Sustainability & Circular Economy

# Modification of carob powder (*Ceratonia siliqua L.*) for by-product valorization in the food industries

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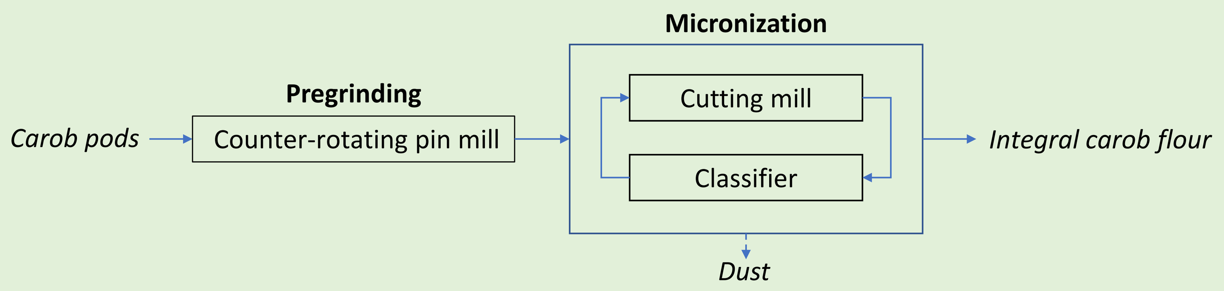
## INTRODUCTION

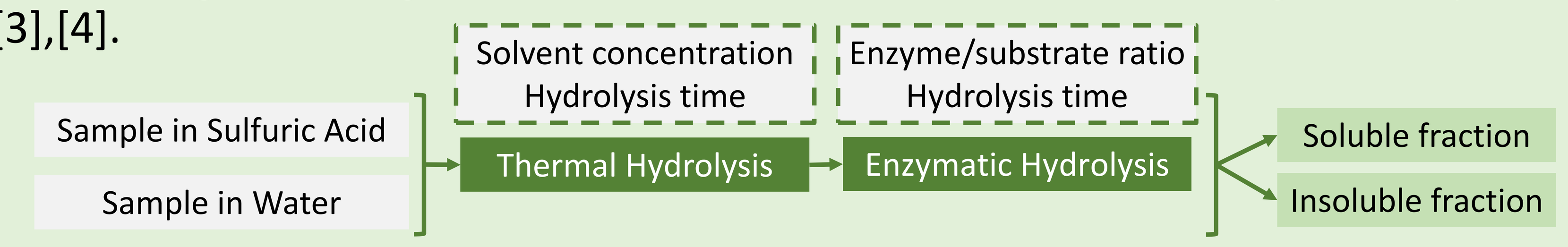
Carob fruit originates from the carob tree, is a leguminous evergreen tree (*Ceratonia siliqua L.*) cultivated in the Mediterranean area, namely in Portugal [1]. Its production is highly relevant, extensive, and sustainable. Carob pods contain various relevant nutrients, which include proteins, carbohydrates, and fibers [2].

## MATERIALS AND METHODS

An innovative milling process was applied to produce an optimized integral carob flour according to **Scheme 1**. The carob flour was sieved with a mesh size of 100 µm to obtain a fraction of flour composed principally of insoluble fiber.




This fraction of flour >100 µm was modified by a two-step acidic/thermal and enzymatic hydrolysis (**Scheme 2**) to improve its functional properties as a result of the cleavage and degradation of cellulose, hemicellulose, and lignin compounds [3],[4].

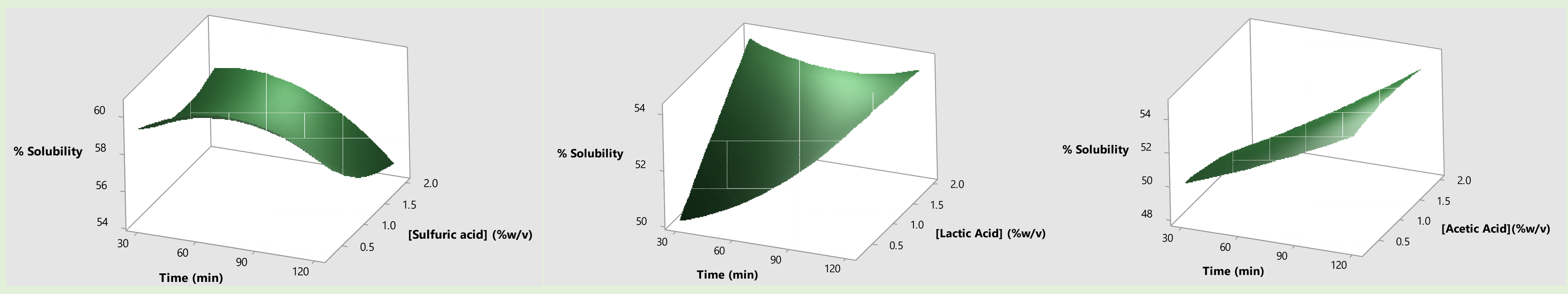



Scheme 1 – Representation of the two-step thermal and enzymatic hydrolysis.

The assessed solvents were sulfuric, lactic, and acetic acids, deionized water at 100 °C, and the enzymes were cellulase and xylanase. Furthermore, it was evaluated how the steps could be minimized in order to decrease process's costs and runtime at industrial level.

## RESULTS AND DISCUSSION

The first trial for the hydrolysis optimization was to optimize the thermal/acidic step. An experimental design using the Box Behnken model was assessed considering the factors: solvent concentration and time of hydrolysis. The results showed that the lower concentration and higher hydrolysis time were optimal for obtaining higher solubility values.




The sulfuric acid was the solvent that presented better solubility results in only 1 h long hydrolysis compared to the results obtained for acetic and lactic acids in 2 h hydrolysis (**Table 1**).

Table 1 – Solubility values obtained for the termal/acidic hydrolysis considering sulfuric, lactic and acetic acids using the Box Behnken model.

| Sulfuric Acid | | | Lactic Acid | | | Acetic Acid | | |
|---|---|---|---|---|---|---|---|---|
| **Concentration % (v/v)** | | | | | | | | |
| 0.2 | 1.1 | 2 | 0.2 | 1.1 | 2 | 0.2 | 1.1 | 2 |
| **Hydrolysis Time (min)** | | | | | | | | |
| 60 | | | 120 | | | 120 | | |
| **% Solubility** | | | | | | | | |
| 60.94 | 57.61 | 59.02 | 53.98 | 54.50 | 53.56 | 54.42 | 55.43 | 53.75 |

For the validation of the previous results, the trial was performed under the same conditions (second trial), and the results confirmed that the best suitable solvent was sulfuric acid. The results did not differ significantly compared to water (control) (**Table 2**), concluding that water would be the optimal solvent for thermal hydrolysis given its eco-friendly and low-cost nature. Thus, water would be used for the following thermal hydrolysis trials.

Table 2 – Solubility values obtained for the termal/acidic hydrolysis considering sulfuric, lactic and acetic acids and water as control.

| Solvent | % Theoretical Solubility | Desirability | % Experimental Solubility |
|---|---|---|---|
| **Sulfuric Acid** | 60.94 | 0.81 | 58.58 ± 0.65 |
| **Lactic Acid** | 54.14 | 0.80 | 56.27 ± 0.87 |
| **Acetic Acid** | 54.80 | 0.90 | 56.07 ± 0.58 |
| **Water** | | | 55.84 ± 0.84 |

The third trial for hydrolysis optimization was to assess and optimize the enzymatic step. For this step, the insoluble mass resultant from the thermal hydrolysis was separated and dried for further hydrolysis. The enzymatic hydrolysis was performed using a mixture of xylanase (48 U/g) e cellulase (90 U/g) at 55 °C and the considered factors were enzyme/substrate ratio and time of hydrolysis.

The lowest enzyme/substrate ratio and time were the optimal results obtained, possibly due to the enzyme reaching maximum activity and saturation at these conditions (**Table 3**).

Table 3 – Solubility obtained for enzymatic hydrolysis using the Full factorial model.

| Enzyme/Substrate Ratio % (v/v) | Hydrolysis time (h) | % Solubility |
|---|---|---|
| **0.5** | 2 | 23.46 ± 1.73 |
| **0.5** | 3 | 22.08 ± 2.36 |
| **1.75** | 2.5 | 17.23 ± 0.66 |
| **3** | 2 | 18.69 ± 1.03 |
| **3** | 3 | 22.57 ± 2.59 |

Overall, this two-step hydrolysis method obtained a ~80 % solubility of the >100 µm carob flour.

This method was further optimized to minimize steps in order to decrease process's costs and runtime at industrial level (fourth trial). A continuous methodology was considered to eliminate the separation and drying steps between hydrolyses. The results obtained for comparing individual two-step hydrolysis and continuous hydrolysis showed that the enzymatic hydrolysis, when applied uninterruptedly after the thermal hydrolysis, does not solubilize the substrate, possibly due to the absence of the drying step which could impair the structure of the carob matrix hindering the enzymes from acting (**Table 4**).

Table 4 – Solubility obtained for continuous hydrolyses and individual two-step hydrolysis.

| Solvent | % Experimental Solubility |
|---|---|
| **Continuous Hydrolysis (Thermal+Enzymatic)** | |
| **Sulfuric Acid** | 50.03 ± 2.49 |
| **Water** | 51.73 ± 3.40 |
| **Individual Two-step Hydrolysis** | |
| **Water** | Total of ~80 (~50 % thermal + ~30 % enzymatic) |

These results showed that when applying continuous hydrolysis, compared to the individual two-step hydrolysis, it is possible to obtain ~50 % carob flour solubility and exponentially reduce the overall process cost (no enzymes and no acidic solvents), ultimately making this methodology greener and more food grade suitable.

## CONCLUSIONS

This study showed that the optimal modification process for carob powder encompasses a single thermal hydrolysis using water to obtain 50 % solubility of the initial carob flour. The resulting liquid by-product can be used to produce sweeteners, fillings, creams, etc., highlighting the importance of food-chain valorization in food industries and the remainder insoluble by-product can be used as granulate, exfoliant, etc.

## ACKNOWLEDGEMENTS

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