RICE BRAN OIL EXTRACTED WITH SUPERCRITICAL CO_2 AND BIOACTIVITY EVALUATION

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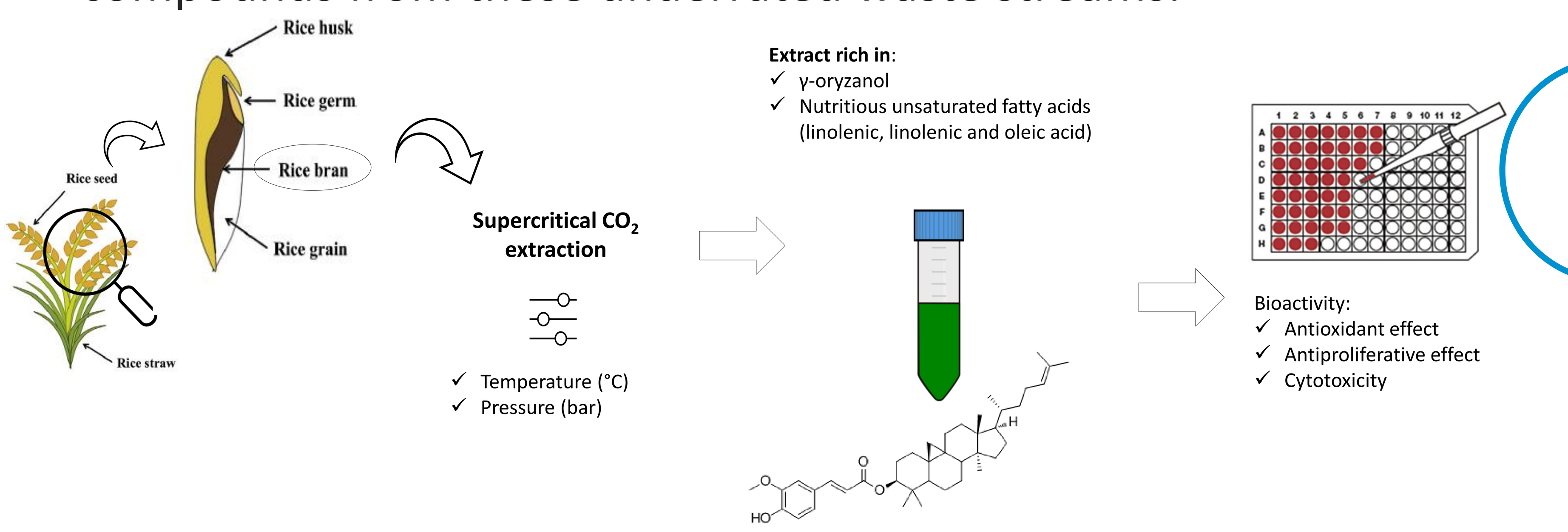
INTRODUCTION

Rice (*Oryza sativa L.*) is the most important staple food crop consumed globally. In terms of production rice is the third most important grain in the world behind wheat and corn. Rice processing involves some milling steps to produce several materials such as rice bran, husk and broken rice. Bran accounts for 5-10% of the harvested rice. This natural raw material is an interesting source of bioactive compounds that show antioxidant, anti-inflammatory, anti-cholesterolemic, anti-diabetic, anti-cancer activities and heart-related health benefits.

AIM

The objective of the present work is to optimize the supercritical fluid extraction (SFE) process to valorize rice by-products to develop novel ingredients containing bioactive compounds from these underrated waste streams.



METHODS

- Response surface methodology was used to model the recovery of γ-oryzanol and fatty acids (FA) from rice bran through SFE to assess the effect of pressure and temperature on the extraction performance (yield and chemical composition of the oil).
- γ-oryzanol and the fatty acids were analyzed by LC-MS/MS and GC-MS respectively.
- A human intestinal cell line (Caco-2) was used to assess the antioxidant effect of the obtained samples. The antiproliferative effect was evaluated in a human colorectal adenocarcinoma cell line (HT29).

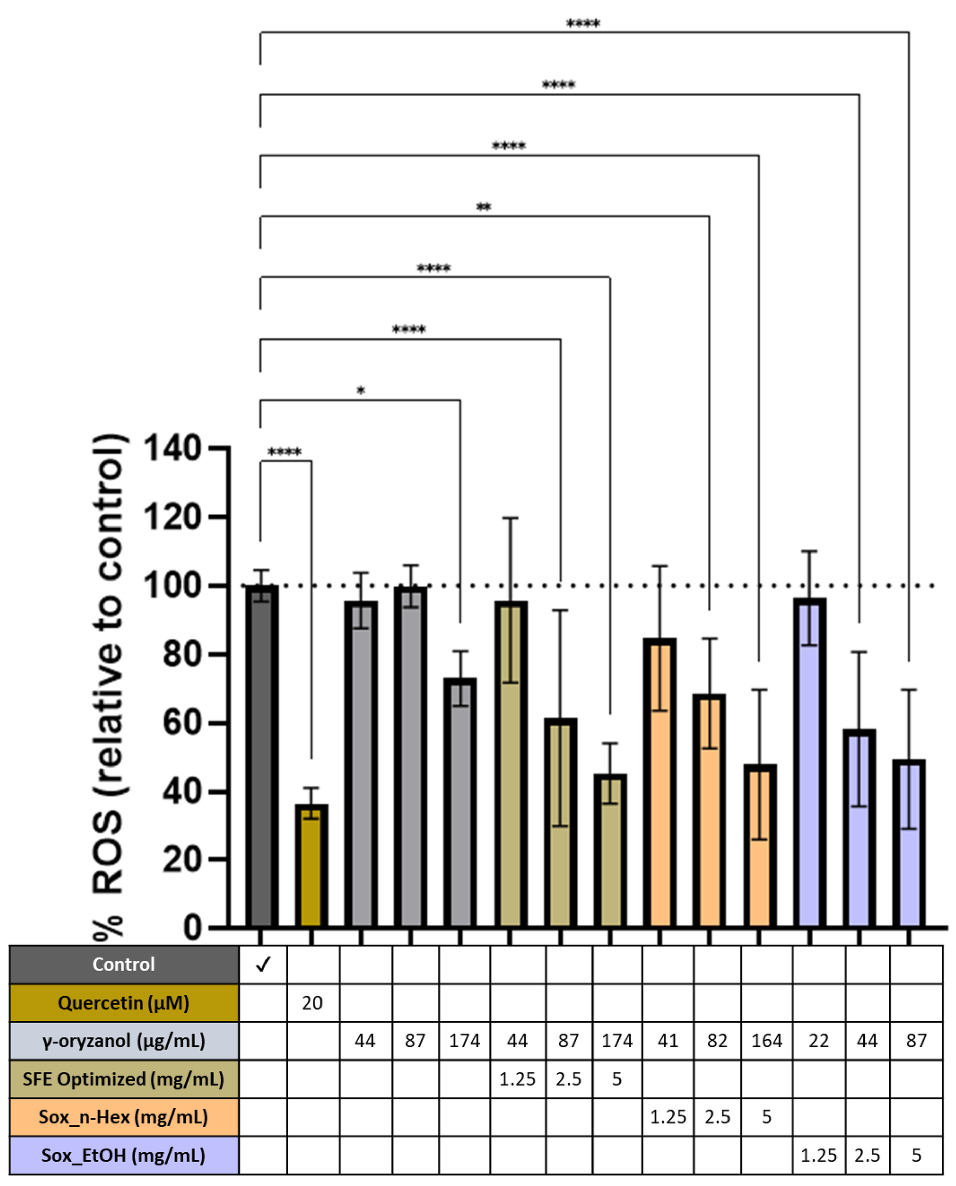
RESULTS & DISCUSION

i. Supercritical Fluid Extraction

After a central composite design of experiments in which the raw material weight (20 g), flow rate (20 g/min), and extraction time (3 h) were fixed, temperature and pressure were varied (40-80 °C, 200-500 bar). The pressure was the most significant SFE parameter with a quadratic effect on **fatty acid extraction** and a positive effect on **γ-oryzanol extraction**. Moreover, the temperature had also a positive effect on the extraction yield. The yield obtained by the optimized SFE method was comparable to conventional Soxhlet extraction with n-hexane (SOX.Hex), ~18 $g_{extract}$/100graw material, and it was 2 times more **selective** for the γ-oryzanol extraction. Moreover, the total FA amount obtained by both methods was similar (~780 mg/$g_{extract}$) and the profile was comparable, being the most abundant compounds **palmitic, oleic and linoleic acids**.

	SFE*	SOX.Hex	SOX.EtOH
Extraction yield (%)	17.3	18.0	24.9
γ-Oryzanol (mg/$g_{extract}$)	36.6	18.57	21.6
Antioxidant activity, ORAC (µmol TEAC/$g_{extract}$)	450 ± 48	480 ± 50	310 ± 10

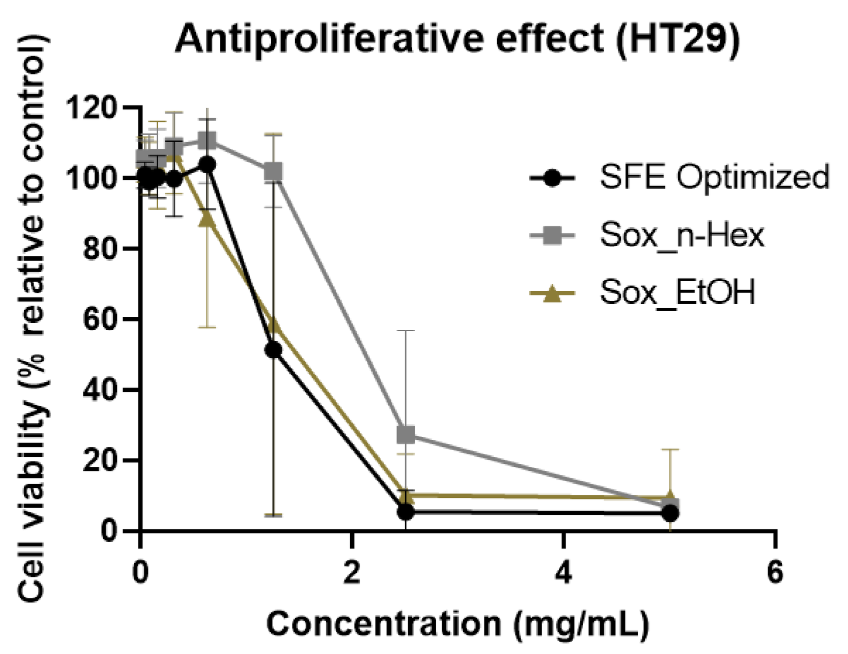
***Optimized SFE conditions:**
Flow rate: 20 g/min
Extraction time: 180 min
Pressure: 500 bar
Temperature: 62 °C



Cellular antioxidant capacity (Caco-2), expressed as % of ROS inhibition relative to the control. The symbol * indicates significance relative to the control.

ii. Bioactivity evaluation

The antioxidant activity was evaluated through ORAC and cell-based assay and results showed that while SOX.EtOH demonstrated a lower scavenging capacity of peroxyl radicals, all samples showed a similar profile in inhibiting reactive oxygen species (ROS) generated by AAPH in Caco-2 cells. In particular, all extracts (1.25-5 mg/mL) showed the capacity to inhibit up to 50% of the intracellular ROS, and this effect was more pronounced than that obtained for the standard γ-oryzanol. The antiproliferative effect was evaluated in HT29 cell line and the extracts SFE and SOX.EtOH showed to be more active in inhibiting cancer cell proliferation than SOX.Hex. In this assay, γ-oryzanol was also tested and showed no antiproliferative effect for the same range of concentrations



EC50 values in HT29 (mg/mL)
SFE: 0.9 ± 0.04[a]
SOX.Hex: 1.5 ± 0.19[b]
SOX.EtOH: 0.9 ± 0.04[a]

CONCLUSIONS

The bioactive-rich extracts obtained from rice by-products using a green extraction strategy, namely SFE, presented high concentrations of γ-oryzanol and other compounds with antioxidant and antiproliferative effect.

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