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Scaling Up Mild Micronization Techniques for Biomass Bioactive Compound Extraction: From High-Pressure Homogenization (HPH) to Disc Mill

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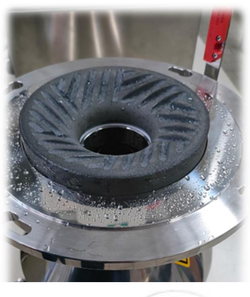
WP 8 - Task 8. 1 .2

Valorization of the waste by biotechnology processes for high value molecules or new products

Introduction

Micronization techniques, including High-Pressure Homogenization (HPH) and Disc Mill, enhance the extraction and bioaccessibility of bioactive compounds from agri-food residues by disrupting cell structures [1]. HPH utilizes high mechanical forces such as cavitation, turbulence, shear, and elongational stresses to micronize plant tissues, while disc mill uses grinding shear forces to present similar effects [1]. These techniques allow for the efficient release of trapped bioactive compounds in cells, thus increasing extraction yields. However, HPH is effective in cell disruption and bioactive compound extraction, its scalability may have some limitations and can be challenging, particularly in large-scale applications. A disc mill could be used as a reliable and significant approach to scaling up HPH due to its robust mechanical action and ability to process large quantities of biomass at high concentration efficiently. Our results demonstrate that after five passes through the disc mill, the outcomes considerably surpassed the optimum conditions results achieved with HPH (5 passes, 80 MPa, 25 °C), as measured by total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP), particle size, chlorophyll, and microscopy analysis. This study indicates the feasibility and effectiveness of disc mill as a scalable approach to replace HPH in large-scale extraction processes.



Experimental section

HPH

Disc Mill

TPC

TFC

Chlorophyll

FRAP

Color analysis

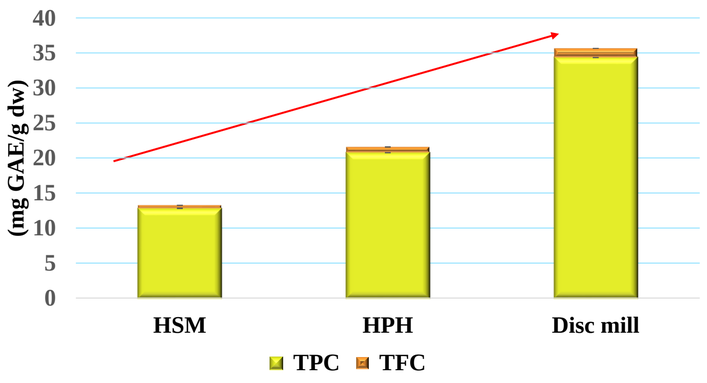
Particle size

Microscopy

Fresh rucola was milled using a laboratory knife grinder and then suspended in pure water at a solid-to-liquid ratio of 1:10 g/mL. This suspension was subsequently subjected to high-shear mixing (HSM) at 20,000 rpm for a duration of 5 min. The sample pretreated with HSM was used as the control. The obtained suspension with concentration of 10 % (w/v) was subjected to HPH under varying conditions, including treatment temperatures ranging from 5 to 25 °C and varying the number of passes through the orifice (np = 1–15). The obtained suspension of fresh rucola with concentration 25 % (w/v) was processed using the disc mill at room temperature (25 °C), with the number of passes varied from 1 to 5. To analyze the effectiveness of each micronization technique and to compare them, different analyses were conducted. The TPC and TFC were measured to determine the concentration of bioactive compounds, and the FRAP assay was used to evaluate antioxidant capacity. Particle size analysis was conducted to assess the level of micronization obtained by each method. Additionally, chlorophyll (a and b) content was quantified to analyze the impact on pigment concentration, and microscopy analysis was performed to demonstrate the morphological changes in the treated biomass for both techniques.

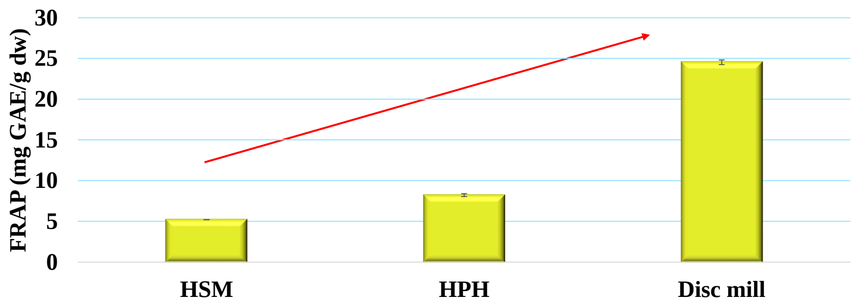
TPC-TFC

The results showed that the disc mill, after five passes at 25°C, achieves significantly higher Total Phenolic Content (34.3 (mg GAE/ g dw)) and Total Flavonoid Content (1.3 (mg GAE/ g dw)) compared to HPH at its optimal fifth pass (20.8 and 0.8 (mg GAE/ g dw)) for TPC and TFC respectively). These findings show that the disc mill is not only effective but also significantly superior for bioactive compound extraction, making it a reliable alternative to HPH in large-scale applications.



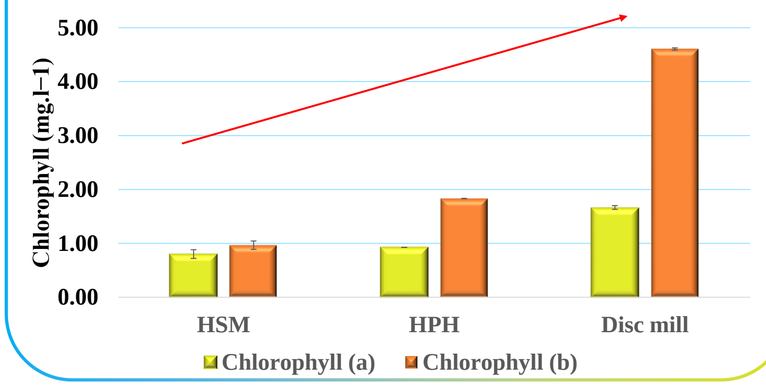
FRAP

The FRAP test results clearly show that the disc mill method presents a significantly higher antioxidant activity (24.5 (mg AAE/g dw)) compared to HPH, which has 8.2 (mg AAE/g dw). This substantial difference may be attributed to the disc mill's ability to more effectively disrupt cell walls, releasing higher amount of antioxidant compounds. This outcome is supported by particle size analysis and microscopy results, which are clear evidence of the enhanced cell wall disruption achieved by the disc mill. Also, The disc mill's ability to maintain a lower operational temperature and apply milder mechanical forces compared to HPH likely contributes to the preservation of these compounds, resulting in higher FRAP values.



Chlorophyll

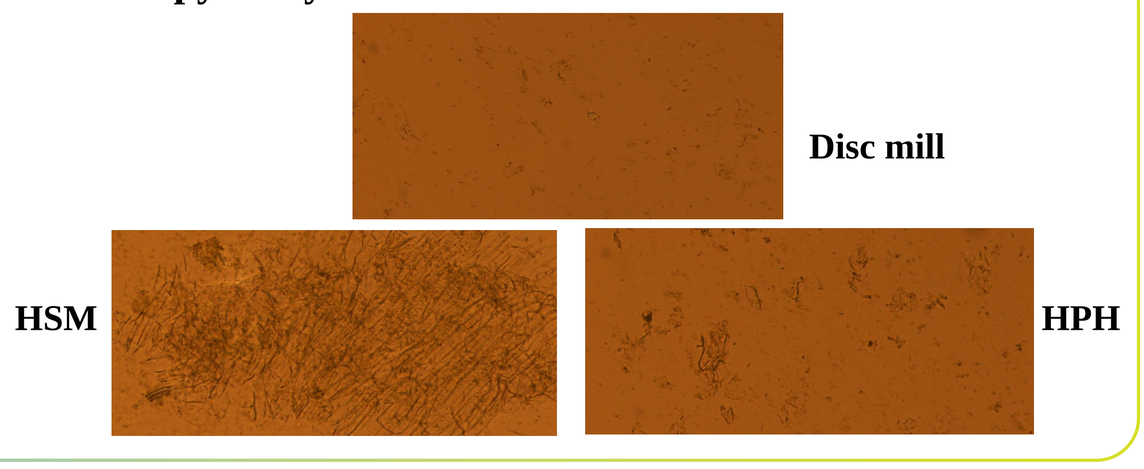
The results of the chlorophyll analysis showed that the disc mill technique significantly outperforms both HPH and HSM in extracting chlorophyll, particularly chlorophyll (b). The disc mill extracted a much higher concentration of chlorophyll (a) and (b), indicating its superior effectiveness in breaking down cell walls and releasing these pigments. These results show that the disc mill is not only efficient in extracting chlorophyll but also efficient at preserving these pigments during the extraction process.



Particle size analysis

Sample	Surface Weighted Mean D[3,2] (μm)	Vol. Weighted Mean D[4,3] (μm)	d(0.1) (μm)	d(0.5) (μm)	d(0.9) (μm)
HSM	**346.6**	**634.7**	**192.4**	**580.9**	**1144**
1	152.9	638.0	137.9	569.7	1145
3	112.6	618.6	85.7	599.7	1209
5	**89.4**	**614.6**	**61.7**	**587.1**	**1165**
10	23.8	100.6	13.8	69.3	214.9
15	19.2	70.6	10.5	52.4	132.0
Disc mill	**81.9**	**400.2**	**70.3**	**334.2**	**825.7**

Microscopy analysis



[1] Carpentieri, S., Ferrari, G., & Donsì, F. (2023). High-Pressure Homogenization for Enhanced Bioactive Recovery from Tomato Processing By-Products and Improved Lycopene Bioaccessibility during In Vitro Digestion. *Antioxidants*, *12*(10), 1855.

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