

S. Nunes¹, A.T. Serra^{1,2}, J.Poejo¹, C. Semedo³, M.R. Bronze^{1,2,3}, A.L. Simplicio^{1,2}, Catarina M.M. Duarte^{1,2}, A.A. Matias^{1,2,3}

¹ Instituto de Tecnologia Química e Biológica- Universidade Nova de Lisboa (ITQB-UNL), Avenida da República, Estação Agronómica Nacional, 2780-157 Oeiras, Portugal; ² Instituto de Biologia Experimental e Tecnológica (IBET), Avenida da República, Apartado 12, 2781-901 Oeiras, Portugal; ³ IMED, Faculdade de Farmácia da Universidade de Lisboa, Av. das Forças Armadas, 1649-019, Lisboa, Portugal

AIM Development of bioactive-rich concentrates (BRC) derived from Portuguese cherries and cactus pear with potential prevention/therapeutic application in IBD.

Introduction

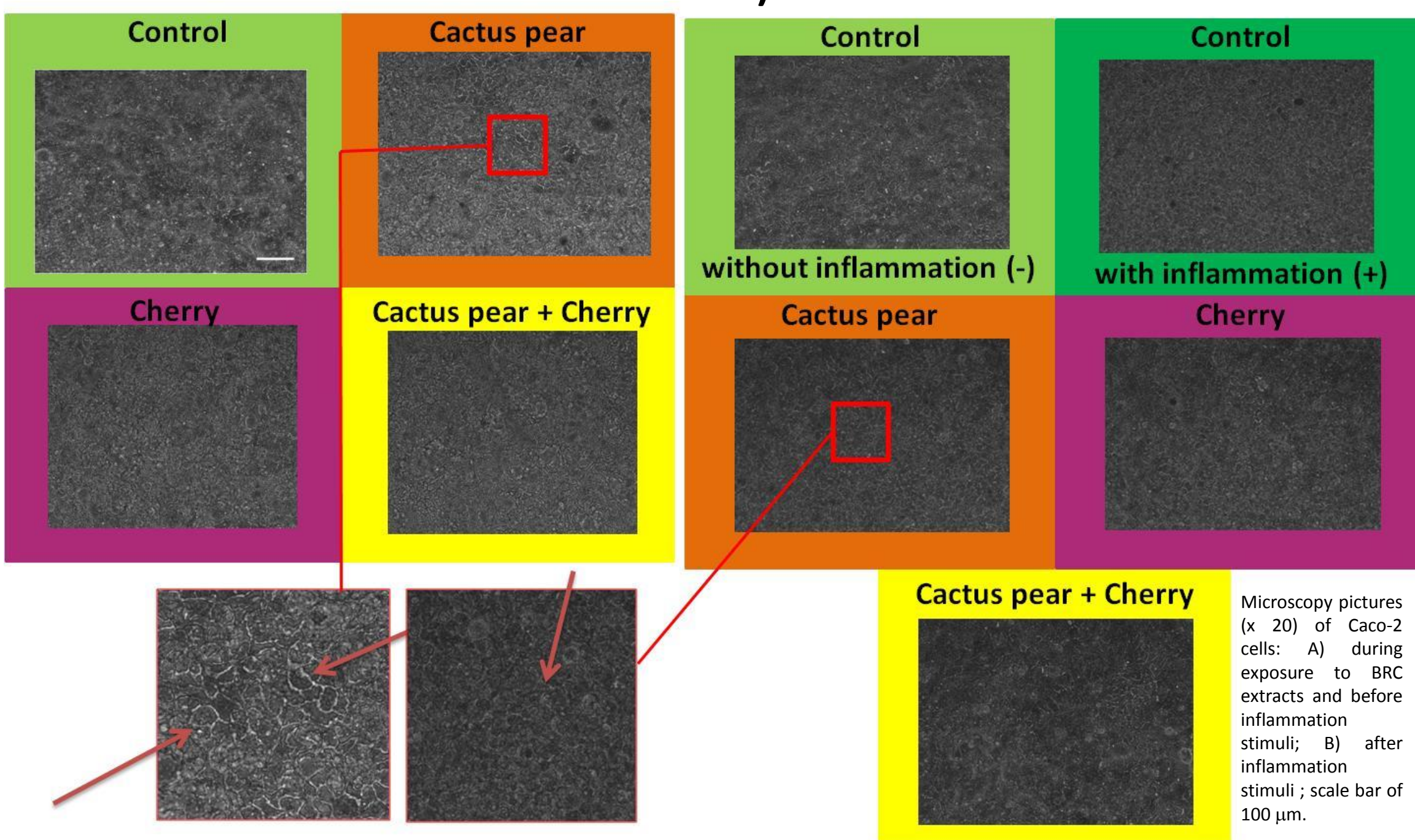
- Inflammatory Bowel Diseases (IBD) are characterized by an uncontrolled inflammatory response. Oxidative Stress plays a major role in the maintenance and amplification of this response.¹
- There are evidences that polyphenols regular consumption can reduce or delay IBD development, due their strong antioxidant capacity.¹
- Sweet Cherries (*Prunus Avium*) and cactus pear (*Opuntia ficus indica*) are known for their high polyphenolic composition and strong antioxidant activity that are correlated with their potential anti-inflammatory activity^{2,3}.

Anti-inflammatory Activity

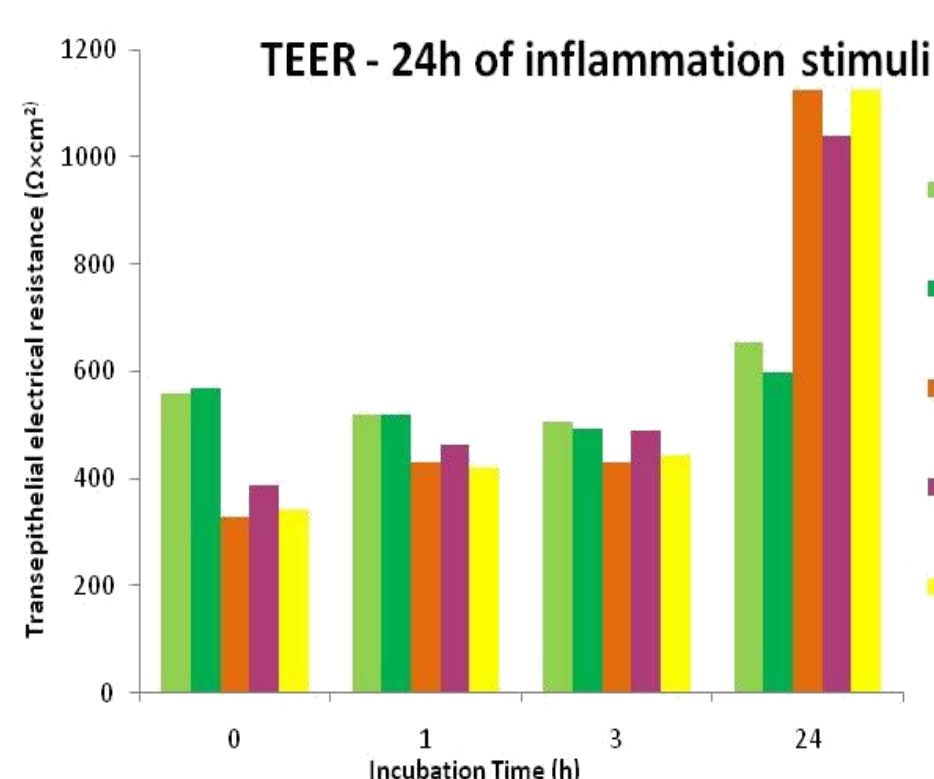
Cell Barrier Integrity

A) Caco2 cells before inflammation stimuli (during incubation with BRC).

B) Caco2 cells after 24h of inflammation stimuli.

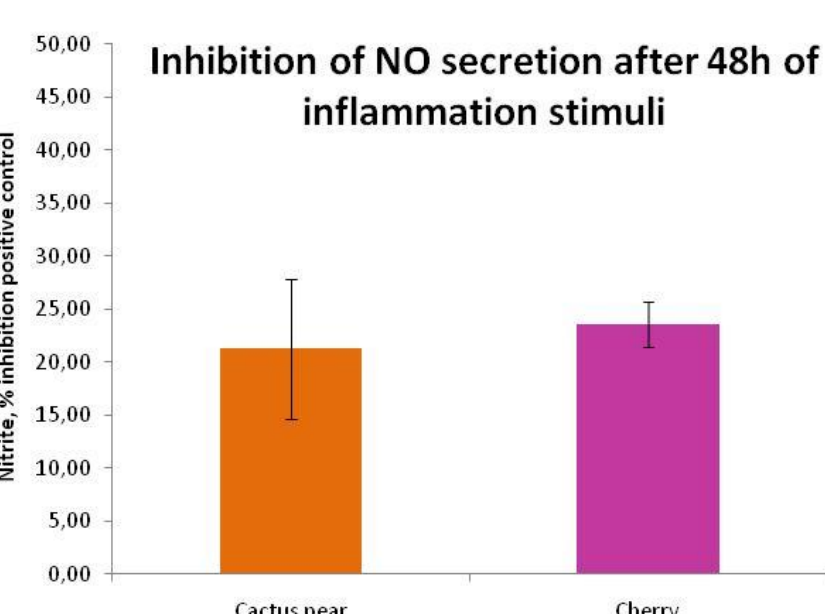


- Before Stimuli: Well defined and marked Tight cellular Junctions with BRC's in apical compartment
- After stimuli: Inflamed untreated Cells (+) were smaller and tight junctions slightly prominent; BRC pre-treated cells with no visible tight junctions; well defined monolayer.



- Time 0: Decrease of TEER during pre-incubation with BRCs could indicate paracellular transport (opening of *tight junctions*).
- After 24h of inflammation stimuli, there is a slight decrease of TEER in control with inflammation what indicates molecular expression and transport.
- Pre-incubation with both BRC leads to an increase in TEER values demonstranting modulation of the cell intestinal barrier response to the induced stimuli.

Inhibition of NO secretion

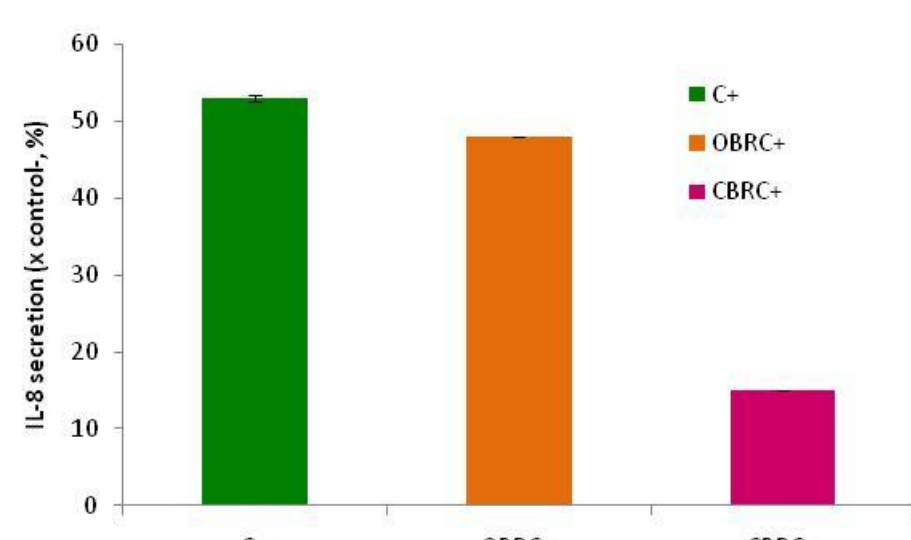


- Nitric oxide is overproduced in case of inflammation¹ and is mainly secreted to basolateral compartment.
- After 24h of inflammation stimuli there are no differences in NO secretion what is consistent with previously reports.⁶

After 48h of inflammation stimuli, Cherry's and Cactus pear's BRC inhibit NO secretion in a similar percentage.: 23 and 21% respectively.

IL-8 Secretion

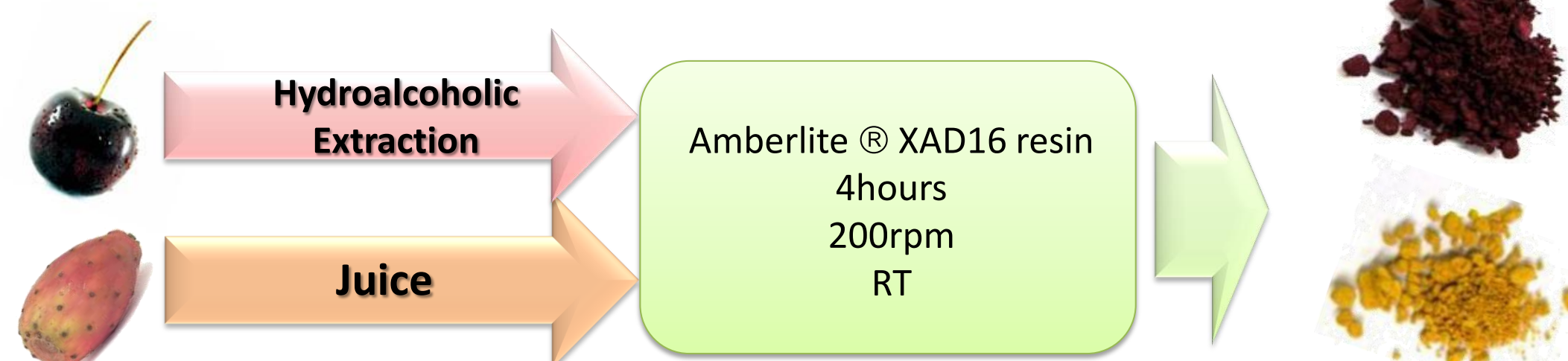
- Stimuli used induced IL-8 secretion
- Pre-incubation (4h) with BRC extracts, namely Cherry BRC could modulate IL-8 secretion, reducing it practically to basal level.



IL-8 secretion by differentiated Caco-2 cells (after our experimental inflammation stimuli), expressed in relative terms to the negative control.

Bioactive rich concentrates

Preparation of Cherry's and Cactus pear's BRC.



Polyphenolic Composition

1. Cherry BRC - high content in anthocyanins that are recognized as anti-inflammatory compounds²:

Cyanidin-3-glucoside (26,82mg/g)
Cyanidin-3-rutinoside (114,8mg/g)
Peonidin-3-glucoside (4,83mg/g)

2. Cactus Pear BRC - high content in flavonoids (pink line – 360nm), namely isoharmnetin (and derivatives), and Betalains. Betalains are known by their anti-inflammatory activity.^{3,5}

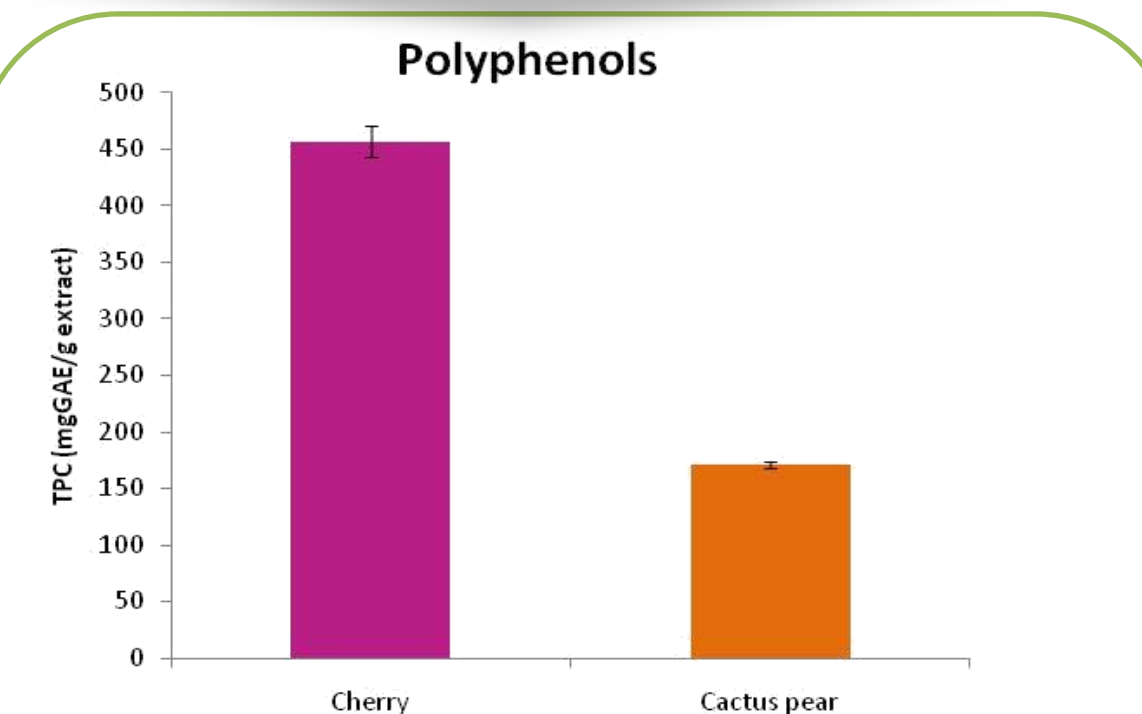


Fig. 1 Total polyphenolic compounds (quantified Folin – Ciocalteu method)

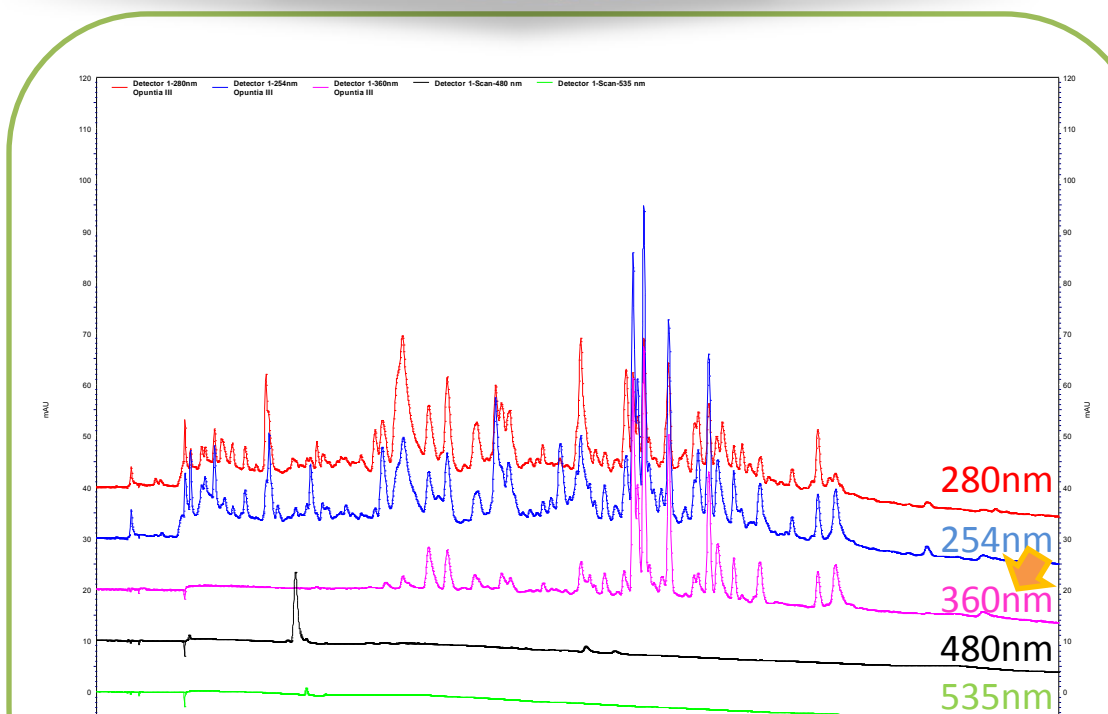
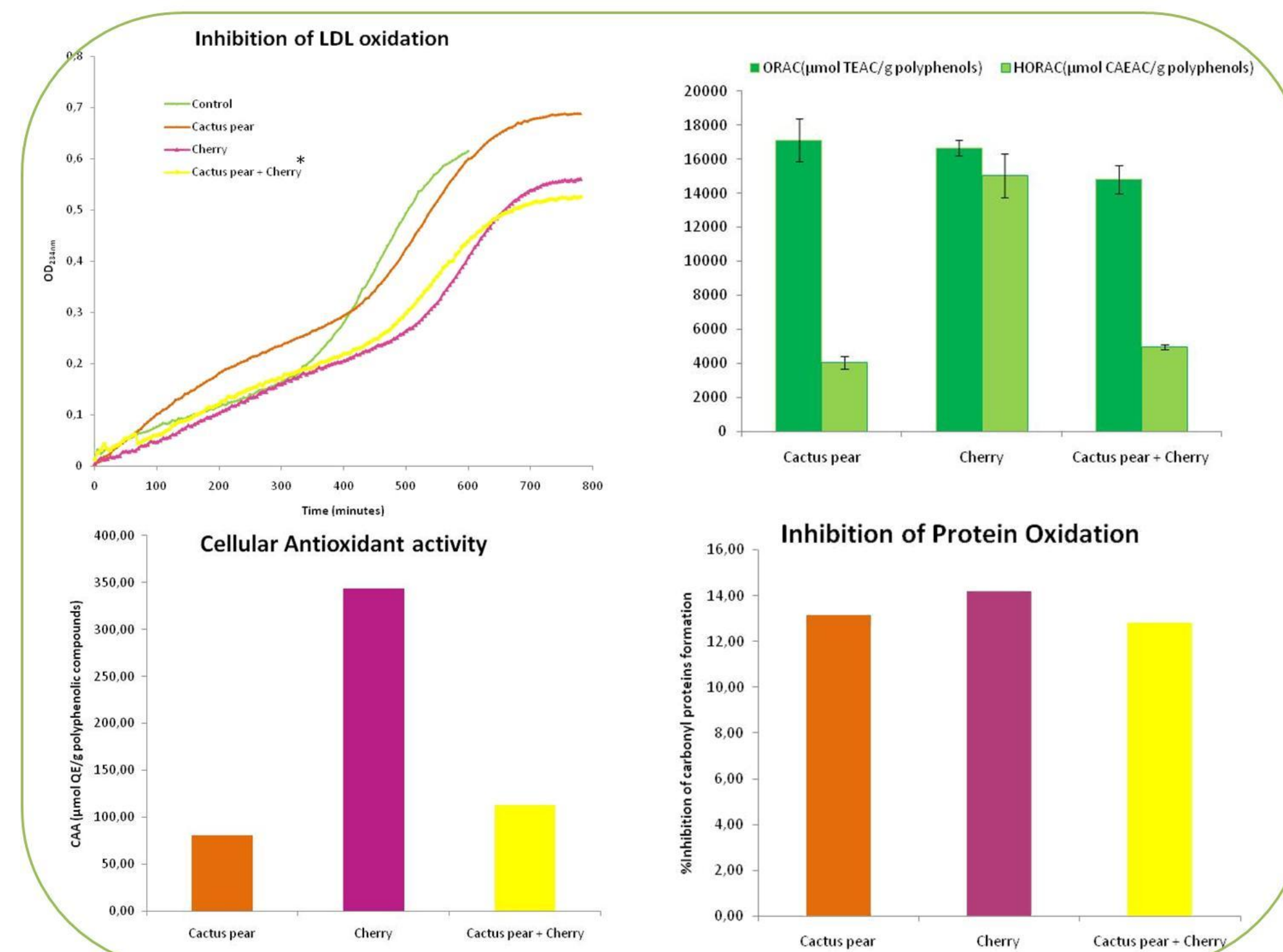


Fig. 2 Cactus Pear's BRC HPLC chromatogram

Antioxidant Activity

- All extracts were characterized in terms of antioxidant activity using different and complementary assays
- A 1:1 (mg GAE) mixture of both extracts were formulated in order to evaluate synergistic action

In vitro chemical assays:
ORAC; HORAC activity.
Ex-vivo chemical assay:
LDL oxidation
In Vitro Cell-based assays:
CAA Value; Protein Carbonyls



*Cherry and cactus pear extracts were mixed in the same polyphenolic proportion.

Cherry BRC, rich in anthocyanins, demonstrate to have:

- Higher antioxidant activity against hydroxyl radical (HORAC);
- Preeminent ability to inhibit the oxidation of LDL;
- More pronounced cellular antioxidant activity against H₂O₂ aggression

Mixture of cherry BRC and cactus pear BRC has:

- Less HORAC than cherry's BRC, WHICH means that this two extracts don't have synergic activities.
- Similar inhibition of LDL oxidation to cherry's BRC, showing the important contribution of anthocyanins present in this EXTRACT.

Conclusion

- Cherry's BRC has higher antioxidant activity.
- Cactus pear's and Cherry's BRC causes an Increase in Intestinal Epithelial Tight Junction Permeability.
- BRCs pre-incubation, namely with Cherry BRC, modulate cell intestinal barrier and IL-8 and NO secretion after pro-inflammatory cocktail,
- Cherry's BRC represents a promising natural anti-inflammatory modulator for IBD.