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Cytoprotective and anti-inflammatory properties of the bioaccessible fractions of encapsulated HPP-treated mango peel extracts

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INTRODUCTION & AIM

Fruit by-products are source of bioactive compounds (carotenoid and phenolic compounds) with antioxidant and anti-inflammatory properties. Ultrasound-assisted extraction (UAE), high-pressure processing (HPP) and encapsulation are sustainable technologies for their extraction and protection.

The aim of this study was to evaluate the total carotenoid compounds (TCC), total phenolic compounds (TPC) and antioxidant capacity before and after *in vitro* digestion, and the cytoprotective and anti-inflammatory effects of the bioaccessible fractions (BF) of mango peel extracts. Additionally, to assess the effect of the encapsulation of the extracts on the outcome of these assays.

METHODS

Peel

Mango Keitt

High-pressure processing (HPP)
200 MPA/25 ºC/5 min

Iso-Lab System, Model FPG7100:9/2C (Stansted Fluid Power LTD) Equipment at ICTAN-CSIC

Ultrasound-assisted extraction (UAE)
Amplitude 36 µm / 10 min

500 Watts-20 kHz
Probe tipe 19 mm

Extract
Ethanol /acetone (64:36)

Without encapsulation (SF1)

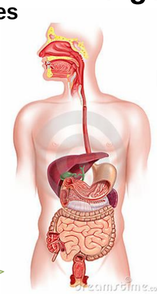
Encapsulation
Arabic gum 8%

Spray-drying (SF2)
150 ºC

Lyophilization (SF3)

***In vitro* gastrointestinal digestión[1]**

Encapsulates
SF1
SF2
SF3



Bioaccessible Fractions (BF)

BF-SF1
BF-SF2
BF-SF3

Analysis of BF (SF1, SF2 and SF3)

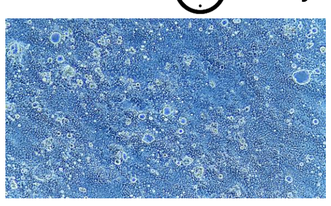
-Individual and Total Phenolic Compounds (TPC)[2] as sum of individuals by HPLC-DAD

-Individual and Total Carotenoid Compounds (TCC)[3] as sum of individuals by HPLC-DAD

-Antioxidant Activity (AA) (FRAP)[2]

BF (Blank (B), SF1, SF2 and SF3)

Caco-2 cells — 9 days



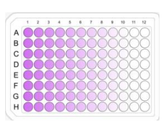
Differentiated

24 h

Cytotoxicity

MTT

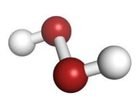
BF dilutions: 1/5, 1/10, 1/20 v/v DMEM



4h, 37°C, 95% RH, 5% CO_2

Stress induction[4]

H_2O_2



5 mM, 2 h

Cytoprotection

- Cell viability (MTT) — Spectrophotometry
- Cell death (Annexin V/propidium iodide)
- Cell cycle (propidium iodide)
- Reduced glutathione (GSH) (CMFDA)

Flow cytometry

Inflammation

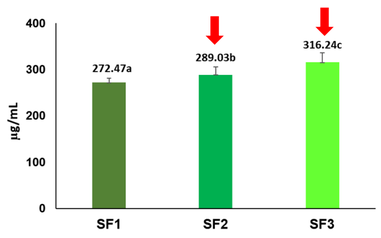
- Interleukin-6 (IL-6)
- Interleukin-8 (IL-8)

ProQuantum immunoassay

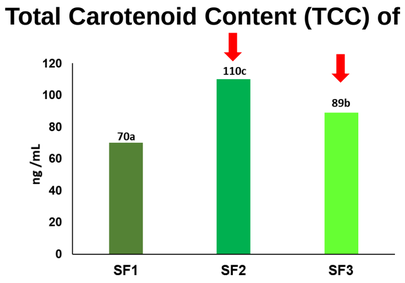
RESULTS & DISCUSSION

Bioaccessible Fractions (BF) composition

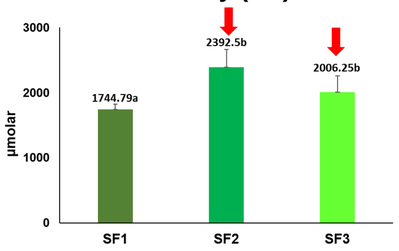
Total Phenolic Compounds (TPC) of BF



Total Carotenoid Content (TCC) of BF



Antioxidant Activity (AA) FRAP of BF



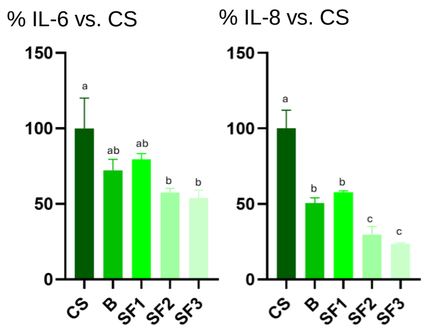
The BF of extracts encapsulated by spray-drying (SF2) and by lyophilization (SF3) presented greater TPC, TCC and AA (FRAP) than the non-encapsulated extract (SF1).

CYTOTOXICITY OF BF (without stress)

Viability (% vs. control)			
	Dilution (v/v) in DMEM		
	1/5	1/10	1/20
B	93.46±15.86[a]	103.28±12.68[a]	129.96±5.80[a]
BF-SF1	126.53±14.63[b]	111.79±10.10[a]	123.22±13.77[a]
BF-SF2	1.09±0.75[c]	111.31±18.82[a]	142.52±8.59[b]
BF-SF3	1.53±2.20[c]	96.40±12.27[a]	105.63±16.59[a]

Different letters (a-c) indicate statistically significant differences ($p < 0.05$)

ANTI-INFLAMMATORY EFFECTS OF BF



All samples showed anti-inflammatory effects, reducing IL-8 levels (vs. CS) and IL-6 levels (only encapsulated ones).

This suggest that the encapsulation helps the bioactives to exert their benefits better, as cell death and GSH assays shown.

CNS: Non-stressed control
CS: Stressed control

CYTOPROTECTION OF BF

Viability (stressed)

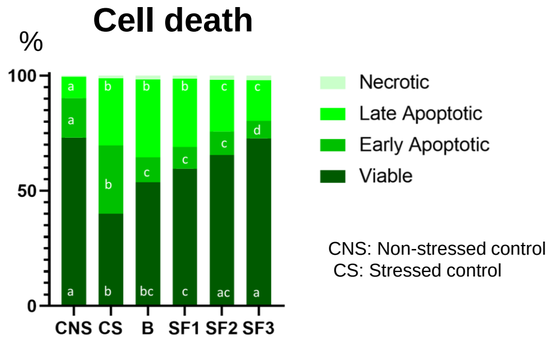
Viability (% vs. control)	
H_2O_2	59.24±1.11[a]
B	74.87±11.50[b]
BF-SF1	104.38±7.40[c]
BF-SF2	115.38±12.79[c]
BF-SF3	97.08±19.53[c]

Dilution 1/10 (v/v) is selected (no damage).

Hydrogen peroxide produced a 41% viability decrease.

All samples recovered control values (higher than the blank).

Cell death

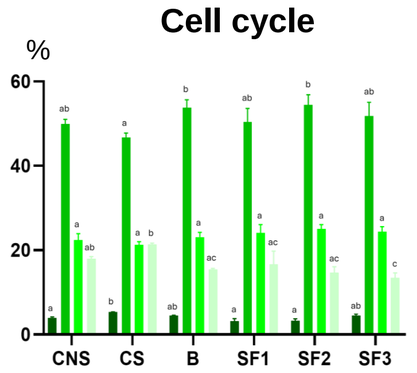


CNS: Non-stressed control
CS: Stressed control

Oxidative stress decreased viability (analogous to the MTT assay), while increased both early and late apoptosis.

The extracts were able to reduce the apoptotic population and BF of SF3 even restore basal viable population.

Cell cycle



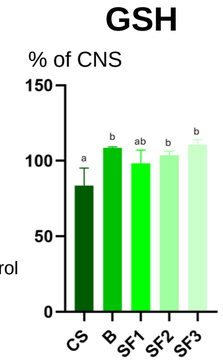
CNS: Non-stressed control
CS: Stressed control

Sub G1 population was increased with H_2O_2.

Basal CNS values were obtained with all samples, although the blank and SF3 were also statistically equal to the CS.

G0/G1 pase was reduced only after CS treatment.

GSH



17% reduction of GSH levels after stress induction. All samples recovered basal levels.

Encapsulated samples (SF2 & SF3) showed a stronger positive effect than the non-encapsulated (SF1).

CONCLUSION

Greater TCC, TPC, AA and cytoprotective and anti-inflammatory effects were observed in encapsulated samples (SF2 and SF3) compared to the non-encapsulated one (SF1), although no differences were observed between spray-drying and lyophilization. Thus, both encapsulation techniques are equaly effective and may enhance the bioactivity of mango peels.

REFERENCES

[1]Brodkorb et al. Nat Protoc, 2019, 14, 991–1014 (INFOGEST); [2]Fernández-Jalao et al. J Food Eng, 2019, 213, 60-68; [3]Estévez-Santiago et al. Food Funct, 2016, 7, 1354; [4]Gómez et al. Food Res Int., 2019, 120, 52-61.

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