Enzyme-assisted *in situ* supercritical fluid extraction of isorhamnetin conjugates from *Opuntia ficus-indica* (L.) Mill

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1. Introduction

Opuntia cladodes (*Opuntia ficus-indica* L. Mill) have been consume to improve the well-being and to prevent cancer and metabolic syndrome complications[1]. Recent studies on *Opuntia ficus-indica* (L.) Mill have proven that isorhamnetin and its conjugates, the most abundant flavonoids in this plant, possess anti-inflammatory, antioxidant, anti-obesity and anti-cancer activities [2-5]. A number of novel techniques have been apply to obtain these type of compounds from plant materials, such as high-pressure pretreatment, microwave-assisted extraction, ultrasonic-assisted extraction and supercritical fluid extraction [6-9]. Furthermore, the degradation and disruption of the cell-wall matrix via enzymatic hydrolysis improve the release of phenolic compounds [9-12]. Enzymes are ideal catalysts to assist in the extraction, modification or synthesis of complex bioactive compounds of natural origin. Enzymes have the ability to degrade or disrupt cell walls and membranes, thus enabling better release and increase the extraction of bioactive compounds. Kim et al. [10] reported that the treatment with enzymes increased the total sugar, uronic acid, and polyphenol content in *O. ficus-indica* extracts that showed an increased radical scavenging activity. However, nowadays the consumers and industries are demanding more natural ingredients and green process.

Supercritical carbon dioxide (SC-CO^2) is an alternative, green, eco-friendly technology with the ability to extract compounds without thermal deterioration and oxidation. Enzymes have been rarely use in highly selective supercritical carbon dioxide (SC-CO^2) fluid extraction. For instance, a combination of α-amylase and SC-CO^2 extraction was employed for single step hydrolysis of black pepper starch and extraction of the oleoresin fraction modifying time and enzyme ratio at fixed SC-CO^2 conditions [10]. Previous studies demonstrated the use of SC-CO2 technology for the selective extraction of bioactive components from *O. ficus-indica*, however it resulted in low extraction yield [8]. A previous study, evaluated the effect of enzymatic hydrolysis of *O. ficus-indica* under optimum conditions at SC-CO^2 using different cocktails as a pretreatment for the release of isorhamnetin conjugates [13]. The use of enzyme under SC-CO^2 conditions can be appropriate as pretreatment to the selectively release of the bioactive compounds with potential health benefits. Further studies should be perform to directly obtain high yield bioactive glycosides without the need of extensive purification processing as they may be extracted within the supercritical fluid extraction process. For this reason, this study evaluated the combination of a stationary SC-CO^2 enzymatic pretreatment process and a dynamic SC-CO^2 process for the green extraction of bioactive components.

2. Results and discussion

In this study multiple objectives were addressed, first determine the optimal process conditions in terms of pressure, temperature and co-solvent amount. Then, evaluated the effect of time during the extraction of isorhamnetin conjugates. Finally, evaluate the effect of different isorhamnetin profiles on the cellular antioxidant capacity in order to determine the more bioactive mixture of compounds. The preliminary studies have shown that during the Rapidase cocktail enzyme increased 3-fold the total isorhamnetin content, followed by Viscozyme with 1.7 fold, compared to control. It is well know that carbohydrate-hydrolyzing enzymes have been use to enhance the extraction of polyphenolics by breaking the cell wall complex. The conditions of 60°C, 100 bar and 20% of ethanol selectively extract a isorhamnetin di-glycoside (IG-5) which has a potent anti-inflammatory effect *in vitro*. Figure 1 shows the effect of the enzyme cocktail on the different isorhamnetin pattern (Left) and the effect of time of extraction on the isorhamnetin profile under the optimal conditions

(Right). The use of Rapidase allowed the selective extraction of IG5 which was the most potent inhibitor of cellular antioxidant capacity.

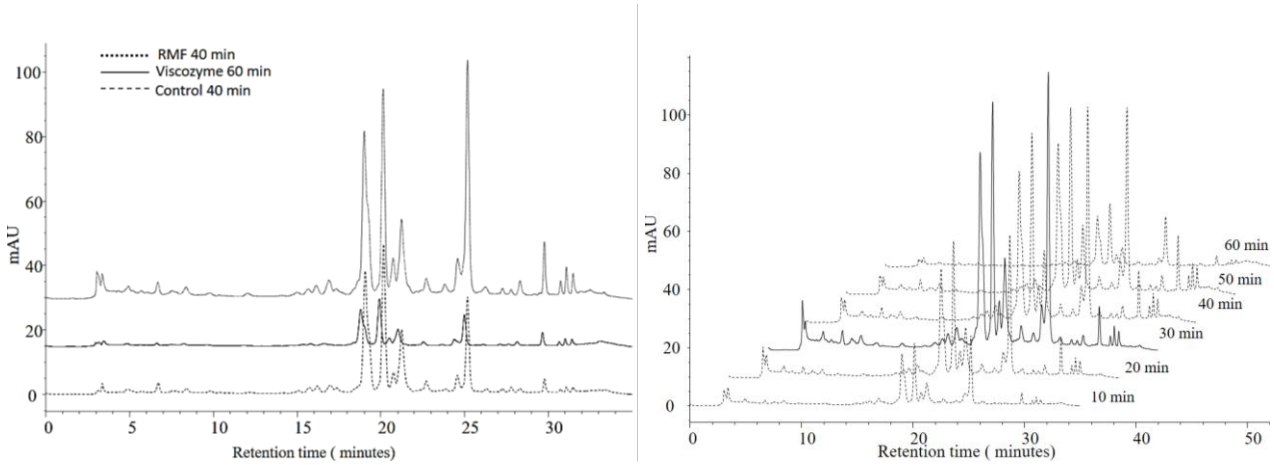

Figure 1. Chromatograms of the effect of enzyme mixture on the bioactive component profile (Left) and the effect of time of extraction under the optimal conditions (Right).

3. Conclusions

The total isorhamnetin content and profile varied significantly depending upon the enzyme used and SC-CO2 conditions applied, affecting their cellular antioxidant capacity. The use of SC-CO2 combined with an enzymatic pretreatment could be used as a green alternative to selectively extract anti-inflammatory compounds of interest for the pharmaceutical and cosmetic industries.

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