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Increased Stability of Bimi® Glucosinolates by Bioencapsulation †

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Abstract: Brassica vegetables are of particular interest not only for their nutritional profile but also as a source of health-promoting bioactives. However, its bitter taste affects its acceptability by the consumer prompting the development of novel varieties with better acceptability such as Bimi®. Elicitors have been used to stimulate the biosynthesis and accumulation of secondary metabolites in plant-foods. Nevertheless, little is known about the response of these new hybrid varieties. To this point, a study was designed to evaluate the effects of elicitors (200 μM salicylic acid (SA), 100 μM Methyl-Jasmonate (MeJA) and their combination) on the composition of Bimi® (*Brassica oleracea* var. italica × *Brassica oleracea* var. *alboglabra*). For this purpose, composition of glucosinolates present in Bimi® samples obtained from experimental farm under Mediterranean climatic conditions, evaluating edible florets and leaves were studied. The plant material was used to elaborate extracts to study the stability and bioaccesibility of the glucosinolates in Bimi® by using gastrointestinal simulated digester system and further RP-HPLC-ESI-MSn analysis. In order to improve the stability, the protective effect of a novel bioencapsulation system using plant material (plasma membrane vesicles obtained from *Brassica oleracea* var. *botrytis*) was also evaluated. Preliminary results are encouraging ongoing research for the development of stable and bioaccessible ingredients from Brassica for health-promotion using natural matrices for encapsulation.

Keywords: sulforaphane; Bimi®, bioaccesibility

1. Introduction

Nowadays, bioactives-enriched foods and functional ingredients have emerged as dietary coadjutants to improve human health [1]. Particularly, the vegetables of the Brassicaceae family are bioactive-rich, not only for their nutritional profile but also for the presence of health-promoters including glucosinolates and isothiocyanates (ITCs) [2]. In broccoli, sulforaphane (SFN) is one of the most ITCs studies, since it has been reported to be an inductor of Nrf2 transcription factor and a phase II detoxification enzymes inductor [3,4]. However, the bitter taste of broccoli hinders its acceptance by the consumer. As a solution, novel varieties of broccoli with less pungency have emerged - such as Bimi®, a new hybrid which is the result of the crossbreeding of broccoli (*Brassica oleracea* var. *italica*) with a green Chinese kale (*Brassica oleracea* var. *alboglabra*). Nevertheless, little is known about the effects of different elicitors (200 μM SA, 100 μM MeJA and their combination) on these new species and its performance as an enriched material for functional ingredients prototypes. In addition, ITCs are usually instable in aqueous solution [5,6]. In this way, microencapsulation techniques emerge as

a solution for increasing its stability. For all that, the aim of this work is to know the effect of elicitors in Bimi® edible parts and leaves and study its stability (with and without microencapsulation) after simulating an in vitro gastric digestion.

2. Methods

2.1. Plant Material and Treatments

Ten Bimi® seeds per treatments were germinated in vermiculite for two days. 5-days old seedlings were transplanted to agricultural soil with a Mediterranean climate from March to May 2019. Plants were irrigated with ¼ Hoagland solution. Different elicitations were performed: (i) 100 μM MeJA dissolved in 0.2% ethanol, (ii) 200 μM SA dissolved in 0.2% ethanol and (iii) a combined treatment (SA + MeJA), and control plants treated only with distilled water. Elicitation was performed 4 days before harvest (90-days after transplanting). Then, the edible part and leaves were sampled for further analysis.

2.2. Preparation of Extracts and In Vitro Gastrointestinal Digestion

100 g of freeze dried material and water at 1:5 (*w*:*v*) ratio were used for extracts elaboration. Samples were microencapsulated with 1g of plasma membrane vesicles obtained from cauliflower [7]. 1 mL from each sample was used for the in vitro gastrointestinal digestion. The protocol was performed as described in Minekus et al. [8].

2.3. Glucosinolates and ITCs Analysis

Freeze-dried and ground samples from the elicitation experiments were analyzed following the procedure of Garcia-Ibañez et al. [9], using a HPLC-DAD-ESI-MSn for identification of compounds. Upon in vitro gastric digestion, the extracts were studied in UHPLC-QqQ-MS/MS to identify metabolites of interest for the evaluation of bioaccessibilty [10].

2.4. Statistical Analysis

Data analysis was performed using an ANOVA and a HSD Tukey as a post-hoc test in RStudio (version 1.2.5). Significant differences were considered when ($p < 0.05$).

3. Results and Discussion

Elicitation experiments (Table 1) revealed no differences in glucoraphanin (GRA) when applied in leaves. However, hydroxyglucobrassicin (HGB) concentrations increased after elicitation with the tree treatments in the edible part of Bimi®. Regarding glucobrassicin (GB), a statistically significant increase ($p < 0.05$) was found in the edible part with the combination treatment and with 100 μM MeJA in leaves. About methoxyglucobrassicin (MGB), a statistically significant increase was analyzed in the edible part after 200 μM SA and combined treatments. In leaves, MGB was quantifiable after treatment with 100 μM MeJA and the combined treatment. For neoglucobrassicin (NGB), a statistically significant increase was found after 100 μM MeJA and the combined treatment elicitation in Bimi® edible part ($p < 0.05$). In leaves an increase was found after 100 μM MeJA elicitation ($p < 0.05$). In previous works performed in other brassica crops, MeJA elicitation increased the concentration of mainly indolic glucosinolates [11]. In addition, in cabbage an effect on aliphatic GLSs was observed, however no effect was observed in Bimi®, what suggest that the elicitor effect is species-specific [12]. About SA, when 100 μM SA spray applied to turnip plants showed differential response between same type GLS [13]. Similar results were observed in Bimi®, since 200 μM SA showed an increase for GB and MGB but a decrease in NGB concentrations ($p < 0.05$).

Table 1. Effect of elicitors in glucosinolates of Bimi® edible part and leaves. Data (mg g D.W.$^{-1}$) shown are average values per treatment (n = 4) ± standard deviation. Different letters indicate statistically significant differences in the one way ANOVA and HSD Tukey test ($p < 0.05$).

	Edible Part				Leaves			
GLS	Control	200 μM SA	100 μM MeJA	SA + MeJA	Control	200 μM SA	100 μM MeJA	SA + MeJA
GRA	4.06 ± 0.12a	3.95 ± 0.06b	4.13 ± 0.08a	3.98 ± 0.04b	1.4 ± 0.04ab	1.67 ± 0.07a	1.10 ± 0.07b	1.52 ± 0.01ab
HGB	*	1.76 ± 0.19a	1.56 ± 0.21a	1.27 ± 0.05a	*	*	*	*
GB	0.94 ± 0.04d	3.73 ± 0.14c	4.79 ± 0.1b	5.63 ± 0.07a	0.72 ± 0.03c	0.75 ± 0.12c	3.9 ± 0.04a	2.26 ± 0.09b
MGB	1.72 ± 0.08b	2.2 ± 0.11a	1.8 ± 0.05b	2.35 ± 0.08a	*	*	1 ± 0.01a	0.71 ± 0.07b
NGB	0.74 ± 0.05c	0.18 ± 0.04d	2.44 ± 0.1b	3.01 ± 0.16a	*	0.19 ± 0.02c	1.63 ± 0.03a	0.5 ± 0.003b

***** *The presence of the GLSs was under limit of quantification by HPLC-DAD-ESI-MSn (<0.02 mg/g D.W.).*
SA: salicylic acid, MeJA: methyl jasmonate, GLS: glucosinolate, GRA: glucoraphanin, HGB: 4-hydroxy-glucobrassicin, GB: glucobrassicin, MGB: 4-metoxy-glucobrassicin; NGB: neoglucobrassicin.

Regarding to the in vitro digestibility assay, an SFN-rich extract was elaborated from the edible part (0.5 ± 0.07 μM) and leaves (1.25 ± 0.1 μM) of Bimi® elicitated with the combined treatment and 100 μM MeJA, respectively. In Figure 1 is shown the SFN concentrations (μM) during 3 h of gastric in vitro digestion. About the extracts obtained from the edible part of Bimi® (Figure 1A), the microencapsulated samples showed a higher SFN concentration in the time course of the experiment ($p < 0.05$). Except at 90 and 120 min when no statistically significant differences were found ($p > 0.05$). Similar results were observed for Bimi® leaves (Figure 1B). However, concentrations from the free and microencapsulated extracts did not show statistically significant differences after 120 min of gastric digestion until 180 min ($p > 0.05$).

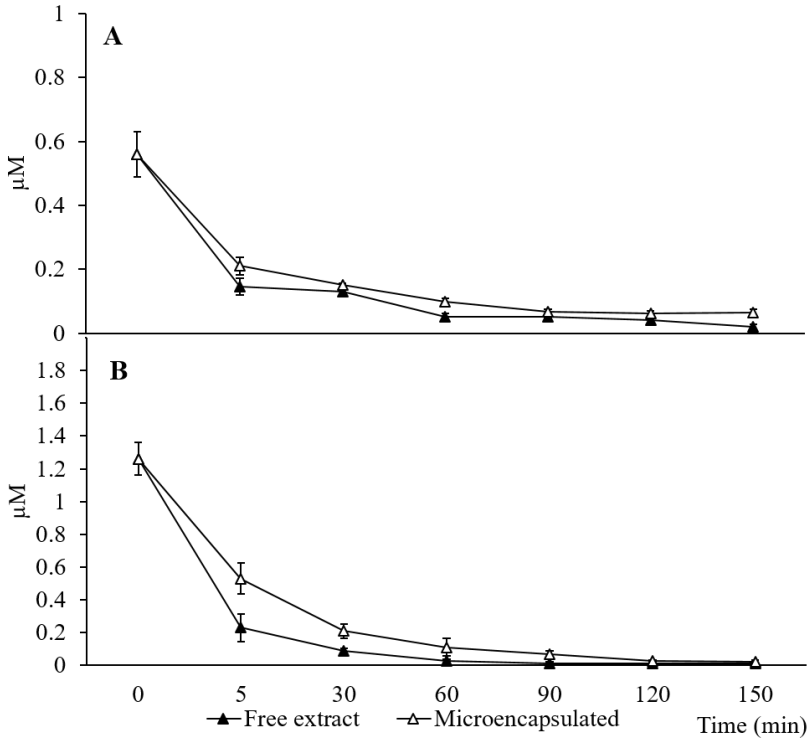

Figure 1. (**A**) Sulforaphane concentrations for an aqueous extract (free and microencapsulated) obtained from the edible part of Bimi® after gastric digestion. (**B**) Sulforaphane concentrations for an aqueous extract (free and microencapsulated) obtained from Bimi® leaves after gastric digestion. Data represented are average values (n = 3) ± standard deviation. A two-way ANOVA was performed and an HSD Tukey test as a post hoc.

4. Conclusions

In summary, elicitors (like SA and MeJA) favored an enrichment in glucosinolates in the edible part of Bimi® and its leaves. In addition, it was demonstrated that the response to elicitation is not

only elicitor-specific but also species-specific. When Bimi® plant material was used for a SFN-rich extract for an in vitro gastric digestion, higher concentrations of SFN were found in the microencapsulated samples, in both the edible part and leaves. This results suggests that plasma membrane vesicles from cauliflower are a suitable vehicle for SFN gastrointestinal delivery. In this way, our research opens a new pathway for optimizing the chain process from field production to food and ingredient development and enhancement of its stability and bioaccesibility in the gastrointestinal tract.

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