Actividades divulgación Proyecto AGROALNEXT_2022

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Provincia	Toledo, España
Fecha	18-20 Octubre, 2023
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Código proyecto	AGROALNEXT_2022/27
Grupo de investigación	CSIC Consejo Superior de Investigaciones Científicas; CEBAS Centro de Edafología y Biología Aplicada del Segura; Laboratorio de Fitoquímica & Alimentos Saludables CyTA (CEBAS-CSIC) LabFAS

INFORME DE LA ACTIVIDAD:

Asistencia y Comunicación a Congreso Internacional (Póster)

Congreso: **Primer Congreso Internacional sobre Estilo de Vida, Dieta y Salud del Vino**.
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Título de la Comunicación:
Sinapines in mustards – metabolite farming for resilient and healthy agrifood products

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RESUMEN/ABSTRACT

SINAPINES IN MUSTARDS – METABOLITE FARMING FOR RESILIENT AND HEALTHY AGRIFOOD PRODUCTS

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Background. A nutritious diet is essential to achieving good health in the modern world, and is crucial in light of the worrisome rise in obesity worldwide. Cruciferous sprouts are rich in nutrients and health-promoting bioactive compounds including glucosinolates (GSLs) and phenolic compounds. Sinapines (sinapic acid choline esters), are a class of compounds that are less studied that other bioactives in cruciferous foods, but have several biological activities (anti-inflammatory, anti-cancer, antibacterial) with an important role in the pathophysiological processes.
Objectives. This research carried out a screening of sinapines in mustard sprouts in order to further biostimulate their composition for establishing protocols of resilient and healthy horticultural food production. Additionally, the bioaccesibility of the sinapic acid derivatives was also evaluated.
Methods. Seeds of white (*Sinapis alba*), red (*Brassica juncea*), black (*Brassica nigra*), and Ethiopian mustards (*Brassica carinata*) untreated and ready for sprouting after sanitation and imbibition were sown under controlled conditions until harvested at day 8. The analysis of the freeze-dried plant material was carried out to study the composition of the sprouts by HPLC-DAD-ESI-MSn identification and quantitation of sinapoyl derivatives. The analysis of variance was carried out, followed by the Tukey multiple comparison test (p-value<0.05).
This study forms part of the AGROALNEXT (Agroalnext_2022_027) programme and was supported by MCIN with funding from European Union NextGenerationEU (PRTR-C17.I1) and by Generalitat Valenciana.
Results and Discussion. The analysis of the sprouts of four varieties of commercially available mustards (white, red, black and Ethiopian), revealed interesting compounds including sinapoylcholine and related derivatives, including feruloyl choline(4-O-8')guaiacyl, and sinapine(4-O-8')guaiacyl, together with characteristic sinapoyl gentibiosides present in Brassicas. The seeds presented much higher amounts of sinapine, than the edible sprouts. Therefore, despite Ethiopian mustard and black mustard having similar and low contents of sinapines and other compounds, black mustard showed higher contents of cinnamoyl-choline esters in sprouts, and the white mustard showed the highest contents of sinapines in seeds and sprouts. The evaluation of the bioaccesibility of the sinapic acid derivatives present in mustard sprouts, for screening purposes and further investigations was also performed revealing reduced bioaccessible fraction in all the tested samples.

Keywords: Priming, elicitor, bioactive compounds, sinapoyl derivatives, Brassica

FOTOS DE LA ACTIVIDAD:

Sinapines in Mustards – Metabolite farming for Resilient and Healthy AgriFood Products

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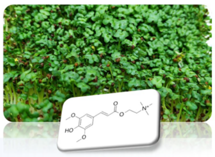
INTRODUCTION

A nutritious diet is essential to achieving good health in the modern world, and is crucial in light of the worrisome rise in obesity worldwide. **Cruciferous sprouts are rich in nutrients and health-promoting bioactive compounds** including glucosinolates (GSLs) and phenolic compounds. **Sinapines (sinapic acid choline esters)**, are a class of compounds that are less studied that other bioactives in cruciferous foods, but have several beneficial properties such as antitumor, neuroprotective, antioxidant, and hepatoprotective effects, making its study relevant [1,2].

Objectives. This research carried out a screening of sinapines in several mustard sprouts in order to select varieties for further biostimulation protocols to enrich their composition in order to obtain resilient and healthy horticultural food products. Additionally, the bioaccessibility of these compounds sinapic acid derivatives was also evaluated in the tested samples, for future studies of bioactivity and 'functionality' on therapeutic targets of interest.



MATERIALS & METHODS

Mustard sprouts development and growth conditions

Seeds of 4 commercial mustards for sprouting were selected: White (*Sinapis alba* L.), Ethiopian (*Brassica carinata* A. Braun), Red (*Brassica juncea* (L.) Czern), and Black Mustard (*Brassica nigra* (L.) W.D.J. Koch; Syn. *Sinapis nigra* L.). 150 g of seeds of each variety were decontaminated, imbibed in aeration for 24h **(Fig. 1.A.)**, and thereafter sown on inert substrate (GrowFelt®, Anglo-Recycling Tech. Ltd., UK), and kept in the dark at 80% relative humidity for 48 hours **(Fig. 1.B.)**. Then, the germinating seeds were transferred to a growth chamber **(Fig. 1.C,D)**, with controlled conditions: Photoperiod 18h/6h; temperature 22°C/18°C; and relative humidity 60%/80%. The sprouts were kept growing until day 8 **(Fig 1.E.)**, harvested at commercially-acceptable size. The growth chamber used LED lighting (Protect BioLED 100W, SysLed Spain, S.L.). Priming and elicitation experiments with the white mustard were carried out using Ethyl-Gallate (Sigma Aldrich, Análisis Vínicos SL, Tomelloso, Spain) as biostimulant.

Identification and characterization of sinapines and their *in vitro* bioaccesibility in mustard sprouts

100 mg of freeze-dried plant material were extracted in MeOH 50% with 2% formic acid, using US bath at room temperature, and overnight extraction at 4 °C. The analysis of sinapic acid derivatives (SAD) was carried out in a HPLC-DAD-ESI-MSn system (Agilent Technologies HPLC 1200, Waldbronn, Germany; coupled with a Bruker mass detector, model UltraHCT, Bremen, Germany) and quantification by acquiring chromatograms at 320nm, and using sinapic acid 98% purity (Sigma Aldrich, Análisis Vínicos SL, Tomelloso, Spain) as external standard. Phytochemical analysis and gastric, intestinal, and gastrointestinal digestions were performed on sprouts powder (500 mg) following INFOGEST static in vitro simulation of food digestion [3].

Statistical analysis

The analysis of variance (One-way ANOVA) was carried out, followed by the Tukey multiple comparison test (mean ± SD). The distribution of the data was verified for normality using the Shapiro-Will test. The software used was the Graph-Pad Prisma 9 version 9.0.0.

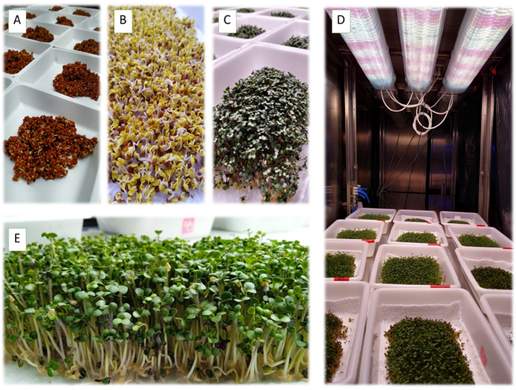


Figure 1. **A**. Seeds distributed upon initial imbibition. **B**. 3-day old germinating seeds after 48h in the dark. **C**. Sprouts growing under controlled conditions. **D**. General view of the LED lighting system and trays of growing sprouts. **E**. White mustard sprouts ready for sampling at 8-days of age.

RESULTS & DISCUSSION

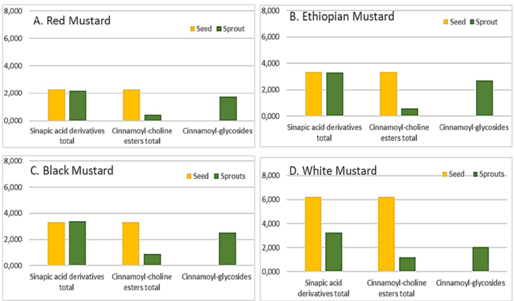


Figure 2. Sinapic acid derivatives (SADs mg/g d.w.), including cinnamoyl-choline esters (sinapines) and cinnamoyl-glycosides in the seeds and sprouts of Red (A), Ethiopian (B), Black (C), and White (D) mustards Contents expressed as average value ± SD..

Organ	Priming (5 mM EG)	Elicitation (5 mM EG)	mg/g dw SAD	mg/g dw SAD digestate	% after digestión simulation
Seeds	0		5.514 ± 0.75	0.217 ± 0.006 *	3.929
	5		5.875 ± 1.14	0.229 ± 0.001 **	3.895
Sprouts	0	0	2.625 ± 0.27	0.039 ± 0.002 ***	1.502
	0	5	2.765 ± 0.28	0.043 ± 0.002 ***	1.570
	5	0	2.673 ± 0.26	0.035 ± 0.002 ***	1.305
	5	5	2.959 ± 0.14	0.036 ± 0.000 ***	1.232

***Table 2.** Bioaccesibility of SADs (Sinapic acid derivatives) of White mustard (Sinapis alba L.) seeds and sprouts. Contents expressed as average value ± SD. The (*) are indicative of statistical differences between the presence of compounds in the digestate vs. the original undigested sample as follows: * p < 0.01, ** p< 0.001, or *** p<0.0001. EG: Ethyl gallate.*

The analysis of the sprouts of four varieties of commercially available mustards presented in **Figure 2** (**A**. Red mustard; **B**. Ethiopian or Abisinian mustard; **C**. Black mustard; and **D.** ,) revealed interesting sinapic acid derivatives (SADs), including the understudied **sinapines or sinapoyl-choline and related derivatives**, such as feruloyl choline(4-O-8')guaiacyl, and sinapine(4-O-8')guaiacyl, together with characteristic SADs of cruciferous species, such as the **sinapoyl gentiobiosides**. The seeds presented much higher amounts of sinapines, than the edible sprouts in the 4 studied varieties. The Ethiopian and black mustard had similar low contents of sinapines and other SADs, in seeds and sprouts, being the red mustard the variety with the lowest contents of SADs. The black mustard showed higher contents of cinnamoyl-choline esters in sprouts, and the white mustard showed the highest contents of sinapines in both, seeds and sprouts. The evaluation of the **bioaccesibility of the sinapic acid derivatives present in white mustard sprouts**, for screening purposes and further investigations was also performed, revealing reduced bioaccessible fraction in all the tested samples.

Concluding Remarks. Sinapines *(*Sinapoyl-choline esters and related forms) were identified in all the varieties of mustards and were particularly abundant in white mustard. The influence of Ethyl-Gallate (EG) as primming and elicitation agent was only positive for the biomass production (data not shown) of the sprouts. The effect as biostimulant for SADs was negligible. Further investigations on natural biostimulants for metabo-farming for bioactives in cruciferous foods and ingredients is guaranteed, including selection of specific organs (seeds or sprouts) according to their contents and bioaccessible/bioavailable fractions of the compounds of interest.

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and authors

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