Article

Grapefruit IntegroPectin isolation via spray drying and via freeze drying: a comparison

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SUMMARY

The comparison of grapefruit IntegroPectin powders isolated via spray drying and via freeze drying in terms of phenolic content, quantification of the most representative flavonoids (naringin and hesperidin), radical scavenging activity, total protein content and pH of the aqueous solutions provides relevant information. Except for the protein content, the two drying methods afford largely similar pectins. Optimization of the spray drying parameters allowed to maximize the yield of isolated pectin that nearly approached (>95%) the quantitative yield obtained via freeze drying.

Keywords: IntegroPectin; grapefruit; citrus; flavonoids; freeze drying; spray drying

1 INTRODUCTION

"IntegroPectin" is the name of a new family of citrus pectins of exceptionally high and broad biological activity obtained via hydrodynamic cavitation of citrus biowaste carried out in water only.[1] Obtained from orange,[2] grapefruit[3] and lemon[4] industrial processing waste, the pectin powders rich in adsorbed flavonoids and terpenes so far described have been isolated via freeze drying.

Widely used in the pharmaceutical and food industries, the latter technique to dry biopharmaceuticals, food and biological materials with little or no degradation requires very low temperature (-54 °C) and pressures (high vacuum) in order to achieve sufficient drying rates.[5] The technology is evolving towards continuous processes capable to reduce the energy consumption, shorten drying times and fit continuous flow synthetic processes. One such technique, combining the characteristics of spray drying and freeze drying, is spray freeze-drying.[6]

Involving the "atomization" of a liquid to create microparticles that following quick solvent evaporation are separated from the drying gas (usually air or inert nitrogen) by means of a cyclone that deposes them in a glass collector situated in the bottom of the device, the spray drying technique has emerged as a less expensive and faster drying technique.[7] The main problem of the spray drying technique, now used in alternative to freeze drying whenever possible, lies in the often low yields (20-70%) of isolated product.[6]

The present work compares grapefruit IntegroPectin powders obtained via spray drying and via freeze-drying in terms of phenolic content, quantification of the most

representative flavonoids, radical scavenging activity, total protein content, and pH of the resulting aqueous solutions. The optimization of the spray drying conditions, furthermore, allowed to maximize the yield of isolated pectin that nearly approached the quantitative yield obtained via freeze drying while preserving its phenolic content.

2 MATERIALS AND METHODS

2.1 Materials. The grapefruit IntegroPectin aqueous extract was obtained via the hydrodynamic cavitation of industrial grapefruit processing waste as previously reported.[3] Gallic acid, naringin, hesperidin, Bradford and Folin-Ciocalteu reagents were purchased by Merck (Darmstadt, Germany). Bovine serum albumin (BSA), and 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) were obtained from Carlo Erba (Milan, Italy). All other chemicals and solvents, purchased from Carlo Erba, were of analytical grade and used without further purification.

2.2 Grapefruit IntegroPectin via freeze drying. A 30 mL sample of the aqueous extract was stored at -80°C (Forma 902 ULT Freezer, Thermo Fischer Scientific, Waltham, MA, USA) overnight and then subjected to freeze drying using a FreeZone 2.5 Liter freeze dry system (Labconco, Kansas City, USA) for 3 days. The powder thereby obtained was accurately weighed. The yield of the freeze drying process was considered as reference (100%). The procedure was repeated in triplicate.

2.3 Grapefruit IntegroPectin via spray drying. A Mini Spray Dryer B-290 (Büchi, Flawil, Switzerland) equipped with an inert loop B-295 was used by setting the following parameters: inlet temperature of 110°C, solution flow of 200 mL/h, nitrogen aspiration of 100%, and cooling temperature (inert loop) of 17°C. After equilibrating the instrument for 10 min with ultrapure water, a 100 mL sample of the grapefruit aqueous extract was processed for 30 min. Finally the instrument was cleaned for 10 min with ultrapure water. The obtained powder was recovered and the yield % was calculated as follows:

$$Yield\ \% = \frac{S\ (mg)}{F\ (mg)}\ x\ 100 \qquad (1)$$

where *S* is the milligram amount of powder obtained after spray drying and *F* the milligram amount of powder obtained after freeze drying the aqueous extract. The process was repeated 3 times and results are reported as mean ± SE.

2.4 DPPH Radical Scavenging Assay. A 2 mL sample of DPPH stock solution (40 µg/mL) was added to a quartz cuvette. Subsequently, 100 µL of ultrapure water or of grapefruit IntegroPectin sample solution (4 mg/mL prepared by dissolving 20 mg of freeze dried or spray dried powder into a 5 mL volumetric amber flask by using ultrapure water as solvent) were added, well mixed and immediately subjected to UV-Vis measurements by using a Shimadzu (Kyoto, Japan) 1700 spectrophotometer.

The DPPH reduction over time was monitored at room temperature by analyzing each sample every 5 min for 1 h. Six DPPH standard solutions in methanol were prepared and analyzed to construct the calibration curve as follows: λ_{max} = 515 nm; linearity range: 4-40 µg/mL µg/mL, regression equation: Abs = 0.018 + 28.59 x [mg/mL], (R = 0.999). Each experiment was performed in triplicate. Results are expressed as residual DPPH percentage over time ± SE. Standard DPPH curves were obtained by analyzing gallic acid standard solutions (0.015-0.050 mg/mL) in ultrapure water according to the operative method used for the freeze dried and the spray dried samples. The residual DPPH % at the 3 selected time points (10, 30 and 60 min)

was used to construct 3 calibration curves helpful to compare the grapefruit-extract powder to a phenolic standard both in terms of extent and rate of DPPH consumption. The experiments aimed at obtaining the calibration curves were performed in triplicate. Results are reported as mean of the equivalent in gallic acid concentration (mg/mL) ± SE for each selected time.

2.5 Flavonoid quantitative analysis. A 5 mL sample of aqueous ethanol ($EtOH/H_2O$ 4:1, v/v) was added to 60 mg of freeze or spray dried grapefruit IntegroPectin powder followed by sonication for 15 min. Afterwards, the obtained dispersion was filtered through a 0.22 µm PTFE syringe filter and brought to volume with fresh $EtOH/H_2O$ (4:1, v/v). Samples were appropriately diluted and then subjected to quantitative analysis by using a HPLC 1260 Infinity Instrument (Agilent Technologies, Santa Clara, USA) equipped with a Quaternary Pump G1311B, a Diode Array Detector 1260 Infinity II and a computer integrating apparatus (OpenLAB CDS ChemStation Workstation). The following conditions were applied: injected volume: 20 µL; column temperature: 25°C. Chromatographic separation was achieved on a Ace Excel Super C18 (5U, 100A, size 125x4.60 mm) reversed-phase column (Advanced Chromatography Technologies, Hyderabad, India). To quantify naringin and hesperidin a mobile phase consisting in 0.1% (v/v) trifluoroacetic acid (TFA) solution in ultrapure water (solvent A) and methanol (solvent B) was used according with the following time program: 0–1 min A:B = 70:30; 1-31 min A:B = 40:60; 31–32 min A:B = 70:30 and 32-35 min A:B = 70:30. The flow rate was set at 1 mL/min and the UV wavelength at 285 nm (DAD investigation 190-800 nm). In these conditions, the retention times of naringin and hesperidin were 14.9 and 16.2 min, respectively. Naringin: linearity range: 5-250 µg/mL, regression equation: Area = 54.05 + 20477.42 x [mg/mL], (R = 0.999). Hesperidin: linearity range: 5-250 µg/mL, regression equation: Area = -69.91 + 26498.71 x [mg/mL], (R = 0.997). Each extraction procedure was performed in triplicate. Results are presented as amount of each selected flavonoid (mg) into 100 mg of powder and reported as means ± SE (standard error).

2.6 Total phenolic content. The total phenolic content was assessed by the Folin-Ciocalteu method.[8] Sample solutions (5 mg/mL) were prepared by dissolving 25 mg of freeze dried or spray dried powder into a 5 mL volumetric amber flask by using ultrapure water as solvent. 50 µL of each clear solution were added to 2 mL of ultrapure water previously loaded into a 15 mL plastic tube. Subsequently, a 130 µL aliquot of Folin-Ciocalteu reagent was added to the tube, mixed well and left to settle for 5 min in the dark. Finally, a 370 µL aliquot of sodium carbonate solution in ultrapure water (0.2 g/mL) was added to each tube, mixed and kept at room temperature in the dark for 2 h. Samples were subjected to UV-Vis measurements by using a Shimadzu 1700 spectrophotometer (Kyoto, Japan) with the appropriate calibration curve and blank. Similarly, six standard solutions of gallic acid in ultrapure water (50-500 µg/mL) were prepared and analyzed to construct the calibration curve as follows: λ_{max} = 760 nm; linearity range: 0.98-9.80 µg/mL, regression equation: Abs = 0.039 + 65.08 x [mg/mL], (R = 0.999). Each experiment was performed in triplicate. Results are expressed as equivalents of gallic acid (mg) found in 100 mg of grapefruit IntegroPectin powder ± SE.

2.7 Total protein content. The total protein content was assessed by the Bradford assay.[9] Sample solutions (10 mg/mL) were prepared by dissolving 10 mg of freeze dried or spray dried powder into 1 mL of ultrapure water as solvent. A 400 µL aliquot of each clear solution was added to 400 µL of ultrapure water previously loaded into a 2 mL plastic vial (Eppendorf, Hamburg, Germany). A 200 µL aliquot of Bradford reagent (B6916, Sigma Aldrich, St. Louis, USA) was thus added and mixed well. The mixture was kept in the dark at room temperature for 30 min. The resulting sample was subjected to UV-Vis measurements by using a Shimadzu 1700

spectrometer (Kyoto, Japan) with the appropriate calibration curve and blank. Similarly, five BSA standard solutions in ultrapure water were prepared and analyzed to construct the calibration curve as follows: λ_{max} = 595 nm; linearity range: 2-7 µg/mL, regression equation: Abs = 0.1700 + 0.0332 x [µg/mL], (R = 0.998). Each experiment was performed in triplicate. Results are expressed as equivalents of BSA (mg) found in 100 mg of grapefruit IntegroPectin powder ± SE.

2.8 pH evaluation of the re-dispersed powders. Sample solutions (5 mg/mL) were prepared by dissolving 25 mg of freeze dried or spray dried grapefruit IntegroPectin powder in 5 mL ultrapure water kept in a volumetric amber flask. The pH of the resulting solutions was measured using a HI 2211 pH/ORP Meter (Hanna Instrument, Woonsocket, RI, USA). Each experiment was performed in triplicate. Results are reported as means ± SE.

2.9 Data analysis. Data were expressed as means ± SE. All differences were statistically evaluated by the Student's t-test with the minimum levels of significance, with $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Spray drying optimization. Since some relevant parameters such as the inlet temperature and the starting solution concentration were not modifiable due to their reliance on the aqueous matrix to be dried, the spray dryer flow rate was the main adjustable instrument parameter. A flow rate range between 50 and 250 mL/h was evaluated (instrument's maximum: 1000 mL/h). The highest applicable process rate turned out to be 200 mL/h. This flow rate allowed to keep a relatively small difference between the inlet and outlet temperatures, and thus an effective and efficient removal of water, at the highest process rate. Eventually, this allowed to achieve a very high IntgroPectin isolated yield of 95.03 ± 6.79% when compared to the 100% yield of the freeze drying process in which no pectin is lost during the drying process. However, while drying of 30 mL of aqueous extract via freeze drying took 3 days, the spray drying allowed to dry 100 mL in 30-50 min (30 min of actual drying procedure of the sample + 10 min of starting equilibration and 10 min of final cleaning).

Table 1. Main features of grapefruit IntegroPectin drying techniques

Freeze drying	Spray drying
Yield %: 100%	Yield %: 95.03 ± 6.79%
30 mL → 3 days	100 ml → 30-50 min
Difficult to scale up and expensive	Easy scale up and low capital and operational costs
Appearance: light yellow, vaporous and needle-like powder	Appearance: light yellow, fine powder

Table 1 summarizes the main features of both drying processes. Figure 1 shows the yellow powders isolated via the two techniques. The visual appearance was clearly different. Whereas the freeze dried sample results in a light yellow, vaporous and needle-like powder, the spray dried sample is made of a light yellow, fine powder.

Figure 1. Freeze dried (left) and spray dried grapefruit IntegroPectin (right).

3.2 DPPH assay. Figure 2 shows the residual DPPH (per cent) as a function of incubation time after sampling the DPHH assay solution containing the IntegroPectin every 5 min for 1 h. Clearly, the spray dried sample (red curve) had a slightly higher antioxidant activity than the freeze dried (black curve) IntegroPectin. This is even more evident when looking at the curve between 20 and 60 min in the semi-logarithmic scale (Figure 2, right).

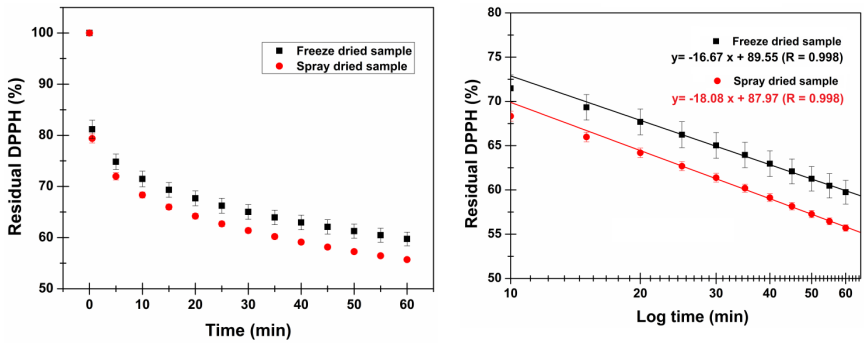

Figure 2. Residual DPPH (%) as a function of incubation time when evaluating freeze (black dots) and spray (red) dried grapefruit IntegroPectin (left plate). Detail of the semi-logaritmic curves and linear curve fitting (linearity range: 20-60 min, right plate).

The slope of the linear plots obtained allows to appreciate the rate at which the two IntegroPectin powders dissolved in solution exert their antioxidant activity. A larger slope points to a quicker consumption rate of the DPHH reagent. Clearly the spray dried samples is a quicker antioxidant agent (angular coefficient of 18.08 *vs.* 16.67 for the freeze dried sample).

To gain a broader molecular view of the antioxidant activity of the newly obtained IntegroPectin powders, we first repeated the DPHH assay with five gallic acid (GA) solutions of known concentration used as standards (Figure 3).

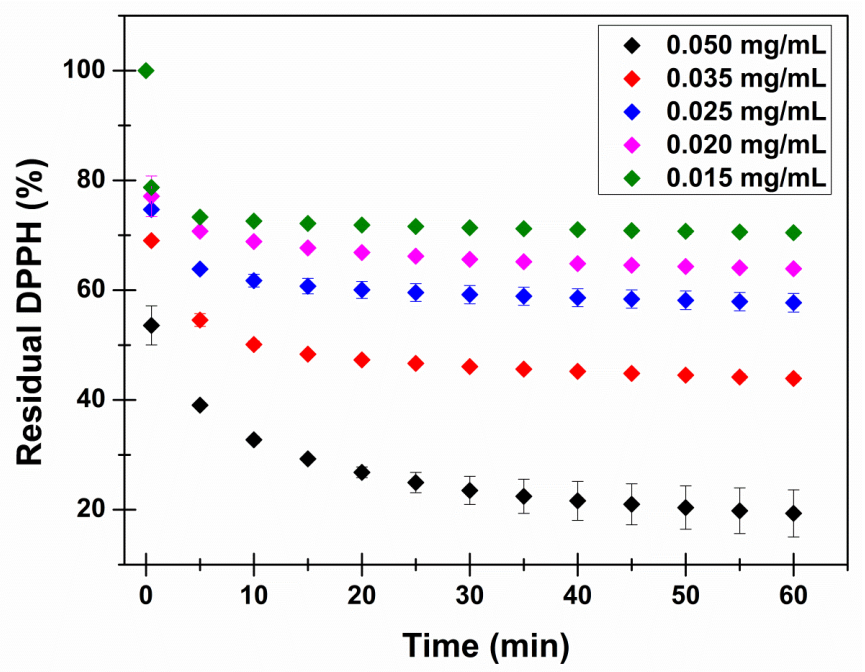

Figure 3. Residual DPPH % as a function of incubation time of gallic acid solutions at different concentrations: 0.050 mg/mL (black dots), 0.035 mg/mL (red), 0.025 mg/mL (blue), 0.020 mg/mL (pink) and 0.015 mg/mL (green).

Hence, the residual DPPH concentration at 10, 30 and 60 min for each gallic acid solution was plotted *vs.* its concentration (Figure 4). Using the outcomes of the resulting calibration curves, and knowing the amount of residual DPPH for the grapefruit IntegroPectin powders at each time, it was possible to evaluate the equivalent antioxidant power compared to a known GA concentration (Table 2).

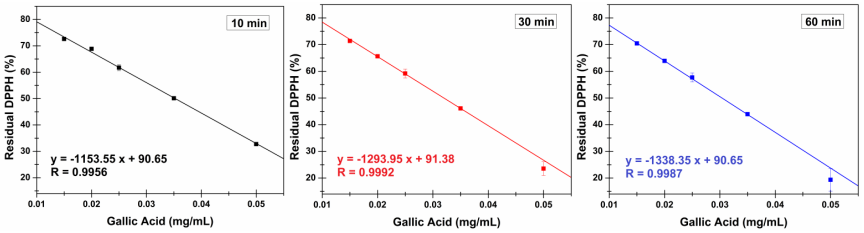

Figure 4. DPPH standard curves for gallic acid standard solutions at fixed time points: 10 min (black line), 30 min (red) and 60 min (blue).

Data in Table 2 confirm what is revealed by the graphs in Figure 2 and Figure 3: the grapefruit IntegroPectin powders and gallic acid, a biophenol of strong antioxidant activity widely used in medical and nutraceutical products,[10] originate a completely different DPPH reduction course.

Table 2. Antioxidant power of freeze dried and spray dried grapefruit IntegroPectin in equivalent gallic acid concentration (mg/mL) ± SE at 10, 30 and 60 min.

Sample	Calibration curve (10 min)	Calibration curve (30 min)	Calibration curve (60 min)
	y = -1153.55 x + 90.65	y = -1293.95 x + 91.38	y = -1338.35 x + 90.65
Freeze dried powder	0.0166 ± 0.0013	0.0204 ± 0.0011	0.0231 ± 0.0009
Spray dried powder	0.0193 ± 0.0005	0.0232 ± 0.0004	0.0261 ± 0.0003

The antioxidant power of GA tends to rapidly reach a *plateau* (Figure 3). The IntegroPectin powders show a steeper curve for both the spray dried and the freeze dried samples (Figure 2, left). This translated in an antioxidant power, expressed as equivalent gallic acid concentration, that is *growing* with time. Again, values in Table 4 confirm the higher antioxidant power of the spray dried powder compared to the freeze dried IntegroPectin.

3.3 Flavonoid content. The flavonoid content in both IntegroPectin powders was evaluated via HPLC-DAD using a method previously reported.[11] Flavonoids and other compounds soluble in aqueous ethanol were extracted as described elsewhere.[12] Chromatograms in Figure 5 (at 285 nm wavelength suitable for the detection of naringin and hesperidin) and the 3D plots in Figure 6 visualizing the whole chromatogram in the entire UV spectrum studied (190-800 nm) show evidence that the two samples originate perfectly overlapping chromatograms. This shows evidence that the higher drying temperatures of the spray drying process do not degrade the valued biophenols contained in the freeze dried IntegroPectin.

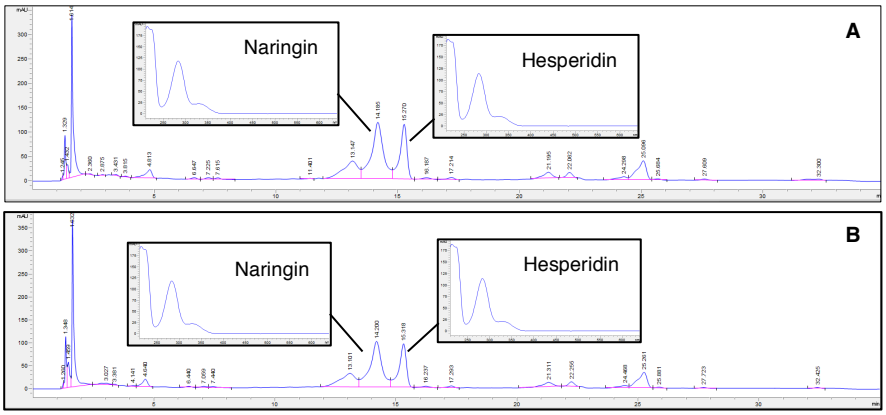

Figure 5. Chromatograms at 285 nm after injection of the phenolic extract from the A) freeze dried and B) spray dried grapefruit IntegroPectin samples. Details of the absorbance spectra of naringin and hesperidin.

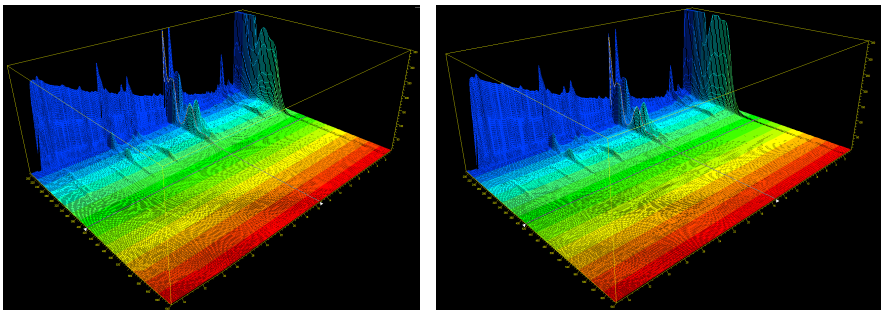

Figure 6. 3D plots after injection of the phenolic extract from the freeze dried (left), and spray dried grapefruit IntegroPectin samples (right).

Indeed, the retention times were identical, and so were the absorption spectra. No biophenol degradation peaks were present in the chromatograms and in the 3D plots of the spray dried IntegroPectin sample. This is in agreement with the higher antioxidant power observed for the spray dried powder, and with the fact that the exposure of the heat-resistant biophenols to the relatively high temperatures of the spray drying process is so brief to not induce their molecular degradation, as previously noted also for resveratrol.[13]

3.4 Total phenol and total protein content. Table 3 shows the outcomes of the Folin-Ciocalteu and Bradford assays alongside the main flavonoid content quantified via HPLC-DAD. The data for the Folin-Ciocalteu and Bradford assays are reported as equivalents of GA and BSA used as standards, respectively.

Table 3. Quantitative results of the HPLC-DAD analysis, Folin-Ciocalteu and Bradford assays for freeze dried and spray dried grapefruit IntegroPectin.

Sample	Total phenolic content[a]	Total protein content[b]	Main flavonoids	
			Naringin[c]	Hesperidin[c]
Freeze dried powder	3.85 ± 0.31	0.246 ± 0.004	3.08 ± 0.03	1.42 ± 0.03
Spray dried powder	4.03 ± 0.07	0.062 ± 0.004	2.87 ± 0.06	1.34 ± 0.03

[a]Folin-Ciocalteu assay, data as mg GA equivalent in 100 mg of recovered powder ± SE; [b]Bradford assay, Data as mg BSA equivalent in 100 mg of recovered powder ± SE; [c]HPLC quantification, data in mg/100 mg

The total phenolic content was slightly higher for the spray dried sample, in accordance with the DPPH assay results. The amount of naringin and hesperidin was approximately the same in both samples (7 and 6 per cent higher in the freeze dried powder). However, the protein content in the freeze dried sample was found to be nearly 4 times higher than in the spray dried sample, in agreement with the cryogenic temperatures of the freeze drying process ensuring retention of the protein tertiary structure revealed by the Bradford assay.[9] In contrast, even though the exposure time to high temperatures is very short, protein denaturation occurs during the spray drying process.[14]

Among the retained proteins in the freeze dried samples there are oxidative enzymes, like polyphenol oxidase and peroxidase, and hydrolytic enzymes that progressively degrade the biophenols present in the IntegroPectn. The inactivation of these enzymes during the spray drying process thus protects the highly bioactive polyphenols from subsequent degradation. Remarkably, a similar behavior was recently reported after comparing phenolic compounds of freeze or spray dried papaya pulp.[15] Also in this case, the spray dried pulp retained a higher amount of phenolic and flavonoid compounds.

3.5 Evaluation of pH. Dissolving equal amounts of the freeze dried and spray dried grapefruit IntegroPectin powders afforded mildly acidic of nearly identical pH (Table 4), showing further evidence that the two techniques chiefly differ in the amount of proteins retained.

Table 4. pH of freeze dried and spray dried grapefruit IntegroPectin dissolved in ultrapure water.

Sample	pH
Freeze dried powder	4.41 ± 0.06
Spray dried powder	4.38 ± 0.03

4. CONCLUSIONS

The comparison of grapefruit IntegroPectin powders isolated via spray drying and via freeze drying in terms of total phenolic content, radical scavenging activity, main flavonoid, total protein content and pH of the aqueous solutions provides valuable information.

First, the optimized spray drying process achieves a very high isolated yield in IntegroPectin (>95%) in comparison to the fully quantitative yield of the freeze drying process with the advantage of shorter duration.

Second, both freeze dried and spray dried IntegroPectin samples have a stronger and quicker antioxidant activity, when compared to a strong biophenol antioxidant such as gallic acid finding multiple medical and nutraceutical applications.[10]

Third, the phenolic chromatographic profiles of the spray dried and freeze dried powders are identical, showing evidence that no biophenol molecular degradation takes place during the spray drying solvent evaporation process despite the exposure to a higher operative temperature.

Fourth, the freeze dried powder has nearly 4 times higher amount of proteins compared to the powder isolated via spray drying.

These results are important in light of forthcoming practical utilization of these new citrus pectins extracted from citrus processing biowaste via hydrodynamic cavitation in water only.[1] For instance, following the discovery of the powerful, broad scope antibacterial activity of grapefruit IntegroPectin,[3] recently cross-linked films of the same new biomaterial showed bactericidal activity against clinical isolates of harmful *Klebsiella pneumoniae*.[16]

The spray drying technique can be readily scaled up, at a small fraction of the costs of the freeze drying process (one ninth of the capital cost and one sixth of the operational cost of the freeze drying industrial process).[17]

We did not conduct the analysis of the citrus terpenes, abundant in the freeze dried IntegroPectin.[18] Pointing to at least partial retention of also these citrus secondary metabolites, however, the spray dried IntegroPectin had a delicate citrus scent. A future investigation will quantify the amount of terpenes in both freeze and spray dried IntegroPectin samples.

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DATA AVAILABILITY

All data are available by contacting the corresponding authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

[1] R. Ciriminna, L. Albanese, F. Meneguzzo, M. Pagliaro, IntegroPectin: a new citrus pectin with uniquely high biological activity, *Biol. Life Sci. Forum* **2021**, *6*, 76. https://doi.org/10.3390/Foods2021-11199

[2] F. Meneguzzo, C. Brunetti, A. Fidalgo, R. Ciriminna, R. Delisi, L. Albanese, F. Zabini, A. Gori, L.B. dos Santos Nascimento, A. De Carlo, F. Ferrini, L.M. Ilharco, M. Pagliaro, Real-scale integral valorization of waste orange peel via hydrodynamic cavitation, *Processes* **2019**, *7*, 581. https://doi.org/10.3390/pr7090581

[3] A. Presentato, E. Piacenza, A. Scurria, L. Albanese, F. Zabini, F. Meneguzzo, D. Nuzzo, M. Pagliaro, D. Chillura Martino, R. Alduina, R. Ciriminna, A new water-soluble bactericidal agent for the treatment of infections caused by Gram-positive and Gram-negative bacterial strains, *Antibiotics* **2020**, *9*, 586. https://doi.org/10.3390/antibiotics9090586

[4] D. Nuzzo, L. Cristaldi, M.Sciortino, L. Albanese, A. Scurria, F. Zabini, C. Lino, M. Pagliaro, F. Meneguzzo, M. Di Carlo, R. Ciriminna, Exceptional antioxidant, non-cytotoxic activity of integral lemon pectin from hydrodynamic cavitation, *ChemistrySelect* **2020**, *5*, 5066-5071. https://doi.org/10.1002/slct.202000375

[5] G.D.J. Adams, I. Cook, K.R. Ward, The Principles of Freeze-Drying, In: *Cryopreservation and Freeze-Drying Protocols. Methods in Molecular Biology*, vol 1257, W. Wolkers, H. Oldenhof, (Ed.s), Springer, New York: 2015; pp.121-143. https://doi.org/10.1007/978-1-4939-2193-5_4

[6] M. B. Adali, A.A. Barresi, G. Boccardo, R. Pisano, Spray freeze-drying as a solution to continuous manufacturing of pharmaceutical products in bulk, *Processes* **2020**, *8*, 709. https://doi.org/10.3390/pr8060709

[7] A. Sosnik, K.P. Seremeta, Advantages and challenges of the spray-drying technology for the production of pure drug particles and drug-loaded polymeric carriers, *Adv. Coll. Interf. Sci.* **2015**, *223*, 40-54. https://doi.org/10.1016/j.cis.2015.05.003

[8] P. Stratil, B. Klejdus, V. Kubáň, Determination of total content of phenolic compounds and their antioxidant activity in vegetables - Evaluation of spectrophotometric methods, *J. Agric. Food Chem.* **2006**, *54*, 607-616. https://doi.org/10.1021/jf052334j

[9] C.L. Kielkopf, W. Bauer, I.L. Urbatsch, Bradford assay for determining protein concentration, *Cold Spring Harb. Protoc.* **2020**, 136-138. https://doi.org/10.1101/pdb.prot102269

[10] S. Choubey, L. Rachel Varughese, V. Kumar, V. Beniwal, Medicinal importance of gallic acid and its ester derivatives: a patent review, *Pharm. Pat. Anal.* **2015,** *4*, 305-315. https://doi.org/10.4155/ppa.15.14

[11] V. De Caro, D. Murgia, F. Seidita, E. Bologna, G. Alotta, M. Zingales, G. Campisi, Enhanced *in situ* availability of aphanizomenon flos-aquae constituents entrapped in buccal films for the treatment of oxidative stress-related oral diseases: biomechanical characterization and *in vitro/ex vivo* evaluation. *Pharmaceutics* **2019**, *11*, 35. https://doi.org/10.3390/pharmaceutics11010035

[12] A. Scurria, M.Sciortino, L. Albanese, D. Nuzzo, F. Zabini, F. Meneguzzo, R.V. Alduina, A. Presentato, M. Pagliaro, G. Avellone, R. Ciriminna, Flavonoids in lemon and grapefruit IntegroPectin, *ChemistryOpen* **2021**, *10*, 1055-1058. https://doi.org/10.1002/open.202100223

[13] G. Di Prima, G. Angellotti, A.G. Scarpaci, D. Murgia, F. D'Agostino, G. Campisi, V. De Caro, Improvement of resveratrol permeation through sublingual mucosa: chemical permeation enhancers versus spray drying technique to obtain fast-disintegrating sublingual mini-tablets, *Pharmaceutics* **2021**, *13*, 1370. https://doi.org/10.3390/pharmaceutics13091370

[14] M. Amdadul Haque, B. Adhikari, Drying and denaturation of proteins in spray drying process, in *Handbook of Industrial Drying*, 4th edition, A.S. Mujumdar (Ed.), Taylor and Francis, London: 2014; pp. 971-982.

[15] W. Faria Gomes, F. Rocha Morais França, M. Denadai, J.K. Santana Andrade, E.M. da Silva Oliveira, E. Sousa de Brito, S. Rodrigues, N. Narain, Effect of freeze- and spray-drying on physico-chemical characteristics, phenolic compounds and

antioxidant activity of papaya pulp, *J. Food Sci. Technol.* **2018**, *55*, 2095–2102. https://doi.org/10.1007%2Fs13197-018-3124-z

[16] E. Piacenza, A. Presentato, R. Alduina, A. Scurria, M. Pagliaro, L. Albanese, F. Meneguzzo, R. Ciriminna, D. F. Chillura Martino, Cross-linked natural IntegroPectin films from Citrus biowaste with intrinsic antimicrobial activity, *Cellulose* **2022**, *29*, 5779–5802. https://doi.org/10.1007/s10570-022-04627-1

[17] B.E. Chavez, A.M. Ledeboer, Drying of probiotics: Optimization of formulation and process to enhance storage survival, *Drying Technol.* **2007**, *25*, 1193-1201. https://doi.org/10.1080/07373930701438576

[18] A. Scurria, M. Sciortino, A. Presentato, C. Lino, E. Piacenza, L. Albanese, F. Zabini, F. Meneguzzo, D. Nuzzo, M. Pagliaro, D. F. Chillura Martino, R. Alduina, G. Avellone, R. Ciriminna, Volatile compounds of lemon and grapefruit IntegroPectin, *Molecules* **2021**, *26*, 51. https://doi.org/10.3390/molecules26010051