

**EPNOE WORKSHOP** 



ROME, SAPIENZA UNIVERSITY

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# POLYSACCHARIDES IN DRUG DELIVERY -

# ON THE ROAD TO INNOVATION!

# Freeze drying versus Spray drying to obtain Grapefruit Integropectin powder

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### INTRODUCTION & SCOPE

IntegroPectin is a new family of citrus pectins of broad biological activity obtained via hydrodynamic cavitation of citrus biowaste carried out in water only. These pectins have already been isolated by freeze drying, a time-consuming and expensive procedure difficult to be scaled up. This work aims to develop a simple, fast, efficient and industrially advantageous procedure for pectins isolation. To this scope the spray drying technique is here proposed and evaluated as, nowadays, it is a greatly appreciated methos for food and pharmaceutical applications also considering an industrial point of view.

## Freeze drying vs. Spray drying







### Spray drying

100 mL of aqueous extract  $\Box$ 

Inlet temperature: 110°C Solution flow: 200 mL/h

Nitrogen aspiration: 100%

Cooling temperature (inert loop): 17°C 🗖

Starting water equilibration: 10 min 🗆

Final water cleaning: 10 min

□ 30 mL of aqueous extract previously stored at -80°C overnight ☐ Freeze drying for 3 days

□ 0.014 mPa

Freeze drying

 $\square \approx -50^{\circ}C$ 

☐ in the dark

HPLC-DAD

60 mg of freeze

dried or spray

dried powder +

5 mL EtOH/H<sub>2</sub>O

4:1 (v/v)

**A)** 

The yield of freeze drying process is assumed to 100% as this\* process does not lead to material loss The yield of spray drying process is referred to the freeze drying one \*\*

Filtration (PTFE;

Hesperidin

HPLC-DAD

analysis

Hesperidin

### Freeze drying Spray drying \*Yield %: 100% \*\*Yield %: 95.03 ± 6.79% $30 \text{ mL} \rightarrow 3 \text{ days}$ 100 ml → 30-50 min Difficult to scale up Easy to scale up Low capital and operational Expensive costs Appearance: light yellow, fine Appearance: light yellow, vaporous and needle-like powder powder pH after water dissolution: pH after water dissolution:



Folin-

Ciocalteu

## Folin-Ciocalteu

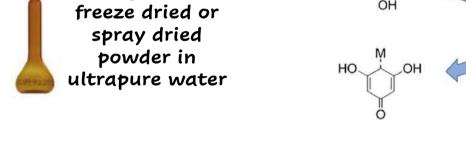
□ 2 h

 $4.03 \pm 0.07$ 

☐ Room temperature

response in presence og

phenolic



1. 50 µL of sample into a plastic tube containing 2 mL of ultrapure water

2. + 130 µL of Folin-Ciocalteu reagent

3. + 370  $\mu$ L of Na<sub>2</sub>CO<sub>3</sub> water solution (1.89M)

Spray dried

5 mg/mL of

☐ In the dark Total phenolic content Sample Freeze dried  $3.85 \pm 0.31$ powder

powder Quantitative results of the Folin-Ciocalteu analysis expressed as equivalent to gallic acid (mg) recovered in 100 mg of powder ± SE

### Chromatograms at 285 nm and 3D plots of the phenolic extract from the A) freeze dried and B) spray dried grapefruit IntegroPectin samples

Sample	Naringin	Hesperidin
Freeze dried powder	3.08 ± 0.03	$1.42 \pm 0.03$
Spray dried	$2.87 \pm 0.06$	$1.34 \pm 0.03$

Sonication

(15 min)

Naringin

Naringin

Quantitative results of the HPLC-DAD analysis expressed as mg recovered in 100 mg of powder ± SE

DPPH

100

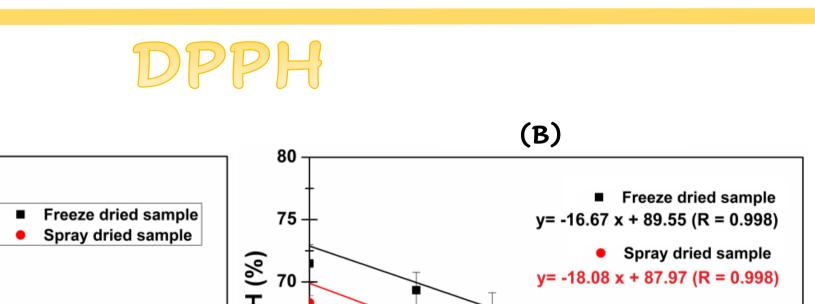
Hesperidin

Naringenine & Naringin

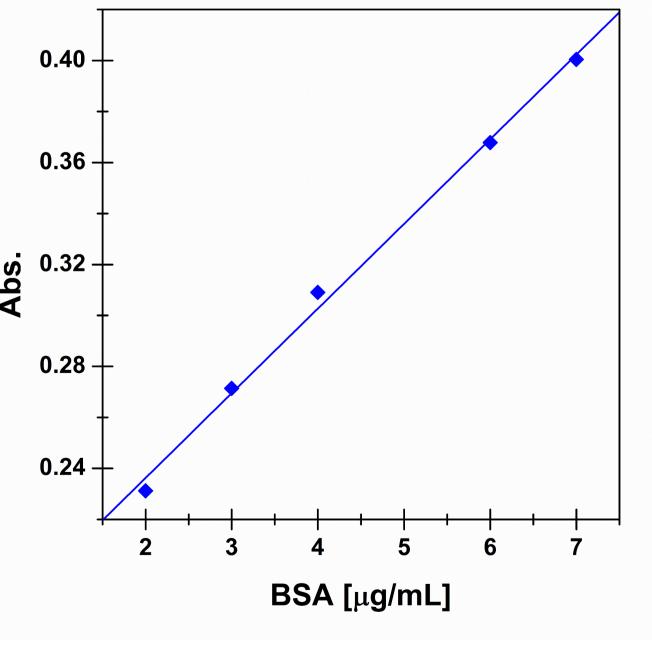
Kaempferol-7-0-

glucuronide

Gallic Acid



## Bradford Assay



The total protein content in the freeze dried sample is nearly 4 times higher than in the spray dried one

☐ The cryogenic temperatures of the freeze drying process ensure protein retention

☐ The even short exposure to high temperatures during the spray drying process may allow to protein denaturation

### Total protein content Sample Freeze dried $0.246 \pm 0.004$ powder

Quantitative results of the Bradford assay investigation expressed as equivalent to bovine serum albumin (mg) recovered in 100 mg of powder ± SE

Bradford assay: bovine serum albumin calibration curve

Spray dried

powder

 $0.062 \pm 0.004$ 

☐ Remarkable Yield (>95%)

Assay

Bradford

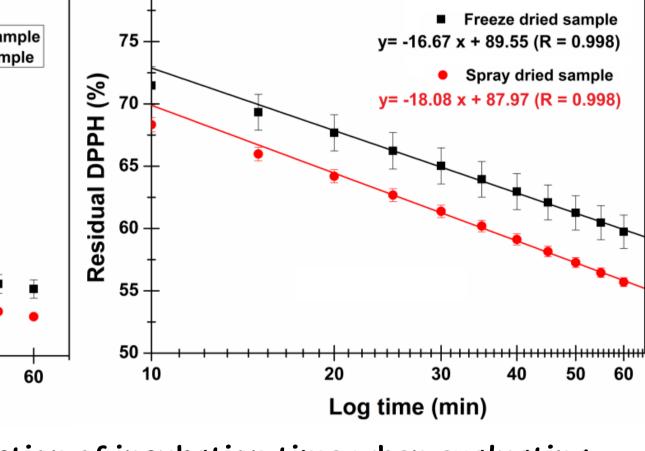
HPLC-

DAD

Conclusions

Time (min

(A)



(A) Residual DPPH (%) as a function of incubation time when evaluating the freeze (black) and spray (red) dried powders. (B) Detail in semilogaritmic scale and linear curve fitting (linearity range: 20-60 min)

Spray dried

powder

Antioxidant power expressed as equivalent			
Calibration curve (60 min)	0.0231 ± 0.0009	0.0261 ± 0.0003	
Calibration curve (30 min)	0.0204 ± 0.0011	0.0232 ± 0.0004	
Calibration curve (10 min)	0.0166 ± 0.0013	0.0193 ± 0.0005	

Freeze dried

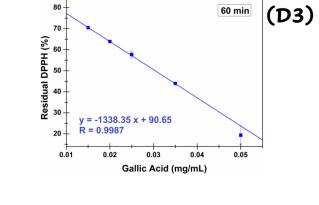
powder

Freeze dried and Spray dried IntegroPectin powders are

superimposable in terms of total phenolic content, amount of some representative polyphenols and chromatographic profile. The Spray dried IntegroPectin demonstrates a slightly higher antioxidant Gallic Acid used as reference: (C) Gallic acid DPPH behavior as power. The Spray Drying methos offers the following further advantages:

☐ Easy to scale up ☐ Possible inactivation of oxidative and hydrolytic enzymes leading to bioactive polyphenols

a function of concentration; (D1-3) DPPH standard curves at fixed time points (10, 30 and 60 min)



10 min (D1)

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Quick procedure

protection from subsequent degradation













