Sequential pressurized liquid extraction and subsequent supercritical antisolvent fractionation of mango seed kernel extracts with antiproliferative activity

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1. Introduction

Mangifera indica L. (Anacardiaceae), commonly named mango, is a native plant to India and Myanmar and it is grown in tropical or subtropical regions. Mango is one of the main products for human consumption worldwide. The nutritional and functional value of the mango fruit constitute the fundamental reason for its wide diffusion as food[1]. Mango is a good source of bioactive phytochemicals with antioxidant and antiproliferative activities[2]. The mango seed kernel is one of the generated wastes during both the fresh consumption and the transformation process of the fruit. Depending on the varieties, kernel represents 45–85% of the seed and approximately 20% of the whole fruit[3]. Moreover, mango seed kernel is reported in literature as a potential source of bioactive compounds. Hence, considering the bioactive potential of this food by-product, a revalorization strategy based on a sequential extraction and fractionation using compressed fluids was optimized in order to obtain polyphenolic-rich extracts from mango seed kernel with the highest antiproliferative activity against HT-29 colon cancer cells. In the first step, kernel was defatted evaluating different non-polar solvents (*n*-hexane, *n*-heptane, *n*-cyclohexane and (+)-limonene) under pressurized liquid extraction (PLE) conditions. In the second step, ethyl acetate and ethanol were used as extraction solvents at different temperatures to obtain polar extracts by an optimized PLE procedure. In the third and final step, the optimum extract was fractionated by supercritical antisolvent procedure (SAF). The optimization of the SAF process was carried out using a response surface methodology (RSM) by Box-Behnken design with three factors: CO_2 pressure (80, 120 and 150 bar), percentage of water in the PLE extract (20, 30 and 50 % H_2O v/v) and PLE extract/SC-CO_2 flow ratio. The selected response was the antiproliferative activity against HT-29 colon cancer cells.

2. Results and discussion

For the first step of the PLE procedure, *n*-heptane was selected due to the good performance exhibited in the extraction of the lipidic content of mango kernel seeds (12.88 %), compared to *n*-cyclohexane (8.15 %), (+)-limonene (2.35 %) and *n*-hexane (8.12 %). After the defatting process, the second step of the PLE procedure was optimized using a central composite design. For this purpose, solvent composition (percentage of EtOH in the mixture EtOH/EtOAc: 0, 50 and 100 % v/v) and temperature (50, 100 and 150 ºC) were evaluated to maximize extraction yield, total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (EC_{50} and TEAC). Optimal extraction yield (12.43 %), TPC (138.37 mg GAE g^{-1}), TFC (1.25 mg Quer g^{-1}) and antioxidant activity (EC_{50} 15.27 µg mL^{-1} and 2.14 mM trolox g^{-1}) were obtained operating at 150 °C and 100 % EtOH v/v. The antiproliferative activity of the optimal PLE extract against HT-29 cells was also evaluated showing a 50 % of cell survival; the IC_{50} value of the extract was 28.67 µg mL^{-1} at 72 h of treatment. Then, the optimal PLE extract was fractionated by SAF process to obtain fractions with improved antiproliferative activity. By employing a RSM it was possible to optimize the most important factors involved in the SAF process. The fraction (extract) obtained operating at 150 bar, 50 % H_20 v/v in the feeding solution and 0.0625 feed/SC-CO_2 flow mass ratio presented the highest antiproliferative activity with 29.15 % of cell survival after 72 h of treatment considering the same IC_{50} concentration than the original PLE extract.

3. Conclusions

In the present work, a sequential extraction and fractionation process based on the use of pressurized liquid extraction and supercritical antisolvent fractionation has been optimized to obtain mango seed kernel extracts with antiproliferative activity against HT-29 colon cancer cells. The SAF process allowed obtaining fractions with improved antiproliferative activity respect to the original PLE extract. Results show the high potential of this strategy for the valorization of the mango by-products.

References

1. Ribeiro, S. M. R. & Schieber, A. Bioactive Compounds in Mango (Mangifera indica L.). in *Bioactive Foods in Promoting Health* (eds. Ronald Ross, W. & Victor, R. P.) 507–523 (Academic Press, 2010). doi:10.1016/B978-0-12-374628-3.00034-7
2. Luo, F. *et al.* Identification and quantification of gallotannins in mango (Mangifera indica L.) kernel and peel and their antiproliferative activities. *J. Funct. Foods* **8,** 282–291 (2014).
3. Jahurul, M. H. a. *et al.* Mango (Mangifera indica L.) by-products and their valuable components: A review. *Food Chem.* **183,** 173–180 (2015).