**Co-precipitation of fluorescein with extracts of mango leaves by supercritical antisolvent process.**

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**Highlights**

* Enhanced solvent extraction was used to extract antioxidant compounds from mango leaves.
* Supercritical antisolvent process was an efficient method to obtain co-precipitated extract with fluorescein.
* Temperature, pressure and CO2 flow rate had remarkable effects on particle formation.

**1. Introduction**

The use of polyphenols from natural resources as fruits, vegetables and nutraceuticals is increasing due to beneficial health effects. In fact, polyphenols possess diverse biological activities as antioxidant, antimicrobial, antidiabetic and anti-inflammatory properties [1]. Otherwise, fluorescein is a fluorescent organic substance which has important optical properties in solution. This characteristic allows it to be used as a marker [2] with interesting active ingredients to be able to track the substance in the organism. In the study reported here, the SAS technique (Supercritical Antisolvent process) optimization was carried out to obtain the co-precipitation of mango leaves extract and fluorescein microparticles. Organic solution containing the solute of interest is sprayed through a nozzle to generate drops of solution into a vessel containing CO2 at supercritical conditions. During mixing, scCO2 is quickly dissolved in the organic solution, causing the precipitation of solutes by antisolvent effect. Afterwards, scCO2 efficiently extracts the organic solvent, allowing to obtain completely solvent-free products.

**2. Methods**

**2.1. Preparation of mango leaves extract**

The extraction of antioxidant compounds of mango leaves was performed by enhanced solvent extraction. The ethanolic extract was obtained using the best conditions of pressure, temperature, CO2 flow rate and solvent flow rate from previous studies [3]. The extract was obtained at 200 bar and 80 °C with 50% CO2 – 50% Ethanol:50% water ratio.

**2.2. Particle co-precipitation with the SAS process**

The SAS technique was used to co-precipitate microparticles of mango extract and fluorescein. A pilot plant built by Thar Technologies® (SAS200) was employed for this purpose. A factorial of mixed levels desing (3\*22 + 2 central points) was used to determinate the effect of pressure, temperature and CO2 flow rate on the co-precipitation of mango leaves extract and fluorescein microparticles. The response variable used as control was the particle size. The conditions of the factorial desing were: temperatures of 35 and 55 °C; pressures of 150 and 200 bar; and CO2 flow rates of 10, 20 and 30 g/min. In all the experiments, the concentration of the extract and fluorescein was 24 mg/ml and 6 mg/ml respectively.

**3. Results and discussion**

Most of the experiments in this work led to the successful co-precipitation of mango leaves extract and fluorescein together. The obtained particles from the extract were spherical, while fluorescein particles were precipitated with an irregular morphology, as show in Figure 1. In general, an increase in the values of the studied parameters led to an increase in the amount of precipitate in the tests performed.



**Figure 1.** SEM Image of mango leaves extract with fluorescein microparticles

In reference to particle size distribution, it has been observed that the particle size of mango extract tended to decrease when pressure was decreased from 200 bar to 150 bar, obtaining particle diameters in the range of microns. In all cases, an increase in temperature led to a decline in both particle size. A decrease in CO2 flow rate led to a smaller particle size in the case of fluorescein, but it happens in the opposite way to the mango leaves extract particles.

**4. Conclusions**

Microparticles of fluorescein with mango leaves extract have been obtained by a SAS process. Parameters that have a marked effect on particle size and size distribution have been evaluated in order to obtain the smallest particles possible. The experiments were successful in most cases. Higher temperature and lower pressure are recommended to obtain smaller particle size.

**References**

1. H. Kim, J.Y. Moon, H. Kim, D.S. Lee, M. Cho, H.K. Choi, Y.S. Kim, A. Mosaddik, S.K. Cho, Food Chem. 121 (2010), 429-436.
2. A. Clemente, R. Jiménez, M. Mar Encabo, M. Lobera, F. Balas, J. Santamaria, Journal of Hazardous Materials. 363 (2019), 358-365.
3. M. Guamán-Balcázar, A. Montes, C. Pereyra, E. Martínez de la Ossa, J. Supercrit. Fluids. 128 (2017), 218-226.