1. Optimized experimental conditions (method A).

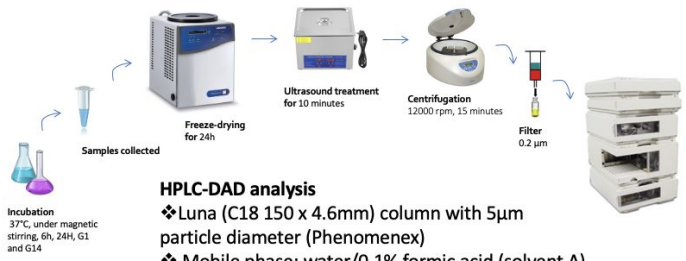
	LA*-G0/ LA-G1/ LA-G14	WA**-G0/ WA-G1/ WA-G14	LWA***-G0/ LWA-G1/ LWA-G14
Activation agent	50 mg	/	50 mg
Lyophilized extract of *P. Granatum peel*	/	100 mg	100 mg
Ultrapure water	10 mL	10 mL	10 mL

***LA**= Activation agent + water
****WA**= Peel pomegranate extract+ water
*****LWA**= Activation agents+ extract + water

Sample after incubation

Figure 1. Experimental methodology for the extraction activation-based on a green approach.

HPLC-DAD analysis



HPLC-DAD analysis
❖Luna (C18 150 x 4.6mm) column with 5µm particle diameter (Phenomenex)
❖ Mobile phase: water/0.1% formic acid (solvent A) and acetonitrile/0.1% formic acid (solvent B)
❖ Flow: 1mL/min. gradient elution
❖ Injection volume: 5µl

Figure 2. HPLC-DAD analysis used for the evaluation of the bioactive molecules formation after different times of incubation.

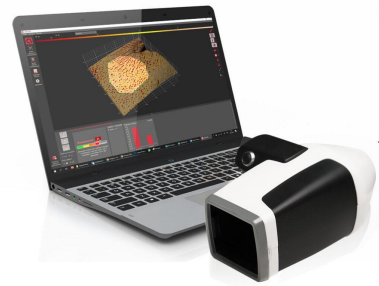
In vitro and *in vivo* evaluation

FRAP
Measurement of the reducing power of the sample against the iron ions.

ABTS
Measurement of the reducing capacity of the sample in reference to a standard (Trolox).

DPPH
Quantification of reducing capacity of the test substance whether it acts through hydrogen or electron transfer.

FOLIN
A spectrophotometric method that evaluates the colorimetric reaction that happened from the interaction between the Folin-Ciocalteau reagent and the extract samples.



Characterization of the extracts

The enzyme cells incubated with the peel extract following method A after different incubation times were subjected to freeze-drying and ultrasound treatment. The pictures obtained for these two-time points showed that the freeze-drying associated with ultrasound treatments led to better-stressed cells, due to it was found an irregular cellular shape, as we can see in *Figure 3 (a-b-c)*.

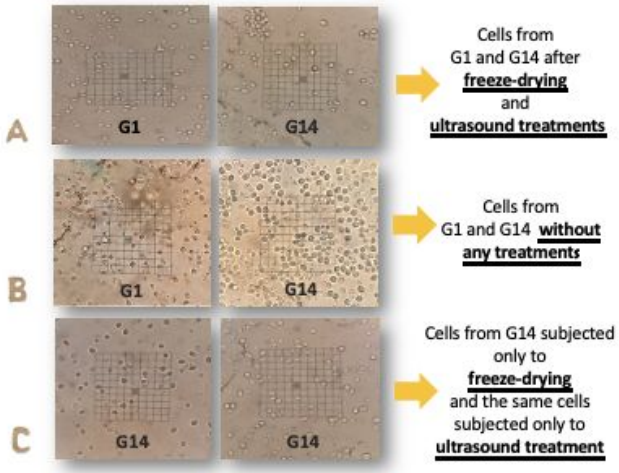


Figure 3-Cells pictures obtained after incubation at different time points (G1 and G14) of method A after freeze-drying and ultrasound treatments using the optical microscope.

The formation of bioactive molecules was monitored by HPLC-DAD. The results allowed the identification and monitoring over time of the loss of signal related to glycosylated molecules, cyanidin 3-glucoside and pelargonidin 3-glucoside, used as standards and present in peel *P. Granatum* extracts (Figure 4).

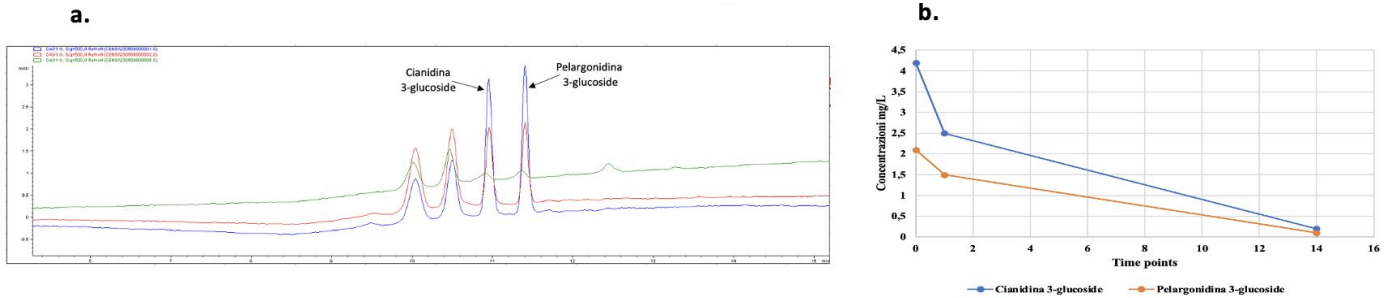


Figure 4. a) Chromatograms of LWA samples at different time points (blue G0, red G1 and green G14); b) Kinetic evaluation of the two standards.

In vitro results

We detected an increase in the total phenol content in samples G0 and G14, which shows that peel *P. Granatum* extract with the activated substrate can increase antioxidant capacity (Figure 5 a-b).

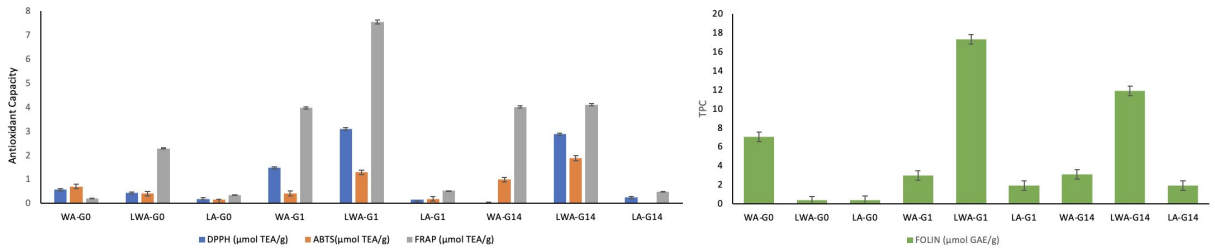


Figure 5. a) Antioxidant capacity and b) total phenolic content results.

Cells study results

The cell results (Figure 6) revealed that all tested sample doses appeared perfectly tolerable by HGF cells, as the cellular vitality rate always exceeds 85%. A statistical reduction in cell toxicity was also detected when treated with samples of fermented extracts.

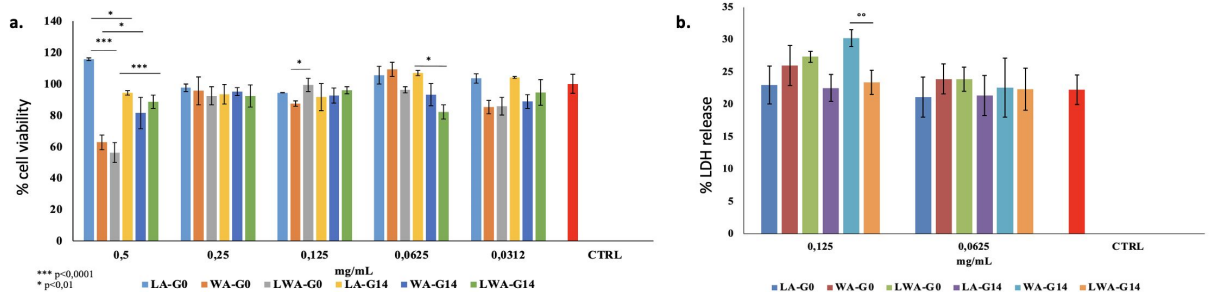
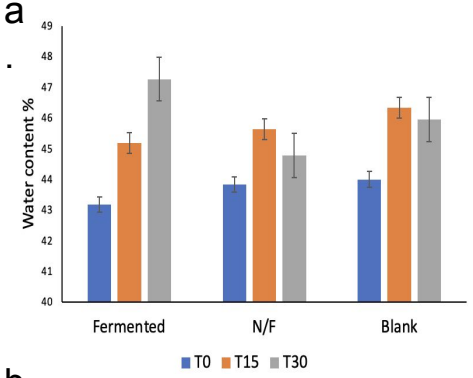


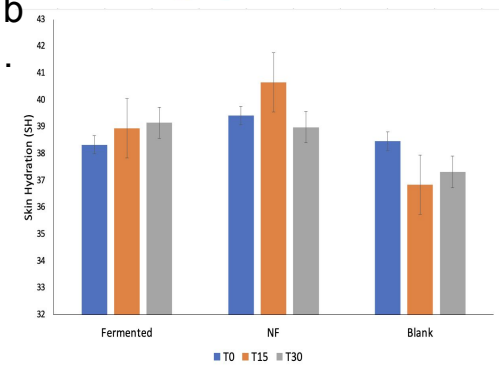
Figure 6. a) MTT (a) and cytotoxicity assay (b).

In vivo results

a.



b.



c.

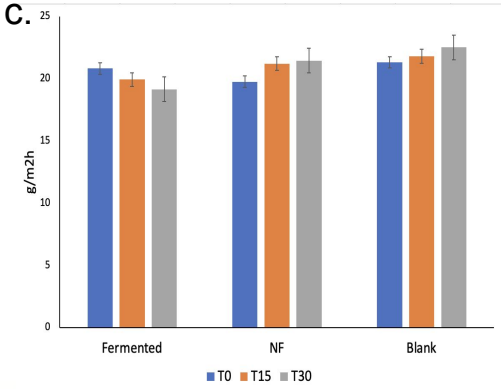
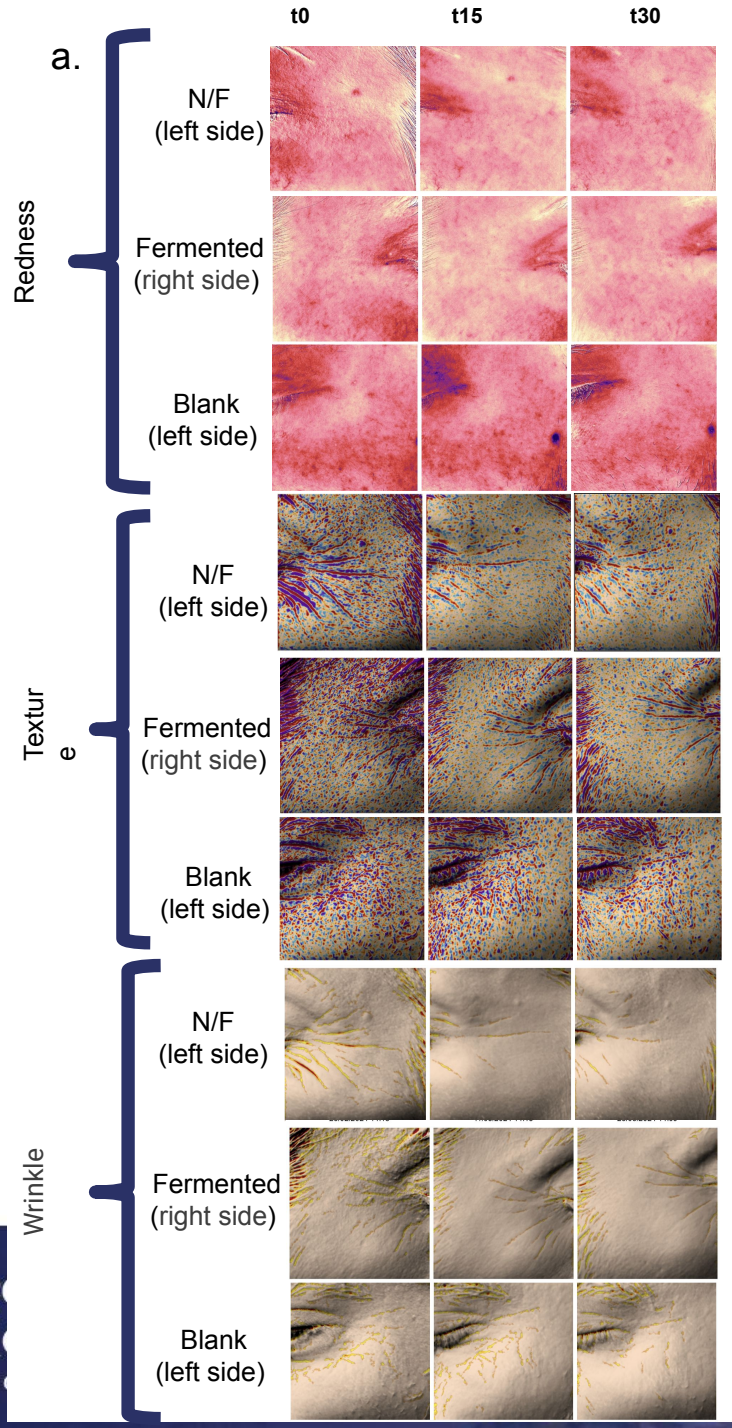
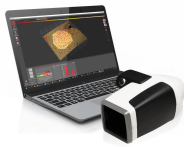


Figure 7. a) Results of the skin surface hydration obtained with the instrument MoistureMeterSC® (Delfin Technologies, Kuopio, Finland); b) Results for deep skin hydration using the instrument MoistureMeterEpid® (Delfin Technologies, Kuopio, Finland) c) Results for Trans Epidermal Water Loss (TEWL) to evaluate the efficiency of the skin barrier. The analysis used a VapoMeter® (Delfin Technologies, Kuopio, Finland).





The *in vivo* evaluation of the formulations with human skin was performed under normal conditions according to the Helsinki Declaration (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and the COLIPA guidelines. Informed consent was obtained from a panel of 20 volunteers (age 26-55) of female and male gender.
Figure 8 shows a decrease in wrinkle length from 47.4 mm (t0) to 21.3 mm (t15) and 7.8 mm (t30). Based on blood vessel dilatation, the skin's redness was evaluated, and results showed a clear reduction in redness, which decreased from 31.05 (t0) to 25.0 after 15 days and 23.16 (t30). The value obtained for the texture showed a 50% texture index reduction, where the rugosity changed from 15.6 µm at t0 to 8.25 µm at t15 and, after 30 days, became 7.7 µm.

b.

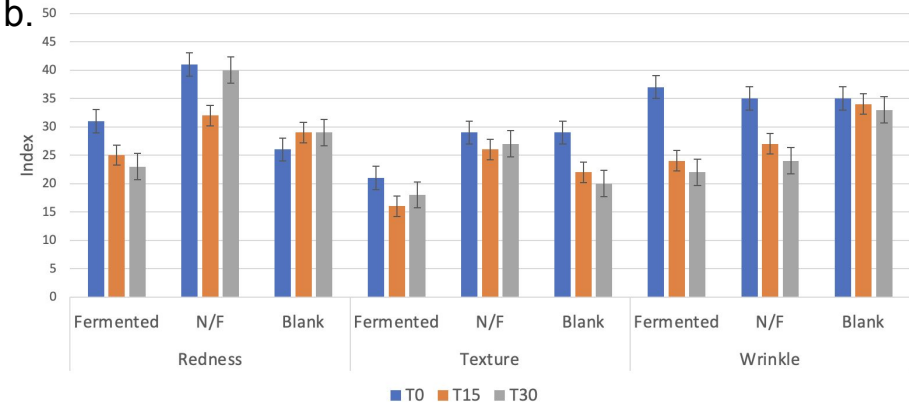
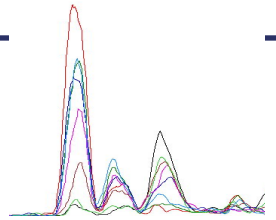


Figure 8. a) Picture of the periorbital area obtained by Antera 3D; b) Results related to the index values obtained for all tested formulations in redness, texture and wrinkle parameters evaluated by using Antera 3D with a software program (Miravex Limited, Antera).

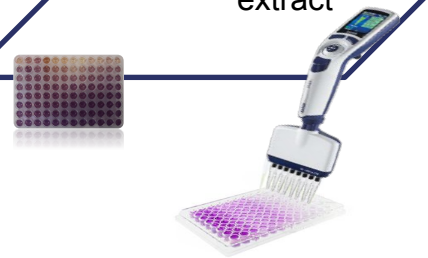
Conclusions

Suitable incubation conditions with antioxidant molecules and peel extract were established

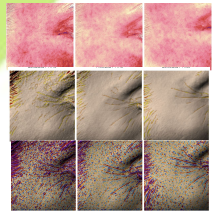
Analytic analysis were developed to evaluate the hydrolytic kinetics for the formation of the aglycone



Antioxidant tests and *in vitro* cells studies confirmed a higher activity of the fermented extract

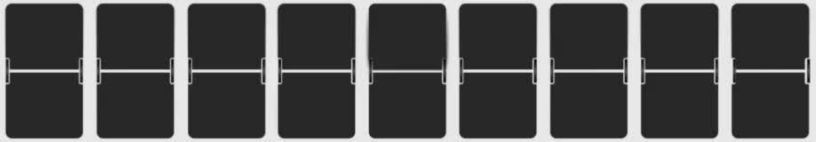


Activation of antioxidant glycosylate molecules in their aglycone form with a natural, economic and sustainable model method, allowing the design of cosmetic formulation, which also demonstrated promising in vivo results.



Maria Rosa Gigliobianco[1], Camilla Elena Di Bella[1, 3] , Lucrezia Di Nicolantonio[2,3], Roberta Censi[2] , Susi Zara[1] , Piera Di Martino[1]

[1]University "G. d'Annunzio" of Chieti and Pescara, Chieti, Italy.
[2]School of Pharmacy, University of Camerino, Camerino, Italy.
[3]Recusol srl, Camerino, Italy.



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