horticulturae

MDPI

Article

Evaluation of the Effects of Process Conditions on the Extraction of Glucosinolates from Broccoli Sprouts

Erika Melissa Bojorquez-Rodríguez [1], Daniel Guajardo-Flores [1], Daniel A. Jacobo-Velázquez [2,3,*] and Sergio O. Serna-Saldívar [1,*]

1 Escuela de Ingeniería y Ciencias, Tecnológico de Monterrey, Av. Eugenio Garza Sada 2501, Monterrey 64849, Mexico
2 The Institute for Obesity Research, Tecnologico de Monterrey, Av. General Ramón Corona 2514, Zapopan 45201, Mexico
3 Escuela de Ingeniería y Ciencias, Tecnológico de Monterrey, Av. General Ramón Corona 2514, Zapopan 45201, Mexico
* Correspondence: djacobov@tec.mx (D.A.J.-V.); sserna@tec.mx (S.O.S.-S.)

Abstract: Glucosinolates and their enzyme-degraded metabolites, such as isothiocyanates, have shown great potential to prevent and treat chronic-degenerative diseases. Broccoli sprouts (*Brassica oleracea* L., var. italica) are an excellent source of glucosinolates. The objective of this study was to evaluate different process conditions (temperature, solvent concentration, and sample:solvent ratio) for the extraction of glucosinolates present in broccoli sprouts. The study evaluated different combinations of temperature (15–64 °C), ethanol concentration (0–100%), and sample:solvent ratio (1:15–1:35 *w*/*v*). The treatment with 40 °C, 50% ethanol/water (*v*/*v*), 1:35 (*w*/*v*) generated the highest extraction of total glucosinolates (100,094 ± 9016 mg/kg DW). The ethanol percentage was the factor that exerted the greatest impact on glucosinolate extraction ($p < 0.05$). In addition, the amount of glucoraphanin in the extract was determined, obtaining a final concentration of 14,986 ± 1862 mg/kg DW of sprouts processed under the optimal extraction conditions studied. The data presented herein demonstrate the relevance of the ethanol concentration during the extraction and the importance of applying high temperature in solid-liquid phase extraction. Under optimal conditions, it was possible to obtain extracts rich in glucosinolates to prepare food supplements, nutraceuticals, and even pharmaceuticals with application in the prevention and treatment of chronic-degenerative diseases.

Keywords: broccoli sprouts; glucosinolate extraction; dietary supplements; functional food ingredients

Citation: Bojorquez-Rodríguez, E.M.; Guajardo-Flores, D.; Jacobo-Velázquez, D.A.; Serna-Saldívar, S.O. Evaluation of the Effects of Process Conditions on the Extraction of Glucosinolates from Broccoli Sprouts. *Horticulturae* **2022**, *8*, 1090. https://doi.org/10.3390/horticulturae8111090

Academic Editors: Marija Banožić and Stela Jokić

Received: 6 November 2022
Accepted: 16 November 2022
Published: 18 November 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Broccoli (*Brassica oleracea* var. italica) is a plant of the Brassicaceae family (formerly Cruciferae), along with cauliflower, cabbage, kale, mustard, turnip, and brussels sprouts [1]. Different studies suggest that ingesting vegetables from this family and its derivatives diminishes the risk of several diseases, including various types of cancer (i.e., colon, breast, and prostate), cardiovascular diseases, and obesity [2–7].

The health benefits of broccoli are attributed to its secondary metabolites, mainly glucosinolates (GSL), which are molecules derived from amino acids. These sulfur-rich compounds are hydrolyzed by the endogenous enzyme myrosinase (β-thioglucosidase glucohydrolase), which yields several degradation products, including isothiocyanates, nitriles, thiocyanates, epithionitriles, and oxazolidines [8]. GSL and their breakdown metabolites play an important role in plant defense as biocidal against different pathogens. Most biological and nutraceutical activities of GSL have been mainly attributed to their hydrolysis products, specifically isothiocyanates. The former compounds are a class of molecules in which R is an alkyl or aryl group [9,10].

It has been demonstrated that GSL have multiple positive effects on several common diseases. Reports have shown neuroprotective, antioxidant, anti-inflammatory, and an-

ticancer effects [11–13]. Other studies have shown the pleiotropic role of this natural compound, thanks to its ability to address different targets and modulate different pathways in neuronal/glial cells [13–15].

Glucosinolates and their enzymatic degradation products (isothiocyanates) occur in all tissues in broccoli plants. Due to the wide application of these compounds, the extraction and quantification of the metabolites from different physiological stages of the plant have been tested, such as seeds, sprouts, and even the fresh plant parts such as leaves, stems, and florets [16,17]. Moreover, it has been found that the physiological process of germination of broccoli seeds to produce young seedlings or sprouts increases their nutritive and phytochemical values [18]. Reports have shown that edible broccoli sprouts constitute a rich source of GSL and other phytochemicals as phenolic compounds [19,20].

Organic solvents with different polarities, such as hexane, acetonitrile, acetone, methanol, and ethanol, have been widely used to extract phytochemicals in several plant models [21–23]. These organic solvents are used in different ratios in combination with water and temperature to yield extracted compounds with the potential to be used for supplements or food ingredients. The extraction method and solvent must be carefully selected, as some are highly toxic and dangerous for humans. Thus, to obtain extracts that can be used in industrial markets, ethanol represents one of the best options due to its comparatively lower side effects and toxicity. This safer solvent extraction can be used in industries such as the cosmeceutical and nutraceutical industries [24].

This work aimed to evaluate the process conditions (temperature, solvent concentration, and weight-volume ratio) for extracting GSL from broccoli sprouts. To achieve this goal, the effects of the extraction conditions were optimized using a central composite design (CCD) combined with response surface methodology (RSM).

2. Materials and Methods

2.1. Chemicals and Plant Material

Sinigrin hydrate, sodium acetate, sulfatase (from Helix pomatia), diethylaminoethyl (DEAE)-sephadex A-25, acetonitrile (HPLC grade), and methanol (HPLC grade) were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). On the other hand, the HPLC-grade ethanol was procured from Desarrollo de Especialidades Químicas, S.A. de C.V (Monterrey, NL, México), whereas the desulfoglucoraphanin was acquired from Santa Cruz Biotechnology (Dallas, TX, USA). Deionized water (18.2 MΩ·cm resistance) used in all protocols was obtained from a Milli-Q Element water purification system (Millipore, Bedford, MA, USA). Broccoli sprouts (*Brassica oleracea* L., var. italica) were obtained from a local producer located in Zacatecas, Mexico. Samples were kept in plastic freezer bags and stored at −20 °C until extracting the compounds.

2.2. Experimental Design

The extraction was performed using an orthogonal CCD to determine the optimal extraction yield conditions. The experiment included the factors temperature, sample:solvent ratio (w/v), and ethanol percentage to estimate the simultaneous effect of these process variables on GSL extraction yield in a quadratic function. The design generated 20 experiments, distributed as follows: 6 replications of the center points, 8 axial points which are identical to the center point except for one factor, and 6 alpha points that are the distance of each individual axial point from the center in the center composite design. A RSM was used to analyze the effect of each process variable on GSL extraction yield. The design matrix is presented in Table 1, whereas Table 2 exhibits the fully coded CCD.

The CCD is a fractional factorial design used in the response surface methodology to reduce the number of experiments compared to a full factorial design. In the CCD, the center points are used to determine the prediction error as identical for all the points located at the same distance from the center of the domain. Therefore, experimental runs are identical to the center points except for one factor, and the inclusion of axial points allows the estimation of curvature, and second-order terms can be estimated. In this study,

there are 6 axial points that go beyond the distance from the center of the design space to form this curvature (alpha points), namely runs 5 and 10 related to temperature; runs 11 and 12 related to sample:solvent ratio; and runs 13 and 14 related to ethanol percentage.

Table 1. Factors and levels used for central composite design analysis for glucosinolate extraction.

Independent Variables	Coded Levels				
	−α	**−1**	**0**	**1**	**α**
x_1 Temperature (°C)	15	25	40	55	65
x_2 Sample:Solvent	1:15	1:20	1:25	1:30	1:35
x_3 Ethanol (%)	0	25	50	75	100

Table 2. Fully coded three-variable central composite design matrix.

Treatment	Temperature (°C)	Sample: Solvent	Ethanol (%)
1	25	1:20	25
2	25	1:30	25
3	25	1:20	75
4	25	1:30	75
5	15	1:25	50
6	55	1:20	50
7	55	1:30	25
8	55	1:20	75
9	55	1:30	75
10	65	1:25	50
11	40	1:15	50
12	40	1:35	50
13	40	1:25	0
14	40	1:25	100
Central Points	40	1:25	50

2.3. Extraction and Desulfation of Glucosinolates (GSL)

A single procedure was performed to extract GSL from the freeze-dried broccoli sprouts. A central composite design was elaborated to evaluate the effects of temperature, solvent concentration, and weight/volume ratio in the extraction yield of GSL. Therefore, four ethanolic extracts (one per replicate) were prepared for each treatment and the control sample.

The extraction of phytochemicals and further desulfation of GSL were performed as previously described [25]. Briefly, broccoli sprouts samples were weighed (1 g) and mixed into different volumes (15, 20, 25, 30, and 35 mL) of ethanol (100, 75, 50, 25, 0%) previously heated for 10 min in an incubator followed by the addition of 50 µL of a 3 mM solution of sinigrin as internal standard (I.S). Samples were incubated for 1 h at 250 rpm in a shaking incubator (VWR, Radnor, PA, USA) at the temperatures stated by the experimental design (15, 25, 40, 55, 65 °C). The extracts were removed from the incubator, left to cool at room temperature, and then centrifuged (SL16R Thermo Scientific, Bremen, Germany) at 18,000× g for 10 min and at 4 °C to sediment wasted solids. The clarified extract (supernatant) was recovered and stored at −80 °C for further glucosinolate analysis.

After the solvent extraction of phytochemicals, GSL were desulphated and purified for analytical essays using disposable polypropylene columns (Thermo Fisher Scientific, Waltham, MA, USA). Polypropylene columns were prepared by adding 0.5 mL of water, followed by 0.5 mL of previously prepared resin Sephadex A-25 and an additional 0.5 mL of HPLC water. Then, 3 mL of clarified broccoli extract supernatant was added to the prepared column and allowed to elute slowly. After removing excess supernatant, columns were washed with 2 × 0.5 mL of HPLC water followed by 2 × 0.5 mL of sodium acetate (0.02 M). Purified sulfatase (75 µL) previously prepared was added to each sample and left

at room temperature overnight (12 h). Desulfoglucosinolates were eluted in vials with a total of 1.25 mL of water (2 × 0.5 mL + 0.25 mL).

2.4. Identification and Quantification of Desulfoglucosinolates

Glucosinolates were assayed using high-performance liquid chromatography coupled with a photodiode array detector (HPLC-DAD). Individual GSL were prepared using a standard curve of desulfoglucoraphanin ranging from 0 to 1250 ppm. The concentrations of total and individual GSL were expressed as mg of desulfoglucoraphanin equivalents per g of broccoli sprouts dry weight (DW), while individual GSL were identified based on retention time as compared with authentic standards and previous reports [22,25–27].

Chromatographic separations were performed on an HPLC system composed of a quaternary pump, an autosampler, and a photodiode array detector (DAD) (1260 Infinity, Agilent Technologies, Santa Clara, CA, USA). Desulfoglucosinolates were separated on a 4.6 mm × 250 mm, 5 µm, C18 reverse phase column (Luna, Phenomenex, Torrace, CA, USA). Separation of desulfoglucosinolates in the HPLC-DAD system was achieved using water (phase A) and acetonitrile (phase B) as mobile phases with a flow rate of 1.5 mL/min and a sequential gradient of 0/100, 28/80 and 35/100 (min/% phase A). The injection volume was 20 µL and compounds were detected at 227 nm. Chromatographic data were processed with OpenLAB CDS ChemStation software (Agilent Technologies, Santa Clara, CA, USA).

2.5. Statistical Analysis

Statistical analyses of chemical analyses were performed using three treatment repetitions. Data represent the mean values of samples and their standard errors. Analysis of variance (ANOVA) and RSM were conducted using JMP software version 16.0 (SAS Institute Inc., Cary, NC, USA). The level of significance of all statistical tests was stated at 95%. Results were subjected to a mean comparison analysis employing Tukey's Honestly-significant-difference (HSD).

3. Results

A three-factor CCD was performed using the GSL concentration as a response using HPLC to obtain the optimum conditions for GSL extraction. The experiments were carried out following the run order shown in Table 2. Ten major GSL were quantified in the extracts as follows: glucoiberin (1); progoitrin (2); glucoraphanin (3); 1-hydroxy-3-indoylmethyl (4); 4-hydroxy-glucobrassicin (5); glucobrassicanapin (6); glucoeurocin (7); gluconasturtiin (8); 4-methoxy-glucobrassicin (9); neoglucobrassicin (10) (Supplementary Figure S1). For most treatments, progoitrin (peak 2) and 4-hydroxy-glucobrassicin (peak 5) were the glucosinolates found in higher proportion in broccoli sprouts, followed by glucoraphanin (peaks 3), neoglucobrassicin (peak 8), and gluconasturtiin (peak 10).

Individual and total concentrations of glucosinolates were quantified in the extract obtained with the extraction conditions under evaluation (15–65 °C, 0–100% ethanol, and 1:15–1:35 sample/solvent) (Table 3). In general, the extraction parameters using 40 °C, 50% ethanol/water (*v*/*v*), 1:35 (*w*/*v*), or 65 °C, 50% ethanol/water (*v*/*v*), 1:25 (*w*/*v*) and 40 °C, 50% ethanol/water (*v*/*v*), 1:15 (*w*/*v*) induced the maximum extraction of total glucosinolates from broccoli sprouts. Interestingly, the extraction using 40 °C, 0% ethanol/water (*v*/*v*), and 1:25 sample:solvent (*w*/*v*) was the one that induced the minimum total concentration (Table 3). The total glucosinolate concentrations ranged from 2131 mg/kg to 100,094 mg/kg DW. The three main glucosinolates detected using the extraction conditions were progoitrin, 4-hydroxy-glucobrassicin, and glucoraphanin, while the two with lower concentrations were neoglucobrassicin and gluconasturtiin.

Table 3. Concentration of total and individual glucosinolates in broccoli sprouts.

Treatment	Glucosinolate Concentration (mg/Kg DW)										
	Glucoiberin	**Progoitrin**	**Glucoraphanin**	**1-Hydroxy-3-indoylmethyl**	**4-Hydroxy-glucobrassicin**	**Glucobrassicanapin**	**Glucoeurocin**	**Gluconasturtiin**	**4-Methoxy-glucobrassicin**	**Neoglucobrassicin**	**Total**
1	3113 ± 43 ^{d,e}	18,366 ± 450 ^{b,c,d}	7015 ± 86 ^{e}	8489 ± 528 ^{b,c}	16,024 ± 760 ^{c,d}	1117 ± 75 ^{e,f}	4141 ± 232 ^{f}	n.d.	429 ± 63 ^{c,d,e}	19 ± 14 ^{e}	58,696 ± 1833 ^{d,e}
2	3746 ± 134 ^{b,c,d,e}	21,482 ± 1264 ^{a,b}	7434 ± 322 ^{d,e}	8475 ± 535 ^{b,c}	17,004 ± 1098 ^{c,d}	1280 ± 150 ^{e}	4443 ± 305 ^{e,f}	n.d.	346 ± 42 ^{d,e,f}	n.d.	64,009 ± 3873 ^{d,e}
3	4040 ± 253 ^{b,c,d}	16,834 ± 783 ^{b,c,d}	11,350 ± 884 ^{b,c}	8970 ± 495 ^{b,c}	20,028 ± 1190 ^{b,c}	1658 ± 98 ^{e}	6088 ± 362 ^{c,d,e,f}	325 ± 27 ^{c,d}	257 ± 8 ^{e,f,g}	222 ± 31 ^{b,c}	69,773 ± 4024 ^{c,d,e}
4	3955 ± 359 ^{b,c,d}	16,274 ± 1714 ^{c,d}	10,285 ± 1123 ^{b,c,d}	8339 ± 926 ^{c}	18,561 ± 1982 ^{c,d}	1406 ± 129 ^{e}	5365 ± 556 ^{c,d,e,f}	201 ± 52 ^{d,e}	113 ± 33 ^{g,h}	57 ± 34 ^{e}	64,555 ± 6789 ^{c,d,e}
5	4361 ± 171 ^{a,b,c}	21,363 ± 184 ^{a,b,c}	11,421 ± 701 ^{b,c}	11480 ± 175 ^{a,b}	24,573 ± 293 ^{a,b}	3061 ± 217 ^{c}	7261 ± 115 ^{a,b,c}	467 ± 25 ^{b,c}	434 ± 17 ^{c,d,e}	262 ± 14 ^{a,b}	84,682 ± 260 ^{a,b,c}
6	2973 ± 288 ^{de}	15,048 ± 618 ^{d}	7040 ± 637 ^{e}	6643 ± 462 ^{c}	13,319 ± 910 ^{d}	1023 ± 29 ^{e,f}	4632 ± 309 ^{d,e,f}	87 ± 19 ^{e,f}	639 ± 14 ^{b,c}	94 ± 15 ^{d,e}	51,498 ± 2990 ^{e}
7	3748 ± 317 ^{b,c,d,e}	15,885 ± 1182 ^{d}	7521 ± 1024 ^{d,e}	6879 ± 461^{c}	14,661 ± 1028 ^{c,d}	891 ± 98 ^{e,f}	4271 ± 310 ^{e,f}	n.d.	620 ± 44 ^{b,c}	n.d.	54,441 ± 4320 ^{d,e}
8	3529 ± 673 ^{bcde}	15,650 ± 3670 ^{d}	9648 ± 1945 ^{c,d,e}	8428 ± 2106^{c}	18,814 ± 4615 ^{c,d}	3109 ± 414 ^{c}	6193 ± 1574 ^{c,d,e}	553 ± 157 ^{b}	518 ± 144 ^{b,c,d}	229 ± 114 ^{b,c}	66,670 ± 15221 ^{c,d,e}
9	4343 ± 231 ^{a,b,c}	17,853 ± 515 ^{b,c,d}	11,734 ± 777 ^{b,c}	9162 ± 296 ^{b,c}	19,632 ± 650 ^{b,c}	2980 ± 130 ^{c,d}	6466 ± 197 ^{b,c,d}	454 ± 24 ^{b,c}	468 ± 14 ^{b,c,d,e}	113 ± 30 ^{c,d,e}	73,204 ± 2821 ^{b,c,d}
10	4711 ± 566 ^{a,b}	24,688 ± 2580 ^{a}	13,352 ± 1566 ^{a,b}	13615 ± 2028 ^{a}	26,975 ± 3839 ^{a}	1831 ± 330 ^{d,e}	8813 ± 1353 ^{a}	943 ± 125 ^{a}	962 ± 124 ^{a}	360 ± 86 ^{a}	96,249 ± 12,550 ^{a}
11	2635 ± 284 ^{e}	23,692 ± 3443 ^{a}	7114 ± 927 ^{e}	12,537 ± 1902 ^{a}	28,481 ± 2023 ^{a}	7611 ± 930 ^{a}	8902 ± 902 ^{a}	622 ± 166 ^{b}	677 ± 163 ^{b}	208 ± 36 ^{b,c,d}	92,480 ± 10271 ^{a,b}
12	5505 ± 952 ^{a}	24,320 ± 1815 ^{a}	14,986 ± 1862 ^{a}	12287 ± 935 ^{a}	27,888 ± 1953 ^{a}	7069 ± 804 ^{a,b}	8229 ± 609 ^{a,b}	n.d.	147 ± 94 ^{f,g}	n.d.	100,094 ± 9016 ^{a}
13	1374 ± 198 ^{f}	329 ± 84 ^{e}	74 ± 40 ^{f}	442 ± 62 ^{d}	317 ± 40 ^{e}	n.d.	n.d.	n.d.	58 ± 20 ^{g,h}	n.d.	2131 ± 430 ^{f}
14	264 ± 46 ^{f}	57 ± 19 ^{e}	788 ± 134 ^{f}	431 ± 79 ^{d}	936 ± 92 ^{e}	n.d.	173 ± 43 ^{g}	n.d.	n.d.	n.d.	2269 ± 307 ^{f}
Central Points	3201 ± 200 ^{c,d,e}	16,232 ± 499 ^{d}	10,056 ± 599 ^{c,d,e}	9070 ± 86 ^{b,c}	18,328 ± 701 ^{c,d}	6392 ± 701 ^{b}	6190 ± 164 ^{c,d,e}	519 ± 45 ^{b,c}	625 ± 35 ^{b,c}	242 ± 18 ^{a,b}	70,853 ± 1738 ^{c,d,e}

Values represent the means of 3 replicates ± standard error. Values with different superscript letters in the same column indicate a statistical difference between the mean concentration of the bioactive compound in the morphotypes evaluated using the LSD test ($p < 0.05$).

Glucoraphanin is the glucosinolate with the most substantial scientific evidence for preventing and treating different chronic and degenerative diseases. The concentration of this glucosinolate ranged from 57 mg/kg to 24,688 mg/kg DW, depending on the extraction conditions. The extraction conditions that maximize the concentration of glucoraphanin in the extract match those that maximize the total GLS concentration, except for the treatment using 65 °C, 50% ethanol/water (*v*/*v*), and 1:25 (*w*/*v*).

Additionally, response surface plots were generated to evaluate the effects of studied factors and their interactions in the response variables. The RSM plots were generated in the function of temperature (15–65 °C), sample:solvent (1:15–1:35), and ethanol (0–100%). The RSM plots are depicted in Figure 1. The *p*-value was used to corroborate each coefficient's significance. As observed, the extraction of glucosinolates is maximized using temperatures in the medium level (not the minimum or maximum ones) (Figure 1A,B). Likewise, ethanol concentration in the medium range maximized glucosinolate extraction, while at minimum or maximum, ethanol concentrations highly reduced the glucosinolate concentration. Otherwise, the higher sample:solvent ratio maximized the total glucosinolate concentration.

To confirm the RSM analysis, ANOVA analysis was employed to corroborate the significance of the factors used on the obtained extraction concentrations. A mathematical model was developed, obtaining the following quadratic equation with a correlation coefficient R2 of 0.94 and a lack of fit with a *p*-value < 0.1.

Quadratic equation:

$$\begin{aligned}\text{GLS concentration} = 53{,}588 + 39.7 \times \text{Temperature} + 310.18 \times \text{Sample:Solvent} + \\ 11{,}462.45 \times \%\text{Ethanol} + 30.38 \times \text{Temperature2} + 256.68 \times \text{Sample:Solvent2} - \\ 273{,}672.2 \times \%\text{Ethanol2} + 15.61 \times \text{Temperature} \times \text{Sample:Solvent} + 743.26 \times \\ \text{Temperature} \times \%\text{Ethanol} - 695.57 \times \text{Sample:Solvent} \times \%\text{Ethanol} + 94.25 \times \\ \text{Temperature} \times \text{Sample:Solvent} \times \%\text{Ethanol}\end{aligned} \quad (1)$$

ANOVA results indicate a strong significance ($p < 0.05$) or difference in extraction rates in relationship with the ethanol concentration on the linear model. Additionally, the parameters that showed significance in the ANOVA tests were the quadratic effects of temperature, sample:solvent and % ethanol. Moreover, their interactions did not show significance ($p < 0.05$). Furthermore, a noticeable trend can be observed in the increase in total glucosinolate concentration when 50% ethanol, 40 °C, and 1:35 (*w*/*v*) sample:solvent ratio was used. Furthermore, a desirability test was performed for factors in the total glucosinolate concentrations, which showed that the predicted optimal conditions for maximizing the total glucosinolate concentration were: 1:35 sample:solvent, 55% ethanol, and 65 °C.

Using the selected combinations, serial extractions were carried out to quantify the total yield of extraction. Results indicate that the combination of 50% EtOH, 40 °C, 1:35 (*w*/*v*) yielded around 85% of total glucosinolates and 83% of glucoraphanin in the matrix after the first extraction step and 8% and 10% more total glucosinolates and glucoraphanin after a second extraction of reused sprouts (Table 4).

Table 4. Extraction yield of total glucosinolates and glucoraphanin from broccoli sprouts.

	Total GLS Yield		**Glucoraphanin Yield**	
Extraction 1	116,687 ± 5594 [a]	85.20%	29,724 ± 993 [a]	83.90%
Extraction 2	14,228 ± 626 [b]	8.10%	2840 ± 201 [b]	10.20%
Extraction 3	3926 ± 268 [c]	2.70%	956 ± 129 [c]	2.80%
Extraction 4	2216 ± 145 [c]	2.30%	822 ± 35 [c]	1.60%
Extraction 5	1959 ± 28 [c]	1.50%	532 ± 51 [c]	1.40%

Concentrations are reported as desulfoglucoraphanin equivalents. Values represent the mean of three replicates ± standard error of the mean. Different superscript letters (a–c) in the same column indicate statistical differences by the Tukey test ($p < 0.05$). DW: Dry weight. GLS: Glucosinolates.

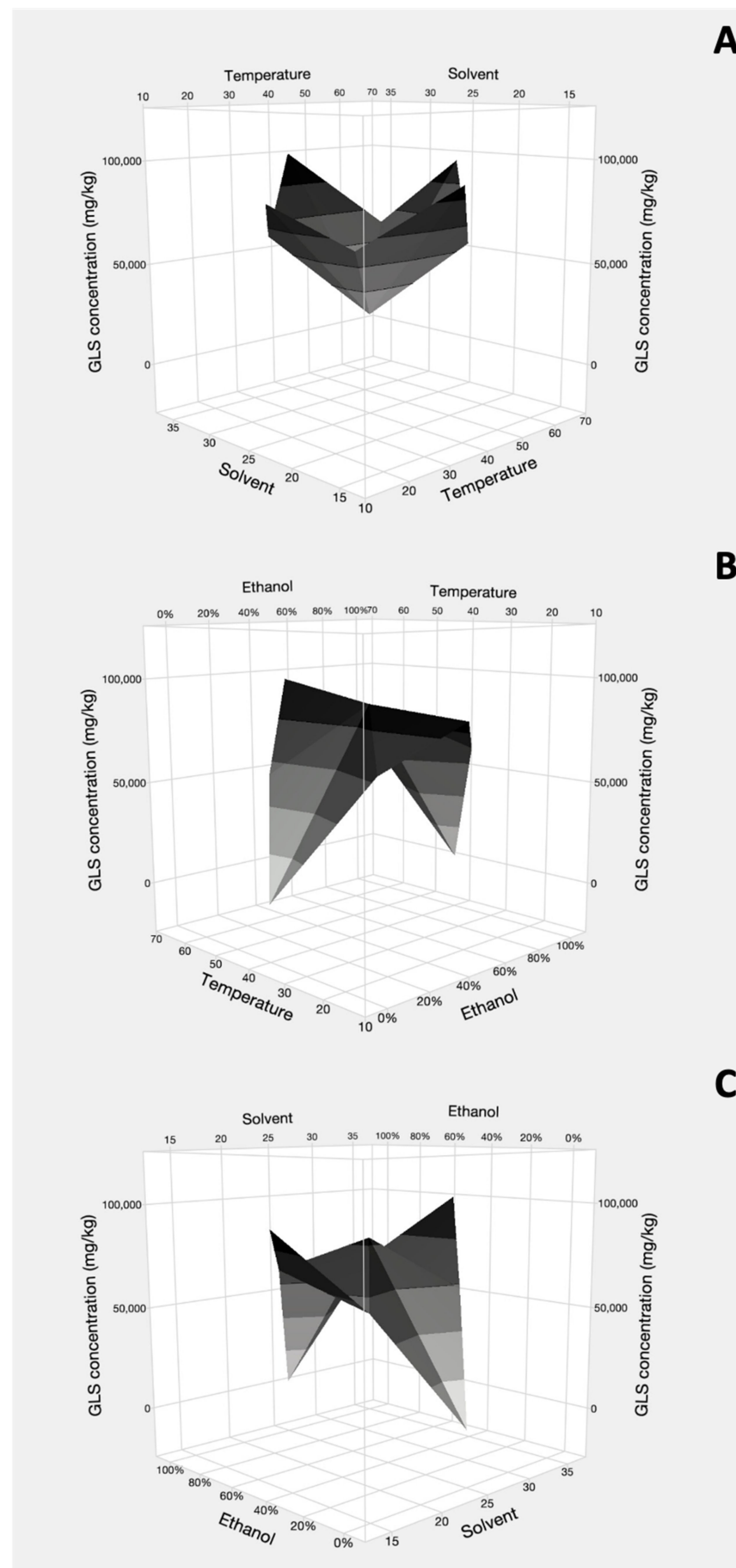

Figure 1. Response Surface graphical analysis for (**A**) Temperature-Sample: Solvent; (**B**) Temperature-%Ethanol; and (**C**) Sample: Solvent-%Ethanol against the Total Concentration of GLS (mg/kg DW) response variable. Significance is established at $p < 0.05$.

4. Discussion

The glucosinolate profile reported herein matches previous reports. However, the proportion of glucosinolates varies depending on the genotype, sprouting time, and other genetic variations [8,28–30].

The most critical factor affecting glucosinolate extraction was the ethanol percentage, which maximized the glucosinolate concentration when using the medium range concentration of 50%. This result could be related to the similar polarity of both hydroalcoholic solvents and the phytochemicals of interest [31]. Normally, methanol is the solvent used for glucosinolate extraction from broccoli sprouts [22,32,33]. Nevertheless, ethanol could be an attractive option as an extraction solvent for several markets, such as the cosmeceutical and nutraceutical markets. Botanical hydroalcoholic extracts used as active ingredients are typically ethanol-based to avoid possible toxicological reactions related to the usage of methanol.

Several ethanol concentrations have been reported to extract glucosinolates from different sources [34–39]. In those reports, the authors mention the importance of applying high temperatures in solid-liquid phase extraction [31]. Campos et al., [38] used RSM to optimize the extraction efficiency for glucosinolates and polyphenols in maca (*Lepidium meyenii*). These authors indicated that the optimal conditions were 70 °C, 1 g: 10 mL sample–solvent ratio, 90 min extraction time and 58% ethanol solution. Under these conditions, they obtained 79% of the total GLS. Furthermore, Moreira-Rodríguez et al., [22] proved the differences between methanol and ethanol (70:30 v/v) and concluded that ethanol extracts showed higher levels of specific glucosinolates (33.33 $\pm$ 0.84 mmol/kg), such as glucoraphanin, whereas methanol extracted slightly higher levels of phenolics. These results proved the synergy between temperature and ethanol concentration to increase the concentrations of individual and total glucosinolates.

In general, when developing conventional extractions, high temperatures facilitate the release of phytochemicals by improving the convection currents and the diffusion rate. At high temperatures, a vibration of the molecules and an increase in the solubility occurs, causing a cell wall breakdown which liberates the internal compounds [40,41]. However, a high rise in temperature can degrade some thermostable compounds.

Extractions must balance the sample and solvent ratio to increase concentration yields [40,42,43]. The sample-to-solvent ratio did not significantly differ between the tested values on the response variables. However, a relevant trend was noticed when the sample-to-solvent increased, where a high GLS concentration rate was observed. In turn, increasing the solvent volume led to a reduction in GSL extraction. This might be explained because of the saturation of the solvent and the limitation of the dissolution of the compounds into the solvent [40,44]. These results prove the importance of finding a balance between the sample and the solvent to reduce the saturation and thus maximize extraction yields.

Finally, the target glucosinolate to obtain a high concentration in the extract was glucoraphanin, considered the most relevant aliphatic glucosinolate as it is the sulforaphane precursor of a highly antioxidant isothiocyanate [27]. The selected treatments for scalability tests were the treatments that showed the highest concentrations of glucoraphanin: 50% etOH, 65 °C, 1:25 (w/v) or 50% etOH, 40 °C, 1:35 (w/v). The extraction using lower temperatures could be easily scalable and bring the first approach to a laboratory pilot scale level. Considering this, the combination recommended for future studies is the use of 50% etOH, 40 °C, and a 1:35 (w/v) ratio. Both treatments led to the accumulation of aliphatic glucosinolates such as glucoraphanin and progoitrin, followed by 4-hydroxy-glucobrassicin, an indole glucosinolate highly present in the extract.

5. Conclusions

The data presented herein demonstrate the relevance of the ethanol concentration during the extraction of glucosinolates and the importance of applying high temperature in solid-liquid phase extraction. Under optimal conditions, it was possible to obtain

extracts rich in glucosinolates to prepare food supplements, nutraceuticals, and even pharmaceuticals with applications in the prevention and treatment of chronic-degenerative diseases. Further studies should be focused on evaluating the residual ethanol in the extracts obtained to ensure the safety of the final product where the bioactive compounds will be incorporated.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8111090/s1, Figure S1: Typical HPLC-DAD chromatograms (shown at 227 nm) of identified desulfoglucosinolates (dsg) from broccoli sprout extracted with 40 °C, 1:35 (*w*/*v*) and 50% ethanol.

Author Contributions: Conceptualization, S.O.S.-S., D.G.-F. and D.A.J.-V.; formal analysis, E.M.B.-R.; investigation, E.M.B.-R.; data curation, E.M.B.-R., D.G.-F. and D.A.J.-V.; writing—original draft preparation, E.M.B.-R.; writing—review and editing, D.A.J.-V.; supervision, S.O.S.-S.; project administration, S.O.S.-S. and D.A.J.-V.; funding acquisition, S.O.S.-S. All authors have read and agreed to the published version of the manuscript.

Funding: This study is based upon research supported by research funds from Tecnológico de Monterrey—"Emerging Food Technologies and Nutraceuticals" and "Bioprocess and Synthetic Biology" research groups as well as The Institute for Obesity Research. Author Erika Melissa Bojorquez-Rodríguez aknowledge the scholarship (#1078169) from the Consejo Nacional de Ciencia y Tecnología (CONACYT, Mexico).

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ilahy, R.; Tlili, I.; Pék, Z.; Montefusco, A.; Siddiqui, M.W.; Homa, F.; Hdider, C.; R'Him, T.; Lajos, H.; Lenucci, M.S. Pre-and post-harvest factors affecting glucosinolate content in broccoli. *Front. Nutr.* **2020**, *7*, 147. [CrossRef] [PubMed]
2. Zhang, N.Q.; Mo, X.-F.; Lin, F.-Y.; Zhan, X.-X.; Feng, X.-L.; Zhang, X.; Lou, H.; Zhang, C.X. Intake of total cruciferous vegetable and its contents of glucosinolates and isothiocyanates, glutathione S-transferases polymorphisms and breast cancer risk: A case–control study in China. *Brit. J. Nutr.* **2020**, *124*, 548–557. [CrossRef] [PubMed]
3. Li, Y.-J.; Ji, Q.-Q.; Wang, Z.; Shen, L.-H.; He, B. *Moringa oleifera* seeds mitigate myocardial injury and prevent ventricular failure induced by myocardial infarction. *Am. J. Transl. Res.* **2020**, *12*, 4511. [PubMed]
4. Su, X.; Wang, S.; Zhang, H.; Yang, G.; Bai, Y.; Liu, P.; Meng, L.; Jiang, X.; Xin, Y. Sulforaphane prevents angiotensin II-induced cardiomyopathy by activation of Nrf2 through epigenetic modification. *J. Cell. Mol. Med.* **2021**, *25*, 4408–4419. [CrossRef] [PubMed]
5. Pereyra, K.V.; Andrade, D.C.; Toledo, C.; Schwarz, K.G.; Uribe-Ojeda, A.; Ríos-Gallardo, A.P.; Quintanilla, R.A.; Contreras, S.; Mahn, A.; Del Rio, R. Dietary supplementation of a sulforaphane-enriched broccoli extract protects the heart from acute cardiac stress. *J. Funct. Foods* **2020**, *75*, 104267. [CrossRef]
6. Peng, N.; Jin, L.; He, A.; Deng, C.; Wang, X. Effect of sulphoraphane on newborn mouse cardiomyocytes undergoing ischaemia/reperfusion injury. *Pharm. Biol.* **2019**, *57*, 753–759. [CrossRef]
7. Silva-Palacios, A.; Ostolga-Chavarría, M.; Sánchez-Garibay, C.; Rojas-Morales, P.; Galván-Arzate, S.; Buelna-Chontal, M.; Pavón, N.; Pedraza-Chaverrí, J.; Königsberg, M.; Zazueta, C. Sulforaphane protects from myocardial ischemia-reperfusion damage through the balanced activation of Nrf2/AhR. *Free Radic. Biol. Med.* **2019**, *143*, 331–340. [CrossRef]
8. Del Carmen Martínez-Ballesta, M.; Moreno, D.A.; Carvajal, M. The physiological importance of glucosinolates on plant response to abiotic stress in *Brassica*. *Int. J. Mol. Sci.* **2013**, *14*, 11607–11625. [CrossRef]
9. Kamal, R.M.; Razis, A.F.A.; Sukri, N.S.M.; Perimal, E.K.; Ahmad, H.; Patrick, R.; Djedaini-Pilard, F.; Mazzon, E.; Rigaud, S. Beneficial health effects of glucosinolates-derived isothiocyanates on cardiovascular and neurodegenerative diseases. *Molecules* **2022**, *27*, 624. [CrossRef]
10. Saha, S.; Hollands, W.; Teucher, B.; Needs, P.W.; Narbad, A.; Ortori, C.A.; Barrett, D.A.; Rossiter, J.T.; Mithen, R.F.; Kroon, P.A. Isothiocyanate concentrations and interconversion of sulforaphane to erucin in human subjects after consumption of commercial frozen broccoli compared to fresh broccoli. *Mol. Nutr. Food Res.* **2012**, *56*, 1906–1916. [CrossRef]
11. Li, Y.; Zhang, T.; Korkaya, H.; Liu, S.; Lee, H.F.; Newman, B.; Yu, Y.; Clouthier, S.G.; Schwartz, S.J.; Wicha, M.S.; et al. Sulforaphane, a dietary component of broccoli/broccoli sprouts, inhibits breast cancer stem cells. *Clin. Cancer Res.* **2010**, *16*, 2580–2590. [CrossRef]
12. Santana-Gálvez, J.; Villela-Castrejón, J.; Serna-Saldívar, S.O.; Cisneros-Zevallos, L.; Jacobo-Velázquez, D.A. Synergistic combinations of curcumin, sulforaphane, and dihydrocaffeic acid against human colon cancer cells. *Int. J. Mol. Sci.* **2020**, *21*, 3108. [CrossRef]

13. Tarozzi, A.; Angeloni, C.; Malaguti, M.; Morroni, F.; Hrelia, S.; Hrelia, P. Sulforaphane as a potential protective phytochemical against neurodegenerative diseases. *Oxidative Med. Cell. Longev.* **2013**, *2013*, 415078. [CrossRef]
14. Bose, C.; Alves, I.; Singh, P.; Palade, P.T.; Carvalho, E.; Børsheim, E.; Jun, S.R.; Cheema, A.; Boerma, M.; Awasthi, S.; et al. Sulforaphane prevents age-associated cardiac and muscular dysfunction through Nrf2 signaling. *Aging Cell* **2020**, *19*, e13261. [CrossRef]
15. Ma, T.; Zhu, D.; Chen, D.; Zhang, Q.; Dong, H.; Wu, W.; Lu, H.; Wu, G. Sulforaphane, a natural isothiocyanate compound, improves cardiac function and remodeling by inhibiting oxidative stress and inflammation in a rabbit model of chronic heart failure. *Med. Sci. Monit. Int. Med. J. Exp. Clin. Res.* **2018**, *24*, 1473. [CrossRef]
16. Ares, A.M.; Bernal, J.; Martín, M.T.; Bernal, J.L.; Nozal, M.J. Optimized formation, extraction, and determination of sulforaphane in broccoli by liquid chromatography with diode array detection. *Food Anal. Methods* **2014**, *7*, 730–740. [CrossRef]
17. Tian, Q.; Rosselot, R.A.; Schwartz, S.J. Quantitative determination of intact glucosinolates in broccoli, broccoli sprouts, Brussels sprouts, and cauliflower by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry. *Anal. Biochem.* **2005**, *343*, 93–99. [CrossRef]
18. Dueñas, M.; Hernández, T.; Estrella, I.; Fernández, D. Germination as a process to increase the polyphenol content and antioxidant activity of lupin seeds (*Lupinus angustifolius* L.). *Food Chem.* **2009**, *117*, 599–607. [CrossRef]
19. Maldini, M.; Baima, S.; Morelli, G.; Scaccini, C.; Natella, F. A liquid chromatography-mass spectrometry approach to study "glucosinoloma" in broccoli sprouts. *J. Mass Spectrom.* **2012**, *47*, 1198–1206. [CrossRef]
20. Park, W.T.; Kim, J.K.; Park, S.; Lee, S.W.; Li, X.; Kim, Y.B.; Uddin, M.R.; Park, N.I.; Kim, S.J.; Park, S.U. Metabolic profiling of glucosinolates, anthocyanins, carotenoids, and other secondary metabolites in kohlrabi (*Brassica oleracea* var. gongylodes). *J. Agric. Food Chem.* **2012**, *60*, 8111–8116. [CrossRef]
21. Lv, C.; Zhang, Y.; Zou, L.; Sun, J.; Song, X.; Mao, J.; Wu, Y. Simultaneous hydrolysis and extraction increased erucin yield from broccoli seeds. *ACS Omega* **2021**, *6*, 6385–6392. [CrossRef] [PubMed]
22. Moreira-Rodríguez, M.; Nair, V.; Benavides, J.; Cisneros-Zevallos, L.; Jacobo-Velázquez, D.A. UVA, UVB light doses and harvesting time differentially tailor glucosinolate and phenolic profiles in broccoli sprouts. *Molecules* **2017**, *22*, 1065. [CrossRef] [PubMed]
23. Farnham, M.W.; Stephenson, K.K.; Fahey, J.W. Glucoraphanin level in broccoli seed is largely determined by genotype. *HortScience* **2005**, *40*, 50–53. [CrossRef]
24. Gil-Chávez, G.J.; Villa, J.A.; Ayala-Zavala, F.; Heredia, J.B.; Sepulveda, D.; Yahia, E.M.; Gonzalez-Aguilar, G.A. Technologies for extraction and production of bioactive compounds to be used as nutraceuticals and food ingredients: An overview. *Compr. Rev. Food Sci. Food. Saf.* **2013**, *12*, 5–23. [CrossRef]
25. Villarreal-García, D.; Nair, V.; Cisneros-Zevallos, L.; Jacobo-Velázquez, D.A. Plants as biofactories: Postharvest stress-induced accumulation of phenolic compounds and glucosinolates in broccoli subjected to wounding stress and exogenous phytohormones. *Front. Plant Sci.* **2016**, *7*, 45. [CrossRef]
26. Torres-Contreras, A.M.; Nair, V.; Cisneros-Zevallos, L.; Jacobo-Velázquez, D.A. Stability of bioactive compounds in broccoli as affected by cutting styles and storage time. *Molecules* **2017**, *22*, 636. [CrossRef]
27. Vallejo, F.; Tomás-Barberán, F.; García-Viguera, C. Health-promoting compounds in broccoli as influenced by refrigerated transport and retail sale period. *J. Agric. Food Chem.* **2003**, *51*, 3029–3034. [CrossRef]
28. Kushad, M.M.; Cloyd, R.; Babadoost, M. Distribution of glucosinolates in ornamental cabbage and kale cultivars. *Sci. Hortic.* **2004**, *101*, 215–221. [CrossRef]
29. Dillard, C.J.; German, J.B. Phytochemicals: Nutraceuticals and human health. *J. Sci. Food Agric.* **2000**, *80*, 1744–1756. [CrossRef]
30. Charron, C.S.; Saxton, A.M.; Sams, C.E. Relationship of climate and genotype to seasonal variation in the glucosinolate–myrosinase system. I. Glucosinolate content in ten cultivars of *Brassica oleracea* grown in fall and spring seasons. *J. Sci. Food Agric.* **2005**, *85*, 671–681. [CrossRef]
31. Deng, Q.; Zinoviadou, K.G.; Galanakis, C.M.; Orlien, V.; Grimi, N.; Vorobiev, E.; Lebovka, N.; Barba, F.J. The effects of conventional and non-conventional processing on glucosinolates and its derived forms, isothiocyanates: Extraction, degradation, and applications. *Food Eng. Rev.* **2015**, *7*, 357–381. [CrossRef]
32. Hanschen, F.S.; Rohn, S.; Mewis, I.; Schreiner, M.; Kroh, L.W. Influence of the chemical structure on the thermal degradation of the glucosinolates in broccoli sprouts. *Food Chem.* **2012**, *130*, 1–8. [CrossRef]
33. Krumbein, A.; Schonhof, I.; Schreiner, M. Composition and contents of phytochemicals (glucosinolates, carotenoids and chlorophylls) and ascorbic acid in selected *Brassica* species (*B. juncea*, *B. rapa subsp. nipposinica var. chinoleifera*, *B. rapa subsp. chinensis* and *B. rapa subsp. rapa*). *J. Appl. Bot. Food Qual.* **2005**, *79*, 168–174.
34. Rebello, N.L. In vitro screening of the sunscreen potential of hydroalcoholic *Erythrina variegata* bark extract. *Int. J. Green Pharm.* **2016**, *10*, S131–S135.
35. Carro, R.T.; Isla, M.I.; Ríos, J.L.; Giner, R.M.; Alberto, M.R. Anti-inflammatory properties of hydroalcoholic extracts of Argentine Puna plants. *Food Res.* **2015**, *67*, 230–237. [CrossRef]
36. Tao, C.; He, B.B. Isolation of intact glucosinolates from mustard seed meal to increase the sustainability of biodiesel utilization. In Proceedings of the ASAE/CSAE Annual International Meeting, Ottawa, ON, Canada, 1–4 August 2004; pp. 6703–6713.
37. Powell, E.E.; Hill, G.A.; Juurlink, B.H.J.; Carrier, D.J. Glucoraphanin extraction from *Cardaria draba*: Part 1. Optimization of batch extraction. *J. Chem. Technol. Biotechnol. Int. Res. Process. Environ. Clean Technol.* **2005**, *80*, 985–991.

38. Campos, D.; Chirinos, R.; Barreto, O.; Noratto, G.; Pedreschi, R. Optimized methodology for the simultaneous extraction of glucosinolates, phenolic compounds and antioxidant capacity from maca (*Lepidium meyenii*). *Ind. Crops Prod.* **2013**, *49*, 747–754. [CrossRef]
39. Sharma, H.K.; Ingle, S.; Singh, C.; Sarkar, B.C.; Upadhyay, A. Effect of various process treatment conditions on the allyl isothiocyanate extraction rate from mustard meal. *J. Food Sci. Technol.* **2012**, *49*, 368–372. [CrossRef]
40. Pham, H.N.T.; Vuong, Q.V.; Bowyer, M.C.; Scarlett, C.J. Ultrasound-assisted extraction of *Catharanthus roseus* (L.) G. Don (Patricia White cultivar) stem for maximizing saponin yield and antioxidant capacity. *J. Food Process. Preserv.* **2018**, *42*, e13597. [CrossRef]
41. Matuszek, K.; Pankalla, E.; Grymel, A.; Latos, P.; Chrobok, A. Studies on the solubility of terephthalic acid in ionic liquids. *Molecules* **2019**, *25*, 80. [CrossRef]
42. Fahey, J.W.; Zalcmann, A.T.; Talalay, P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* **2001**, *56*, 5–51. [CrossRef]
43. Villalobos, V.V.; Padilla, C.R.; Naccha, J.R.; Bracamonte, O.H.; Angulo, J.V.; Alama, O.B. Optimization of maca (*Lepidium meyenii*) glucosinolates extraction by genetic algorithms and response surface. *Sci. Agropecu.* **2016**, *7*, 275–284. [CrossRef]
44. Akbari, S.; Abdurahman, N.H.; Yunus, R.M.; Fayaz, F. Microwave-assisted extraction of saponin, phenolic and flavonoid compounds from *Trigonella foenum-graecum* seed based on two level factorial design. *J. Appl. Res. Med. Aromat. Plants* **2019**, *14*, 100212. [CrossRef]