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Blanching impact on pigments, glucosinolates, and phenolics of dehydrated broccoli by-products

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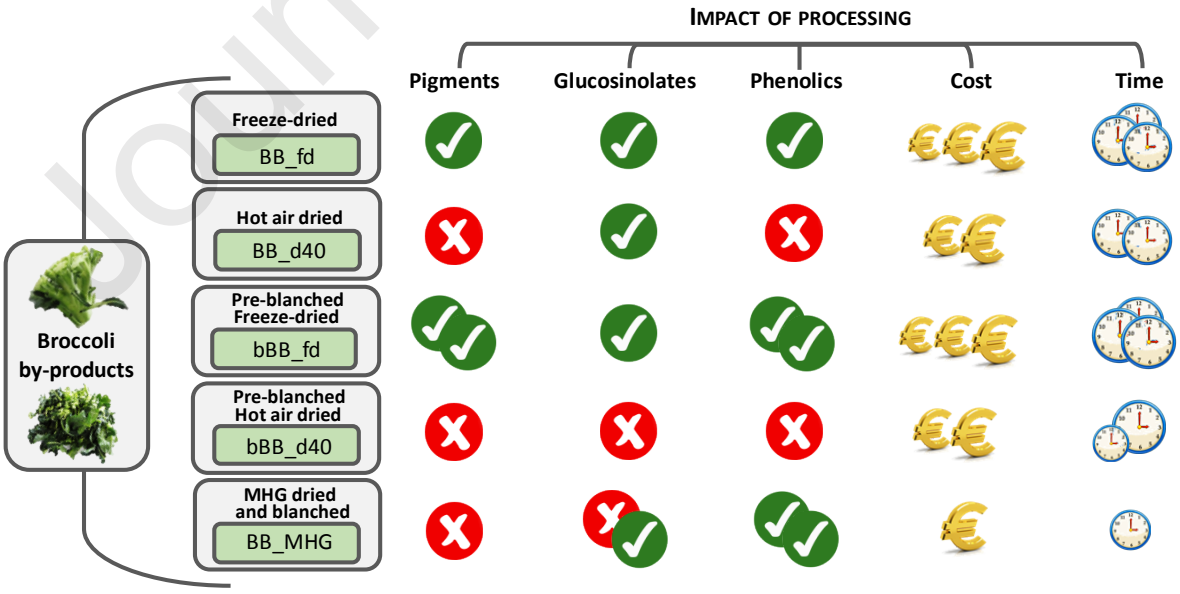
Graphical Abstract:



Highlights

- Broccoli by-products are a source of pigments, glucosinolates, and phenolics.
- Air-drying at 40 ºC retains glucosinolates, 70% of phenolics and 49% of pigments.
- Blanching before freeze-drying improves extractability of pigments and phenolics.
- Blanching before air-drying reduces to less than 50% all compounds.
- Microwave (MHG) dehydration retains glucoraphanin and promotes phenolics extraction.

Abstract

Because of high water content, the valorisation of broccoli by-products requires dehydration that can preserve bioactive compounds. Blanching pre-treatment has been reported to improve the drying rate of broccoli. As a thermal treatment, it promotes also enzyme inactivation. Therefore, in this study, the impact of pre-dehydration blanching step, freeze-drying, air-drying at 40ºC, and microwave hydrodiffusion and gravity (MHG) dehydration on the levels of pigments, glucosinolates, and phenolics, was evaluated by UHPLC-DAD-ESI/MS^n. When compared to freeze-drying, a technique known to preserve compounds, a pre-blanching step increased the extractability of both pigments and phenolics, while air-drying only retained 49% of the pigments and 70% of phenolics, both without affecting glucosinolates. However, when air-drying was preceded by blanching, less than 50% of compounds were retained. On the other hand, MHG dehydration increased the phenolics extractability by 26%, particularly that of kaempferol derivatives while also retaining the amount of the glucosinolate glucoraphanin, when compared to freeze-drying. Nevertheless, only 23% of indole glucosinolates were recovered and pigments were severely reduced, with lutein accounting only for 32% and only chlorophyll *b* was observed in trace amounts after MHG dehydration. Therefore, to valorise broccoli by-products as ingredients, different drying technologies may be used when targeting different composition richness: freeze-drying is suitable for pigments and glucosinolates, air-drying is suitable for glucosinolates, while MHG promotes the extractability of phenolic compounds.

Keywords: *Brassica* by-products; valorisation; microwave hydrodiffusion and gravity; food ingredients; ultra-high efficiency liquid chromatography; glucoraphanin.

1. Introduction

Broccoli frozen-food industry produces stalks, leaves, and inflorescences remains that account for 45% of the initial broccoli heads. These by-products share broccoli nutrients and their bioactive compounds, namely pigments, glucosinolates, and phenolic compounds (Ares, Nozal, & Bernal, 2013; Ferreira, Passos, Cardoso, Wessel, & Coimbra, 2018; Liu, Zhang, Ser, Cumming, & Ku, 2018; Raiola et al., 2017; Thomas, Badr, Desjardins, Gosselin, & Angers, 2018), which, due to their wide applications in industries, could make them valuable. However, due to the high moisture content, the valorisation of broccoli by-products as functional food ingredients requires their stabilization and/or processing to inhibit enzymes and prevent microbial growth that result in the degradation of the products (Oliviero, Verkerk, & Dekker, 2013). In this regard, stabilization by dehydration is a well-known strategy to overcome these drawbacks, also allowing the storage for long periods and the reduction of shipping weights.

For high quality products, freeze-drying is the dehydration technique of choice because it combines low temperature and pressure to sublimate water (Duan, 2017), preserving cell structure when fast freezing methods are employed (Jha, Xanthakis, Chevallier, Jury, & Le-Bail, 2018). Still, due to the high energy demand of freeze-drying (Duan, 2017), air-drying has been the most applied technique for drying broccoli (Doymaz & Sahin, 2016; Jin, Sman, & van Boxtel, 2011; Mrkic, Cocci, Rosa, & Sacchetti, 2006; Mrkic, Redovnikovic, Jolic, Delonga, & Dragovic-Uzelac, 2010; Xu, Jin, Zhang, & Chen, 2017). Nevertheless, regardless its convenience, depending on the temperature (20ºC to 100ºC), air-drying may promote the degradation of some broccoli bioactive compounds, namely indole glucosinolates (Mrkic et al., 2010), ascorbic acid (Jin et al., 2011; Mrkic et al., 2006), and pigments (Zhang et al., 2018). Reduction of dehydration temperature and/or time were shown to partially preserve the contents of phenolic compounds and antioxidant

activity of broccoli. These conditions can be achieved by increasing air flow-rate (Mrkic et al., 2006), by combining air-drying with zeolites to absorb the released water (Oliviero et al., 2013), air-drying with microwaves to promote fast heating and simultaneous blanching effect (Salim, Gariépy, & Raghavan, 2017; Vadivambal & Jayas, 2007), or by microwave hydrodiffusion and gravity (MHG) to dehydrate, while having a blanching effect, and simultaneously extracting water soluble bioactive compounds (Ferreira et al., 2018). The negative impact of dehydration on broccoli active compounds can also be minimized by a blanching pre-treatment, which is reported to improve the drying rate (Doymaz & Sahin, 2016), and inactivate lipoxygenase (Morales-Blancas, Chandia, & Cisneros-Zevallos, 2002), myrosinase (Oliviero et al., 2013; Rungapamestry, Duncan, Fuller, & Ratcliffe, 2008), and peroxidase (Gonçalves et al., 2009), i.e, the enzymes related with the deterioration of carotenoids, glucosinolates, and phenolic compounds, respectively.

This work aimed to evaluate the impact of dehydration and blanching on broccoli by-products bioactive compounds, namely pigments, glucosinolates, and phenolic compounds, making use of oven-drying and MGH technologies, combined with or without a blanching pre-treatment.

2. Material and methods

2.1. Broccoli by-products and chemicals

Two batches of broccoli by-products were used. The first batch consisted of non-blanched stalks and leaves from fresh broccoli heads (harvested in March 2018). These by-products represented 31% of broccoli head. The second batch of broccoli by-products comprised a mixture of stalks, leaves, and inflorescence remains provided by Monliz SA, Portugal, in January 2015 (Ferreira et al. 2018). These by-products represented 45% of

broccoli head and were obtained along the freezing line in the industrial process and after the thermal blanching step (33% of by-product).

All reagents used were of analytical grade or higher available purity.

2.2. Dehydration and blanching of broccoli by-products

To evaluate the effect of dehydration and blanching, broccoli by-products (BB) from the first batch were divided in two groups (**Figure 1**). One group (300 g) was not submitted to blanching, while other was blanched in a domestic microwave, which consisted of three treatments at 800 W for 2 min each, with intercalated mixing, according to Silva et al. (2013). A part of the non-blanched group (BB) and of the blanched broccoli by-products group (bBB) was air-dried at 40ºC (VENTI-Line, VL-115, VWR) until constant weight (64 h for BB_d40 and 40 h for bBB_d40, respectively). Alternatively, other part of BB and bBB was frozen with liquid nitrogen and freeze-dried at -45 to -50ºC and less than 150 mTorr (benchtop K, VirTis with Vacuumbrand pump) for one week (samples BB_fd1 and bBB_fd, respectively).

To evaluate the effect of simultaneous blanching and dehydration, the second batch of broccoli by-products was dehydrated by a new technique, MHG (BB_MHG, 10 min bellow 100ºC, and 35 min at 100ºC) or alternatively, it was freeze-dried (BB_fd2, **Figure 1**), as described by Ferreira et al. (2018).

All dehydrated broccoli by-products (BB_d40, bBB_d40, BB_fd1, bBB_fd, BB_fd2, and BB_MHG) were placed in the dark. Before analysis, the samples were milled in a cooled analytical grinder (Yellowline, A10 IKA).

2.3. Pigments characterization

Pigments were analysed by ultra-high-performance liquid chromatography (UHPLC), using a photodiode-array detector (DAD) coupled with electrospray

ionization/tandem mass spectrometry detection (ESI/MS^n) after extraction according to Guzman, Yousef, & Brown (2012), with some modifications. Dehydrated broccoli by-products (0.2 g) were extracted with 5 mL of 0.1% butylated hydroxytoluene in pure ethanol, for 24 h under agitation, at room temperature, in the dark. After each extraction, the supernatants were separated by gravity settling, filtered through a 0.2 μm filter (cellulose acetate, 30 mm syringe filters, imChem, France) into an amber vial, and stored at −20°C until injection. Triplicate extractions were performed for each sample.

UHPLC-DAD-ESI/MS analyses were carried out on an Ultimate 3000 (Dionex Co., San Jose, CA, USA) apparatus equipped with an autosampler, a quaternary pump, an ultimate 3000 Diode Array Detector (Dionex Co., San Jose, CA, USA), and an automatic thermostatic column compartment (Silva, Abreu, Silva, & Cardoso, 2019). It was coupled to an ion trap MS equipped with an ESI source (Thermo LTQ XL MS, Thermo Scientific, San Jose, CA, USA). Control and data acquisition were carried out with the Thermo Xcalibur Qual Browser data system (Thermo Scientific, San Jose, CA, USA). Nitrogen above 99% purity was used, and the gas pressure was 520 kPa (75 psi). The instrument was operated in positive-ion mode and the full scan covered the mass range from *m/z* 100 to 2000. ESI needle voltage was set at 4.80 kV and an ESI capillary temperature of 275ºC.

The compounds were separated using a Hypersil GOLD C18 column (100 mm length; 2.1 mm i.d.; 1.9 μm particle diameter, end-capped from Thermo Scientific, USA), at 30ºC. Gradient elution was carried out with a mixture of 0.1% (v/v) formic acid in water (solvent A) and 30% (v/v) methanol in acetonitrile (solvent B), with a flow rate of 0.200 mL/min in a linear gradient. The solvent gradient started with 5% of solvent B, reaching 40% at 14 min, 100% at 16 min, and being maintained for two minutes before returning to the initial conditions at 20 min. UV-Vis spectral data for all peaks were accumulated in the range 200–700 nm, while the chromatographic profiles were recorded at 450 and 655

nm for carotenoids (xanthophylls and carotenes) and chlorophylls analysis, respectively. The identification of pigments was performed by comparison of retention times, absorption spectra, and MS data with standards and literature. Quantification was performed by peak integration, using the external standard method, with the exact (chlorophyll a, chlorophyll b, lutein, β-carotene and fucoxanthin) or structurally-related standard compounds (chlorophyll b in the case of other chlorophylls and lutein in the case of other xanthophylls (**Supplementary material**, **Table 2S**).

2.4. Glucosinolates and phenolic compounds characterization

Phenolic compounds and intact glucosinolates were simultaneously analysed by UHPLC-DAD-ESI/MS^n after extraction of 100 mg of dehydrated broccoli by-products in 3 mL of 70% methanol for 30 min under stirring at 70ºC, in the dark, according to Domínguez-Perles, et al. (2010). After each extraction, the supernatants were cooled down in a cool water bath, separated by gravity, filtered through a 0.2 μm filter into an amber vial, and stored at −20°C until injection. Triplicate extractions were performed for each sample.

The chromatographic apparatus used was the same as described for pigments characterization. The instrument was operated in negative-ion mode and the full scan covered the mass range from *m/z* 100 to 2000. ESI needle voltage was set at 5.00 kV and an ESI capillary temperature of 275ºC. CID–MS/MS and MS^n experiments were simultaneously acquired for precursor ions using helium as the collision gas with collision energy of 25–35 arbitrary units.

Gradient elution was carried out with a mixture of 0.1% (v/v) formic acid in water (solvent A) and acetonitrile (solvent B), with a flow rate of 0.200 mL/min in a linear gradient. The solvent gradient started with 5% of solvent B, reaching 40% at 14 min, 100% at 16 min, maintained for two minutes before returning to the initial conditions. UV-Vis

spectral data for all peaks were accumulated in the range 190–700 nm, while the chromatographic profiles were recorded at 227 nm for glucosinolates and 280 and 320 nm or 340 nm for phenolic compounds. The identification of glucosinolates and phenolic compounds was performed by comparison of retention times, absorption spectra, and MS data with standards and literature. Glucosinolates were quantified using sinigrin (Sigma-Aldrich, Germany) and response factors according Buchner, R. (1987) (**Supplementary material, Table 3S**). Quantification of phenolic compounds was performed by peak integration, using the external standard method, with structurally-related standard compounds (**Supplementary material, Table 4S**). Esters of hydroxycinnamic acids with gentiobiose and esters of hydroxycinnamic acids with quinic acid were quantified as 5-*O*-caffeoylquinic acid; quercetin derivatives were quantified as quercetin-7-*O*-galactoside; and kaempferol glucosides, monoacylated kaempferol glucosides and diacylated kaempferol glucosides were quantified as kaempferol.

2.5. Estimation of energy consumption

Energy consumption was measured or estimated for all processing techniques to compare their efficiencies. Energy consumption of oven was quantified with power meter (DANIU, Intertek). Energy consumption during microwave blanching was calculated by the input of microwave power with time and energy consumption during MHG dehydration was estimated according López-Hortas, et al. (2019) data. For estimation of freeze-drying energy consumption, steps of freezing, sublimation and vacuum pump were considered, summing the energetic consumption of all equipment.

2.6. Statistical analysis and principal component analysis

Differences among dehydrated products from the first batch (BB_fd1, BB_d40, bBB_fd, and bBB_d40) and from the second batch (BB_fd2 and BB_MHG) were analysed through One-Way ANOVA followed by Tukey's multiple comparisons tests and unpaired Student's t-test, respectively, in GraphPad prism, Trial Version 6.01 (GraphPad Software, Inc. La Jolla, CA, USA). Differences were deemed significant at p-value<0.001.

Principal component analysis (PCA) was applied to all compounds identified (pigments, glucosinolates, and phenolic compounds) using the values of µg/g of dry weight. The data matrix comprised 6 samples of dehydrated broccoli by-products (3 repetitions) and 64 compounds, thus given a matrix with 18 columns and 64 rows. The data matrix was analysed using MetaboAnalyst (https://www.metaboanalyst.ca) (Chong, Wishart, & Xia, 2019). Data were normalized after auto scaling: mean-centring and division by the standard deviation of each variable.

3. Results and discussion

To evaluate the impact of dehydration and blanching on pigments, glucosinolates, and phenolic compounds, broccoli by-products from batch 1 were freeze-dried (BB_fd1 and bBB_fd) or air-dried at 40ºC (BB_d40 and bBB_d40), in the absence or presence of a blanching pre-treatment (bBB_fd and bBB_d40). In addition, to assess the effect of simultaneous blanching and dehydration, broccoli by-products (batch 2) were either dehydrated by the new technology MHG (BB_MHG) or freeze-dried (BB_fd2). Note that freeze-drying was used for comparison purposed as this is the elected dehydration technique to preserve compounds and the cells structure (Duan, 2017; Jha et al., 2018). The potential of each processing technique to dehydrate and valorise broccoli by-products was studied after estimation of energy consumption and by means of PCA.

3.1. Impact of dehydration and blanching on pigments

The first batch of broccoli by-products (BB_fd1) was much richer in pigments (8.26 mg/g dry weight, dw) than the second (BB_fd2, 0.206 mg/g dw) (**Table 1**), and differences are probably attributed to a multitude of factors, including the different agronomic operations, cultivars, edaphoclimatic conditions, and previous processing (Björkman et al., 2011; Neugart et al., 2018). In this regard, one must highlight that the first batch was obtained in March as fresh broccoli and promptly cut, while the second batch was provided by the industry in December, after cutting, washing, thermal processing, and/or cooling, which could affect this kind of compounds (Cai et al., 2016). Nevertheless, in general, the same pigment derivatives were found in the two batches (**Table 1**), as depicted in bellow for bBB_fd sample (**Figure 2**).

3.1.1. Carotenoids

The main carotenoid found in the first batch of broccoli by-products was β-carotene (up to 1.54 mg/g dw). This amount is comparable to the values reported for *Brassica* leaves (Lefsrud, Kopsell, Wenzel, & Sheehan, 2007; Müller, 1997), but much higher than previously reported values for broccoli inflorescences (Reis et al., 2015; Guzman et al., 2012; Zhang et al., 2018) and broccoli sprouts (Luo et al., 2019). Lutein was the main xanthophyll (up to 904 µg/g dw), but neoxanthin and violaxanthin, luteoxanthin- and lutein-like structures were also found in moderate amounts (105 µg/g dw, 12.1 µg/g dw, 69.4 µg/g dw, 62.8 µg/g dw, respectively). As for β-carotene , the content of lutein herein found is comparable to literature values reported for *Brassica* leaves (Lefsrud et al., 2007) and higher than those described for processed broccoli by-products (Liu et al., 2018). In the second batch, carotenoids were about 50 folds lower than in the first batch and

opposing to the last, lutein was its major carotenoid, representing about 65% of the total carotenoids.

Air-drying at 40ºC reduced by 43% the amount of carotenoids recovered from broccoli by-products, when compared to freeze-drying (BB_d40 vs BB_fd1, **Table 1**). These results are consistent with literature data. In fact, air-drying of broccoli inflorescences was previously shown to reduce about 62% the contents of the main carotenoids (lutein and β-carotene), independently of dehydration temperature (60-80ºC), due to cell wall disruption and further degradation of these compounds along dehydration (Zhang et al., 2018). On the other hand, our results showed that the application of a blanching pre-treatment before air-drying, which reduced the time of dehydration of the by-products by about 40%, had no significant effect on the amounts of carotenoids (bBB_d40 vs BB_d40). Nevertheless, in the freeze-dried products, this blanching step promoted the increase of carotenoids extraction (67%), suggesting that plant cell disruption promoted by the blanching step only improves extractability (Reis et al., 2015; Murador, da Cunha, & de Rosso, 2014; Oliveira, Brandão, & Silva, 2016; Podsędek, 2007) when softer dehydration technique, i.e. freeze-drying, is used (Duan, 2017; Jha et al., 2018). MHG dehydration, which was used in the second batch, reduced the carotenoids from 31 µg/g dw to 16 µg/g dw (BB_fd2 vs BB_MHG). Regardless of whether the total quantities were lower than those observed in the first batch, the carotenoid loss caused by this treatment was in the same range as that observed in lower temperature (40ºC) air-dried samples.

3.1.2. Chlorophylls

The main chlorophylls identified in broccoli by-products were chlorophylls *a* (up to 3.57 mg/g dw) and *b* (up to 0.766 mg/g dw). Chlorophyll derived compounds were also

found, namely pheophytins *a* and *b*, pyropheophytin *a*, pheophorbide *a*, pyropheophorbide *a*, chlorophyllides *a* and *b*, and pheophytin *a* allomer. These compounds were naturally present (Luo et al., 2019) or could have been produced by the acidic conditions promoted by cellular lysis, by the temperature of blanching and/or dehydration used (Gonçalves et al., 2009), or by the light exposure (Yasuda, Oda, Ueda, & Tabata, 2019). The ratio of chlorophylls *a* to *b* was 4.8, which is in accordance with broccoli head values (Guzman et al., 2012). Total chlorophylls *a* and *b* were also in accordance with the data observed for broccoli by-products: 4.62 mg/g dw and 0.80 mg/g dw, respectively (Liu et al., 2018).

Air-drying reduced the amount of chlorophylls by 54% when comparing to freeze-drying but kept the same chlorophylls constituents. The sequential application of blanching and air-drying reduced even further the total chlorophylls contents (by 60%). Nevertheless, in the freeze-dried products, this blanching step promoted the increase of chlorophylls *a* and *b* extraction (8%), as observed by other authors (Cai et al., 2016). Moreover, the blanching step promoted the formation of chlorophylls derived compounds, mainly by the loss of Mg^{2+} (demetallization) of chlorophyll *a* into pheophytin *a* and, in lower extend, of chlorophyll *b* into pheophytin *b*, as observed for broccoli florets blanched before frozen (Murcia, López-Ayerra, Martínez-Tomé, & García-Carmona, 2000). Other chlorophylls derived compounds were observed after blanching, namely pyropheophytin *a*, pheophorbide *a*, chlorophyllides *a* and *b*, and pyrophaeophorbide *a*, which formation has been associated to thermal processing (Ferruzzi & Blakeslee, 2007; Schelbert et al., 2009). In particular, the formation of pheophorbide occurred after breadmaking of doughs with incorporated fresh plant material from *Brassica* species (Klopsch et al., 2019).

After MHG dehydration, total chlorophylls amount was not significantly different from freeze-dried by-product. Nevertheless, chlorophyll *b* was reduced to traces and chlorophyll *a* was not detected, in accordance with favourable degradation of chlorophyll

a into pheophytin *a* and other derived compounds (Canjura, Schwartz, & Nunes, 1991; Murcia, et al., 2000). Pheophytins *a* and *b* were the main chlorophylls, followed by pyropheophytin *a* and pheophorbide *a*, which have been associated to light brown color (Gauthier-Jaques, Bortlik, Hau, & Fay, 2001), in accordance with the color of the samples obtained. On the other hand, chlorophylls reduction was also due to hydrodiffusion of these compounds, as collected extracts were green coloured (Ferreira et al., 2018).

3.2. Impact of dehydration and blanching on glucosinolates

Glucosinolates were analysed after 70% methanol extraction and UHPLC-DAD-ESI/MS^n analysis using an elution program that allowed to identify both glucosinolates (**Figure 3**) and phenolic compounds (**Figure 4**). In opposition to pigments, the first batch of broccoli by-products presented lower amounts of total glucosinolates than the second (5.00 mg/g dw and 7.85 mg/g dw, respectively). Differences between glucosinolates and pigments could be attributed agronomic operations, cultivars, edaphoclimatic conditions, and previous processing (Alanís-Garza, Becerra-Moreno, Mora-Nieves, Mora-Mora, & Jacobo-Velázquez, 2015; Bell & Wagstaff, 2017).

Four glucosinolates were identified: glucoraphanin, an aliphatic compound, and 3 indoles (glucobrassicin, 4-methoxyglucobrassicin, and neoglucobrassicin) (**Table 2**). Glucoraphanin was the main glucosinolate, representing 74% and 48% of total glucosinolates in the first and second batches, respectively. The profile of glucosinolates in these broccoli by-products was similar to that observed for broccoli stalks, leaves, and inflorescences by-products (3 to 15 mg/g dw) (Dominguez-Perles, Moreno, Carvajal, & Garcia-Viguera, 2011; Liu et al., 2018) and broccoli heads (Bell & Wagstaff, 2017; Latté, Appel, & Lampen, 2011).

Air-drying at 40ºC did not impact significantly the total amount of glucosinolates. However, when broccoli by-products were blanched before air-drying, levels of total

glucosinolates decreased by 49%. Glucobrassicin and 4-methoxyglucobrassicin were the most affected ones, decreasing by 61 and 64%, respectively. As observed for pigments, promotion of cell disruption by blanching (Alanís-Garza et al., 2015) led to glucosinolates degradation at 40ºC, owing to non-enzymatic degradation (Hanschen et al., 2012), and/or residual myrosinase activity due to partial blanching inactivation (Oliviero et al., 2013), responsible for the degradation of glucosinolates into isothiocyanates.

MHG dehydration reduced to 23% the amount of total glucosinolates. Glucoraphanin amount was not affected by this dehydration, but indole glucosinolates glucobrassicin, 4-methoxyglucobrassicin, and neoglucobrassicina were reduced by 85, 75, and 65%, respectively, in accordance with the observed in other dehydration processes at high temperatures (Lafarga, Bobo, Viñas, Collazo, & Aguiló-Aguayo, 2018; Mrkic et al., 2010; Tabart, Pincemail, Kevers, Defraigne, & Dommes, 2018). As previously demonstrated by our group, from the total glucosinolates initially accounted in freeze-dried by-products, 50% were lost due to degradation along MHG dehydration, while the remaining were hydrodiffused and recovered in the aqueous extracts (Ferreira et al., 2018).

3.3. Impact of dehydration and blanching on phenolic compounds

The first batch of broccoli by-products presented lower amounts of total phenolic compounds than the second batch (2.20 mg/g dw and 10.6 mg/g dw, respectively), thus following a similar tendency to glucosinolates and contrasting with that observed for pigments. The main phenolic compounds found in broccoli by-products comprised glycosylated flavonoids (quercetin and kaempferol), either non-esterified or esterified with one or two hydroxycinnamic acids (caffeic, methoxycaffeic, sinapic, ferulic, and coumaric acids), as well as hydroxycinnamic acids esterified with carbohydrates (gentiobiose) or with quinic acid, which overall could be divided in six groups (**Figure 4**, **Table 3**).

Notably, the 42 compounds identified were previously reported to occur in broccoli heads (Cartea, Francisco, Soengas, & Velasco, 2011; Vallejo, Tomás-Barberán, & Ferreres, 2004), broccoli sprouts (Sun et al., 2013), and other *Brassica* (Fiol et al., 2012; Francisco et al., 2009; Llorach, Gil-Izquierdo, Ferreres, & Tomás-Barberán, 2003). However, the exception is for the isomers with parent ions at *m/z* 807 and 817 and the compound with parent ion at *m/z* 563, which were herein tentatively assigned to kaempferol derivatives, according to their elution order, absorbance spectra and MS-MS data. As observed in **Table 3**, albeit both batches of broccoli contained the same phenolic compounds, their specific concentrations were variable between them, a fact that has been reported before and accepted to depend on plant part and environmental factors (Cartea et al., 2011).

Air-drying reduced the amount of total phenolics by 30%. Individually, 19 phenolic compounds had a reduction in their abundance (17% to 79%), 19 were not significantly affected, while 4 of them increased their availability with air-drying, namely kaempferol-3-caffeoylsinapoylsophorotrioside-7-sophoroside (47%), kaempferol-3-methoxycaffeoylsinapoylsophorotrioside-7-glucoside (47%), 1,2,2′-trisynapoylgentiobioside (27%), and sinapoyldiferuloylgentiobiose (23%). To our knowledge, this is the first report of air-drying impact on each phenolic compound of *Brassica*. It was only reported the impact of air-drying (50-100ºC) on kaempferol, when analysed by HPLC as aglycon after hydrolysis of the parent glycosides, showing contrasting results as kaempferol increased by 12% to 22% after air-drying (Mrkic et al., 2006). However, these authors also observed that total polyphenols decreased by 13% to 56% when analysed after reaction with Folin–Ciocalteau reagent, a colorimetric assay, indicating the reduction of antioxidant compounds in general, including the most reactive phenolic compounds.

The sequential blanching and freeze-drying of broccoli by-products resulted in an incremented recovery of phenolic compounds (51%), compared to freeze-dried by-products. Despite that, two monoacylated kaempferol glucosides, namely kaempferol-3-sinapoylsophorotrioside-7-sophoroside (33%) and kaempferol-3-sinapoylsophorotrioside-7-glucoside (72%), were significantly reduced. The increase of total phenolic content was already observed for broccoli subjected to blanching (Alanís-Garza et al., 2015; Cai et al., 2016), although also without individual identification of phenolic compounds. Moreover, this increase was hypothesized as a result of cell lysis (Cai et al., 2016) with release of bound phenolics and consequent promotion of their extraction (Gonzales et al., 2015; Harbaum, Hubbermann, Zhu, & Schwarz, 2008). However, when blanching preceded air-drying, the amount of recovered phenolic compounds decreased by 71%. In this case, only kaempferol-3-sinapoylsophorotrioside-7-glucoside was not affected by the dehydration process. These results showed that, for phenolics preservation, air-drying did not benefit from blanching pre-treatment, possibly due to the promotion of oxidation processes.

Similarly to what was observed when blanching preceded freeze-drying, MHG dehydration increased the amount of recovered phenolic compounds (26%). However, the impact of MHG dehydration was different for each group of compounds. Among esters of hydroxycinnamic acids and quinic acid, feruloylquinic acid isomers increased 2.2 folds by increase of isomer 5-feruloylquinic acid from trace amounts. Regarding kaempferol derivatives, kaempferol glucosides increased 74% and kaempferol glucosides esterified with hydroxycinnamic acids increased 3 folds. These results can be explained by the higher stability of kaempferol derivatives to thermal treatments (Wu, Zhao, Haytowitz, Chen, & Pehrsson, 2019). On the other hand, esters of hydroxycinnamic acids and gentiobiose, which represent 59% of total phenolics, decreased by about 17%. It should

be noted that the decrease of these compounds after drying cannot be associated only to degradation, but also to their hydrodiffusion to the aqueous extracts recovered during MHG drying, where these compounds were detected (**Supplementary Material Figure 1S and Table 1S**).

3.4. Impact of dehydration and blanching techniques on broccoli by-products valorisation

The potential of each processing technique to dehydrate and valorise broccoli by-products was studied after estimation of energy consumption and by means of principal component analysis (PCA). As observed in **Table 4**, MHG dehydration was the faster processing technique and the one that spent less energy (1350 J) to dehydrate broccoli by-products. Oven air-drying needed 53 to 83 more time and 32 to 51 more energy than MHG dehydration, depending on the application or the absence of a blanching pre-treatment, respectively. Freeze-drying was the most time and energy consuming technique. The scale up of these technologies to industrial level can be adopted according the composition needed, this is, the amount of pigments, glucosinolates, and phenolics. However, it should be reminded that presently the biggest MHG apparatus only allow to process a volume of broccoli by-products of 70 L (MAC70) (Périno et al., 2016).

PCA was applied to all dehydrated samples to determine the main sources of variability based on samples pigments, glucosinolates, and phenolic compounds. **Figure 5.a** shows the scores plot (PC1 x PC2) of the data provided where six groups were obtained. These two axes contain 88.8% of the total variance (PC1 =77.4% and PC2 = 11.4%). According to the loadings plot (**Figure 5.b**) samples with lower amount of compounds were separated from samples with high amount of compounds by PC1. Blanched samples (bBB_fd; bBB_d40; and BB_MHG) were separated from non-blanched samples (BB_fd1; BB_d40; and BB_fd2) by PC2, mainly due to chlorophyll derivatives

contribution. These results corroborate that the different processing techniques allows to obtain dehydrated broccoli by-products with different characteristics and the processing techniques should be chosen according both final composition required and environmental concern (energy and time expenditures).

4. Concluding remarks

Dehydrated broccoli by-products were shown to represent a source of pigments, including carotenoids (16 to 2689 µg/g dw) and chlorophylls (139 to 5569 µg/g dw), glucosinolates (2502 to 7846 µg/g dw), and phenolic compounds (2203 to 15987 µg/g dw). Depending on the blanching pre-treatment and dehydration process applied, products with different profiles were obtained.

Air-drying at 40ºC did not impact glucosinolates but only retained 49% of the pigments and 70% of phenolics. Air-drying benefited from a previously blanching step, by reduction of dehydration time. However, this step also promoted higher degradation of compounds along air-drying. MHG dehydration preserved the glucosinolate glucoraphanin, increased the total amount of phenolic compounds by 26%, with special contribution of kaempferol glucosides, but had a negative impact on pigments by reducing the content of lutein and promoting the degradation of chlorophylls *a* and *b* into their derivatives. In comparison with freeze-drying, both air-drying and MHG dehydration technologies impacted pigments, glucosinolates, and phenolics at different extents. Nonetheless, these technologies provide benefits by stabilizing by dehydration broccoli by-products in a more energetically friendly way, making them ready to be milled and used to prepare flours, as potential food ingredients, promoting broccoli by-products valorisation.

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FIGURES

Figure 1. Scheme of broccoli by-products processing.

Figure 2. Representative chromatograms from 1-25 min at 450 nm and 655 nm, from the first batch of broccoli by-products after freeze-drying with a pre-blanching step (bBB_fd). The numbers in the chromatograms correspond to the UHPLC-DAD-ESI-MS peaks described in Table 1.

Figure 3. Representative chromatogram from 1.5-12 min at 227 nm (for glucosinolates detection), from the first batch of broccoli by-products after freeze-drying with a pre-blanching step (bBB_fd). Greek letters (glucosinolates) and numbers (phenolic compounds) in the chromatogram correspond to the UHPLC-DAD-ESI-MS^n peaks described in Table 2 and 3, respectively.

Figure 4. Representative chromatogram from a) 1–25 min of phenolic compounds (at 320 nm), from the first batch of broccoli by-products after freeze-drying with a pre-blanching step (bBB_fd). b) Inset shows expanded region of the chromatogram with retention time of 4–16 min. The numbers in the chromatogram correspond to the UHPLC-DAD-ESI-MS^n peaks described in Table 3.

Figure 5. PCA of the pigments, glucosinolates and phenolic compounds from dehydrated broccoli by-products: **a)** scores scatter plot (PC1 vs PC2) and **b)** PC1 vs PC2 loadings.

Figure 1

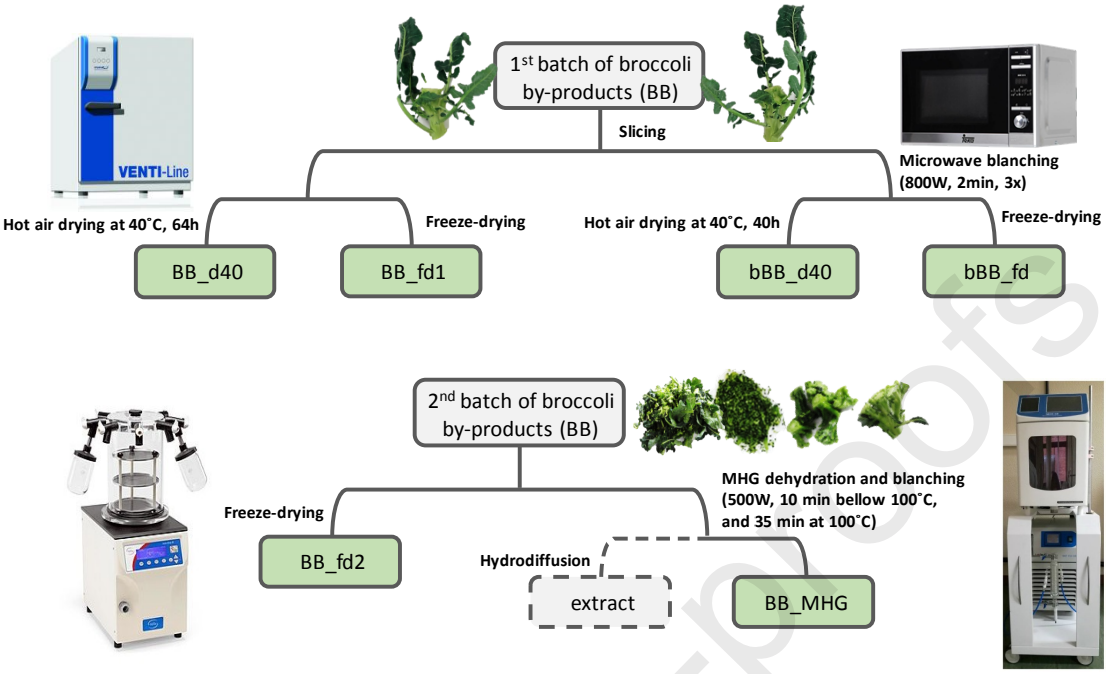


Figure 2

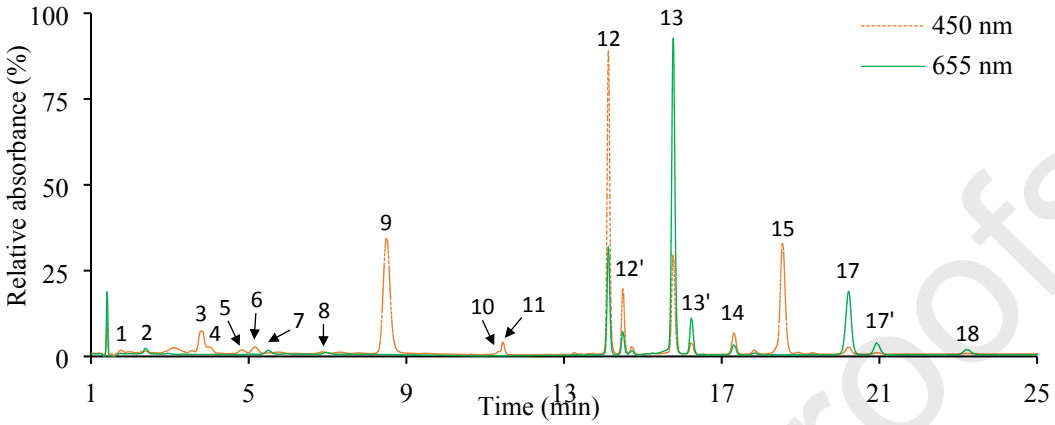


Figure 3

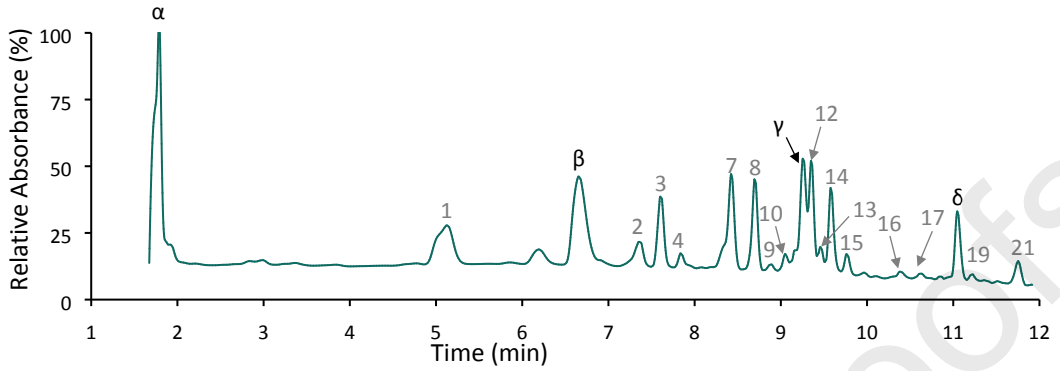


Figure 4

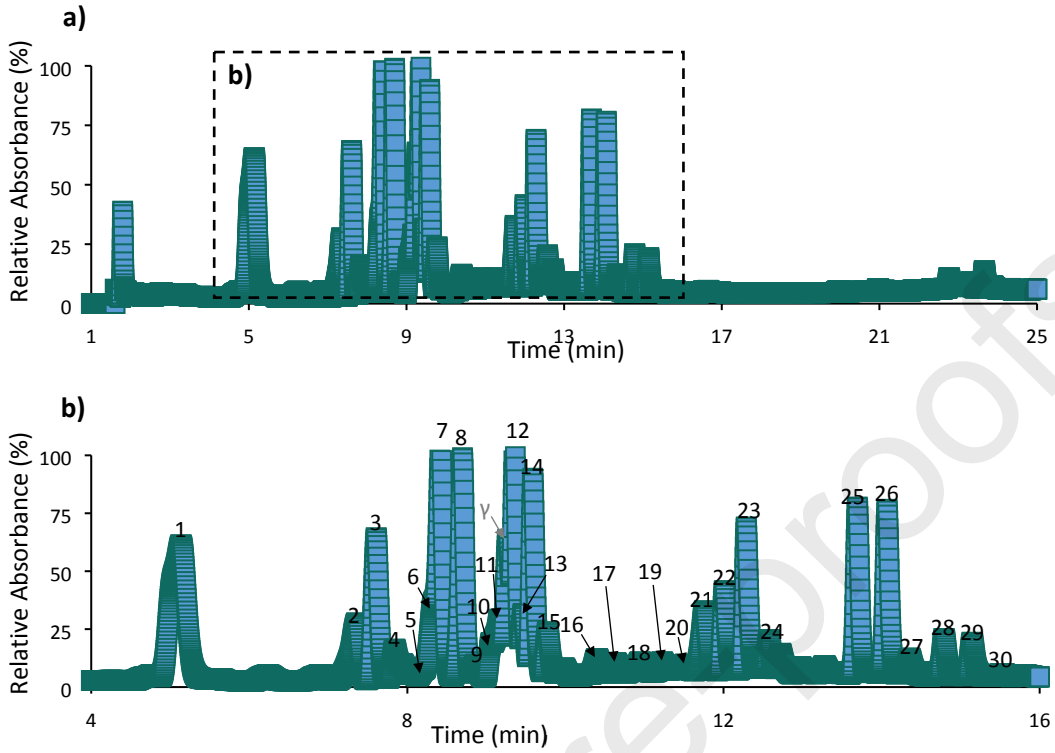
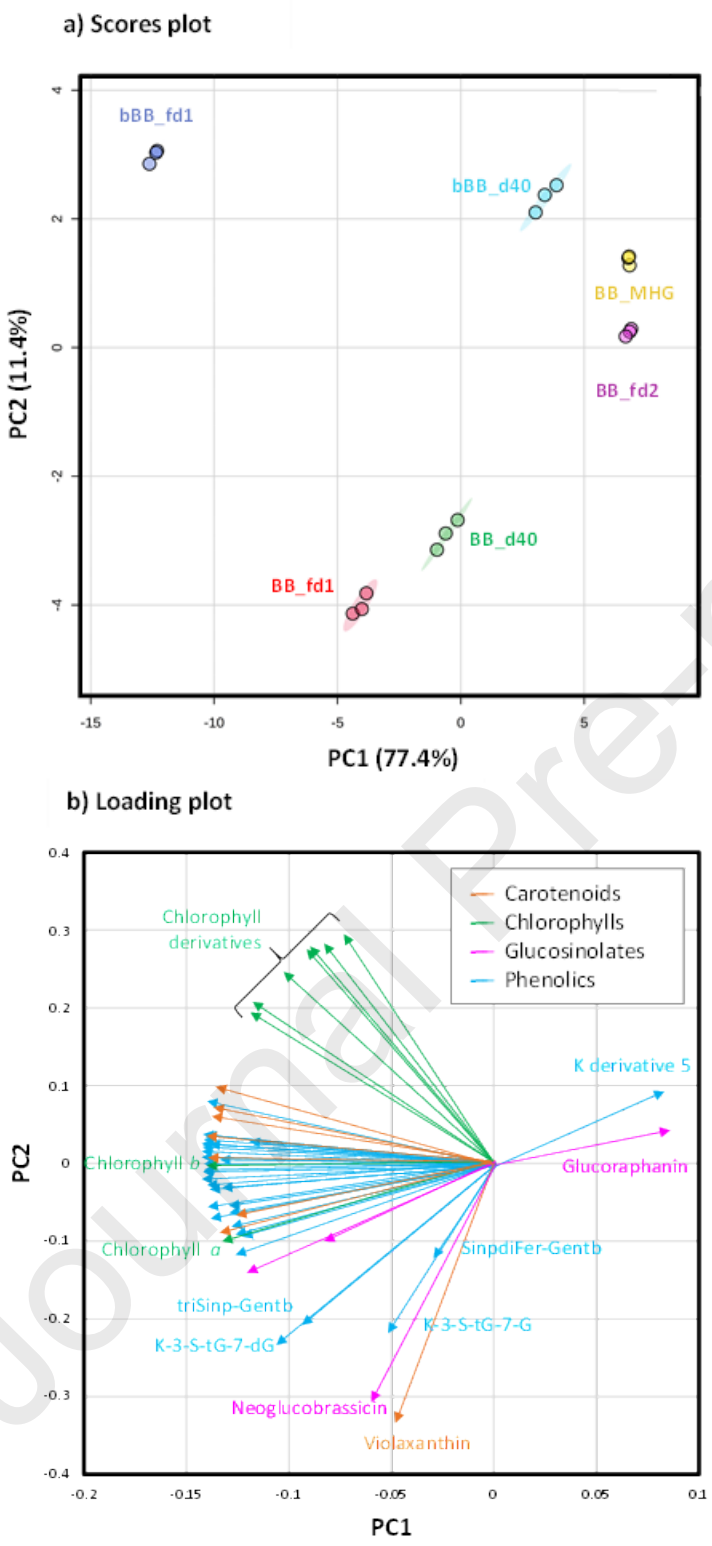


Figure 5



TABLES

Table 1. Identification and quantification of pigments from dehydrated broccoli by-products analysed by UHPLC-DAD-ESI-MS.

Table 2. Identification and quantification of glucosinolates from dehydrated broccoli by-products analysed by UHPLC-DAD-ESI-MSn.

Table 3. Identification and quantification of phenolic compounds from dehydrated broccoli by-products analysed by UHPLC-DAD-ESI-MSn.

Table 4. Energy consumption for each processing technique used to dehydrate broccoli by-products.

Table 1

P	RT (min)	$[M+H]^+$	λmax	Probable Compound	Batch 1				Batch 2	
					BB_fd1	BB_d40	bBB_fd	bBB_d40	BB_fd2	BB_MHG
1	1.8	630	461, 601, 648	Chlorophyllide b	-	-	0.3 ± 0.0	tr	tr	-
2	2.3	616	415sh, 430, 590, 619, 663	Chlorophyllide a	tr	7.0 ± 0.1^{d}	42.4 ± 0.1^{a}	9.35 ± 0.24^{c}	tr	tr
3	3.8	602	414, 437, 465	Neoxanthin	98.2 ± 0.1^{b}	55.3 ± 0.0^{d}	104.6 ± 0.9^{a}	39.3 ± 0.2^{e}	2.7 ± 0.0′	1.8 ± 0.0″
4	4.1	602	417,439,469	Violaxanthin	146.0 ± 0.0^{a}	76.1 ± 0.0^{b}	12.1 ± 0.2^{c}	4.7 ± 0.0^{d}	2.5 ± 0.0′	2.3 ± 0.0′
5	4.9	602	399, 422, 448	Luteoxanthin alike	11.0 ± 0.0^{c}	7.7 ± 0.0^{e}	26.1 ± 0.1^{a}	8.2 ± 0.0^{d}	-	-
6	5.2	602	399, 422, 448	Luteoxanthin alike	14.9 ± 0.0^{c}	11.3 ± 0.0^{d}	43.3 ± 0.7^{a}	11.0 ± 0.1^{d}	-	-
7*	5.5 & 5.9	594	407, 506, 536, 608, 664	Pheophorbide a	-	tr	49.9 ± 0.1^{c}	38.5 ± 0.1^{c}	3.0 ± 0.5′	5.8 ± 0.6′
8	6.9	536	405, 507, 538, 606, 665	Pyrophaeophorbide a	-	tr	20.7 ± 0.1^{b}	10.7 ± 0.0^{c}	4.0 ± 0.1′	1.9 ± 0.3″
9	8.5	569	423sh, 446, 473	Lutein	503.3 ± 1.1^{c}	308.2 ± 0.3^{d}	903.7 ± 2.1^{a}	298.8 ± 1.0^{d}	19.8 ± 0.3′	6.3 ± 1.6″
10	11.3	569	418sh, 442, 469	Lutein alike	13.5 ± 0.0^{b}	6.5 ± 0.1^{d}	13.1 ± 0.1^{b}	7.9 ± 0.2^{c}	1.9 ± 0.0′	2.1 ± 0.1′
11	11.5	569	417sh, 439, 467	Lutein alike	34.0 ± 0.1^{c}	17.6 ± 0.2^{d}	49.7 ± 0.0^{b}	17.7 ± 0.2^{d}	2.6 ± 0.0′	2.0 ± 0.0″
12*	14.1 & 14.5	908	462, 599, 648	Chlorophyll b	530.5 ± 0.9^{c}	262.7 ± 0.1^{d}	766.3 ± 0.7^{a}	234.6 ± 0.3^{e}	33.1 ± 0.1	tr
13*	15.8 & 16.2	894	412sh, 430, 537, 580, 617, 663	Chlorophyll a	3492.3 ± 1.5^{ab}	1609.8 ± 0.0^{c}	3573.4 ± 1.5^{a}	835.0 ± 2.6^{d}	111.0 ± 0.3	-
14*	17.3 & 17.9	886	434, 526, 598, 652	Pheophytin b	tr	-	103.0 ± 0.1^{b}	48.2 ± 0.1^{c}	tr	15.0 ± 1.2
15	18.6	537	427sh, 452, 476	β-carotene	788.2 ± 4.5^{c}	428.9 ± 1.0^{e}	1536.1 ± 1.9^{a}	540.4 ± 4.5^{d}	1.1 ± 0.1′	1.1 ± 0.3′
16	19.5	888	402, 500, 530, 607, 665	Pheophytin a allomer	-	-	-	-	tr	tr
17*	20.2 & 20.9	872	408, 505, 535, 608, 664	Pheophytin a	80.9 ± 1.0^{d}	26.7 ± 0.0^{e}	917.6 ± 0.9^{a}	416.1 ± 0.1^{c}	23.2 ± 0.0″	90.1 ± 6.5′
18	23.3	814	409, 507, 537, 608, 665	Pyropheophytin a	-	-	95.9 ± 0.1^{a}	35.7 ± 0.0^{c}	1.0 ± 0.0″	26.1 ± 1.9′
				Total Carotenoids	1609.2 ± 5.3^{c}	911.7 ± 1.3^{d}	2688.7 ± 4.1^{a}	963.0 ± 5.3^{d}	30.6 ± 0.4′	15.6 ± 1.5″

Total Chlorophylls	4103.7 ± 0.4^{c}	1906.1 ± 0.1^{d}	5569.4 ± 0.9^{a}	1628.2 ± 3.0^{e}	175.3 ± 0.9′	138.9 ± 10.5′
Total Pigments	**5712.9 ± 5.7^{c}**	**2817.8 ± 1.4^{d}**	**8258.1 ± 3.2^{a}**	**2556.2 ± 3.7^{e}**	**205.9 ± 1.3′**	**154.5 ± 12.0′**

P – peak number; RT – retention time (min); $[M+H]^+$ – positive molecular ion (*m/z*); λ_{max} - maximum wavelengths (nm); BB_fd1 – batch 1 broccoli by-products submitted to freeze-drying; BB_d40 – batch 1 broccoli by-products submitted to air-drying at 40ºC; bBB_fd – batch 1 broccoli by-products submitted to blanching, followed by freeze-driying; bBB_d40 - batch 1 broccoli by-products submitted to blanching, followed by air-drying at 40ºC; BB_fd2 - batch 2 broccoli by-products submitted to freeze-drying; BB_MHG - batch 2 broccoli by-products submitted to microwave hydrodiffusion and gravity (MHG); *epimers eluting in different RT; sh –indicate a shoulder in λ_{max}; - not detected; tr – traces were detected. Values are expressed as µg/g of broccoli by-product dry weight. In each line different letters (a,b,c, and d) or different symbols (′ and ″) mean significant differences ($p < 0.001$).

Table 2

P	RT	Probable compound [M-H]⁻: Main MS² [M-H]⁻	Batch 1				Batch 2	
			BB_fd1	BB_d40	bBB_fd	bBB_d40	BB_fd2	BB_MHG
α	1.8	**Glucoraphanin** **436:** 372(100); 259(2)	2.41 ± 0.13^{a}	2.24 ± 0.12^{a}	2.34 ± 0.08^{a}	1.42 ± 0.24^{b}	5.80 ± 0.18′	5.64 ± 0.14′
β	6.7	**Glucobrassicin** **447:** 259(100)	1.02 ± 0.03^{b}	1.21 ± 0.08^{ab}	1.36 ± 0.04^{a}	0.52 ± 0.05^{c}	1.28 ± 0.03′	0.20 ± 0.00″
γ	9.3	**4-methoxyglucobrassicin** **477:** 259(100); 274(60)	0.65 ± 0.04^{a}	0.66 ± 0.00^{a}	0.77 ± 0.00^{a}	0.28 ± 0.03^{b}	0.42 ± 0.00′	0.11 ± 0.00″
δ	11.1	**Neoglucobrassicin** **477:** 446 (100); 259(2)	0.92 ± 0.06^{a}	1.05 ± 0.04^{a}	0.47 ± 0.01^{b}	0.28 ± 0.01^{b}	0.34 ± 0.00′	0.12 ± 0.00″
		Total (μg/g)	**5.00 ± 0.23^{a}**	**5.15 ± 0.05^{a}**	**4.93 ± 0.04^{a}**	**2.50 ± 0.33^{b}**	**7.85 ± 0.15′**	**6.06 ± 0.14″**

P – peak number; RT – retention time (min); [M-H]⁻ : Main MS² [M-H]⁻ – molecular ion and main product ions observed with percentage in brackets (m/z), other ions were found although not discriminated; BB_fd1 – batch 1 broccoli by-products submitted to freeze-dried; BB_d40 – batch 1 broccoli by-products submitted to air-drying at 40ºC; bBB_fd – Batch 1 broccoli by-products submitted to blanching, followed by freeze-drying; bBB_d40 - Batch 1 broccoli by-products submitted to blanching, followed by air-drying at 40ºC; BB_fd2 - Batch 2 broccoli by-products submitted to freeze-drying; BB_MHG - Batch 2 broccoli by-products submitted to microwave hydrodiffusion and gravity (MHG);Values are expressed as mg/g of dry weight. In each line different letters (a,b,c, and d) or different symbols (′ and ″) mean significant differences ($p < 0.001$).

Table 3

P	RT	[M-H]⁻: Main MS^2 [M-H]⁻	λ_{max}	Probable compound	Batch 1				Batch 2	
					BB_fd1	BB_d40	bBB_fd	bBB_d40	BB_fd2	BB_MHG
Quercetin glucosides										
2	**7.4**	**949:** 787 (100)	228, 266, 316, 343sh, 368sh#	Q-3-C-dG-7-G	66.9 ± 1.2^{b}	60.1 ± 0.9^{b}	80.3 ± 0.6^{a}	50.8 ± 0.9^{c}	46.3 ± 0.2′	47.4 ± 0.5′
9	**8.9**	**993:** 831 (100); 787 (40)	243, 275sh, 317, 368sh	Q-3-S-dG-7-G	263.5 ± 6.3^{a}	193.0 ± 5.1^{b}	304.7 ± 13.4^{a}	176.1 ± 3.3^{b}	128.1 ± 2.4′	133.9 ± 2.1′
Kaempferol glucosides										
2	**7.4**	**817:** 655 (100)	228, 266, 316, 343sh, 368sh#	K derivative	14.6 ± 0.7^{b}	10.6 ± 0.5^{b}	22.7 ± 0.4^{a}	5.0 ± 0.5^{c}	2.4 ± 0.1′	3.0 ± 0.3′
2	**7.4**	**807:** 609 (100)	228, 266, 316, 343sh, 368sh#	K derivative	10.0 ± 0.5^{b}	7.2 ± 0.4^{b}	15.5 ± 0.3^{a}	3.4 ± 0.4^{c}	1.6 ± 0.1′	2.0 ± 0.2′
3	**7.6**	**771:** 609 (100)	228sh, 265, 320sh, 349, 368sh#	K-3-dG-7-G	476.9 ± 5.0^{b}	222.9 ± 3.2^{c}	855.5 ± 23.6^{a}	162.0 ± 17.6^{c}	17.1 ± 0.9″	36.1 ± 0.6′
3	**7.6**	**817:** 655 (100)	228sh, 265, 320sh, 349, 368sh#	K derivative	15.8 ± 0.2^{b}	7.4 ± 0.1^{c}	28.4 ± 0.8^{a}	5.4 ± 0.6^{c}	0.6 ± 0.0″	1.2 ± 0.0′
3	**7.6**	**807:** 609 (100)	228sh, 265, 320sh, 349, 368sh#	K derivative	55.5 ± 0.6^{b}	26.0 ± 0.4^{c}	99.6 ± 2.8^{a}	18.9 ± 2.1^{c}	2.0 ± 0.1″	4.2 ± 0.1′
4	**7.9**	**933:** 771 (100)	229sh, 265, 355, 368sh	K-3-dG-7-dG	227.5 ± 6.5^{a}	73.0 ± 5.7^{b}	221.6 ± 15.5^{a}	42.5 ± 4.5^{b}	13.6 ± 1.7″	47.1 ± 2.8′
5	**8.2**	**563**	222sh, 239, 331	K derivative	tr	tr	tr	tr	50.1 ± 0.6″	64.0 ± 0.7′
17	**10.6**	**609:** 447 (100)	244, 269, 344, 368sh#	K-3-G-7-G	1.9 ± 0.2^{a}	1.8 ± 0.1^{a}	2.8 ± 0.1^{a}	0.5 ± 0.0^{b}	1.0 ± 0.1′	0.9 ± 0.0′
19	**11.4**	**609:** 429 (100)	245, 267, 355, 368sh	K-3-dG	13.0 ± 0.9^{ab}	17.2 ± 1.5^{ab}	27.9 ± 1.7^{a}	8.0 ± 0.2^{b}	2.8 ± 0.5′	0.6 ± 0.3′
Monoacylated kaempferol glucosides										
7	**8.4**	**963:** 801 (100); 771 (20)	238, 269, 332, 368sh	K-3-MC-dG-7-G	797.3 ± 23.8^{b}	575.7 ± 35.2^{b}	1875.1 ± 64.2^{a}	475.0 ± 47.0^{b}	38.5 ± 0.8″	112.1 ± 2.9′
8	**8.7**	**933:** 771 (100); 609 (5)	243, 268, 335#	K-3-C-dG-7-G	731.5 ± 3.3^{b}	412.8 ± 30.2^{c}	1324.6 ± 54.9^{a}	335.0 ± 35.6^{c}	29.4 ± 1.6″	66.5 ± 3.7′
8	**8.7**	**1095:** 771 (100); 609 (37)	243, 268, 335#	K-3-C-tG-7-G	151.9 ± 0.7^{b}	85.7 ± 6.3^{c}	275.2 ± 11.4^{a}	69.6 ± 7.4^{c}	6.1 ± 0.3″	13.8 ± 0.8′
10	**9.1**	**1301**	241, 270, 332, 368sh	K-3-S-tG-7-dG	197.0 ± 6.8^{a}	161.8 ± 14.6^{ab}	132.8 ± 12.5^{b}	63.2 ± 3.0^{c}	9.9 ± 1.7′	17.6 ± 0.8′

11	**9.2**	**1139:** 815 (100); 609 (29)	240, 269, 333, 368sh	K-3-S-tG-7-G	387.1 ± 14.5^{a}	81.8 ± 8.0^{b}	108.2 ± 8.1^{b}	116.4 ± 9.7^{b}	18.7 ± 5.2″	110.6 ± 1.9′
12	**9.3**	**977:** 815 (100); 609 (15)	239, 268, 334, 368sh	K-3-S-dG-7-G	823.0 ± 23.5^{a}	390.7 ± 20.8^{b}	851.8 ± 26.5^{a}	199.1 ± 11.5^{c}	tr	23.9 ± 4.4
13	**9.5**	**1109**	243, 268, 338, 368sh	K-3-F-tG-7-G	257.2 ± 10.0^{a}	84.0 ± 5.1^{b}	222.8 ± 14.1^{a}	46.6 ± 2.4^{b}	8.4 ± 1.3′	15.5 ± 1.2′
14	**9.6**	**947:** 785 (100); 771 (75)	242, 268, 333, 368sh	K-3-F-dG-7-G 1	687.2 ± 11.5^{b}	353.7 ± 20.2^{c}	1175.7 ± 17.0^{a}	242.0 ± 16.7^{d}	3.4 ± 0.4″	10.0 ± 1.1′
15	**9.8**	**917:** 755 (100);771 (60)	239, 367, 320, 348sh, 368sh$^{\#}$	K-3-Co-dG-7-G 1	107.9 ± 8.7^{b}	64.9 ± 3.0^{c}	153.9 ± 4.3^{a}	31.2 ± 3.1^{d}	2.4 ± 0.7″	17.6 ± 1.1′
17	**10.6**	**917:** 755 (100)	244, 269, 344, 368sh$^{\#}$	K-3-Co-dG-7-G 2	30.8 ± 2.6^{a}	29.9 ± 2.3^{a}	46.1 ± 2.2^{a}	8.1 ± 0.6^{b}	16.3 ± 2.1′	15.0 ± 0.6′
17	**10.6**	**947:** 785 (100); 609 (2)	244, 269, 344, 368sh$^{\#}$	K-3-F-dG-7-G 2	21.5 ± 1.9^{a}	21.0 ± 1.6^{a}	32.3 ± 1.5^{a}	5.7 ± 0.4^{b}	11.4 ± 1.5′	10.5 ± 0.4′
Diacylated kaempferol glucosides										
20	**11.5**	**1493:** 977 (100); 1169 (71)	244, 270, 340, 368sh	K-3-MC/S-tG-7-dG	41.7 ± 2.9^{b}	33.7 ± 3.1^{bc}	90.7 ± 3.7^{a}	11.2 ± 0.0^{c}	tr	1.5 ± 0.3
21	**11.8**	**1463:** 1139 (100)	242, 270, 328, 367sh$^{\#}$	K-3-C/S-tG-7-dG	87.8 ± 1.4^{c}	128.7 ± 4.7^{b}	191.2 ± 6.2^{a}	41.7 ± 3.6^{d}	16.7 ± 0.6″	91.2 ± 1.9′
21	**11.8**	**1331:** 1169 (100)	242, 270, 328, 367sh$^{\#}$	K-3-MC/S-tG-7-G	275.9 ± 4.5^{c}	404.6 ± 14.6^{b}	601.1 ± 19.4^{a}	131.1 ± 11.2^{d}	52.6 ± 2.0″	286.6 ± 6.0′
22	**12.0**	**1301:** 1139 (100)	242, 269, 330, 368sh$^{\#}$	K-3-C/S-tG-7-G	296.7 ± 2.8^{b}	246.4 ± 8.0^{b}	556.8 ± 35.3^{a}	105.1 ± 10.8^{c}	40.5 ± 0.7″	58.8 ± 0.5′
22	**12.0**	**1507**	242, 269, 330, 368sh$^{\#}$	K-3-dS-tG-7-dG	147.2 ± 1.4^{b}	122.2 ± 4.0^{b}	276.1 ± 17.5^{a}	52.1 ± 5.4^{c}	20.1 ± 0.3″	29.2 ± 0.2′
23	**12.3**	**1345:** 977 (100)	241, 269, 330, 368sh$^{\#}$	K-3-dS-tG-7-G	668.7 ± 23.1^{b}	466.6 ± 22.5^{c}	1164.3 ± 21.2^{a}	244.7 ± 29.3^{d}	88.8 ± 4.9″	148.0 ± 1.2′
23	**12.3**	**1477:** 1153 (100)	241, 269, 330, 368sh$^{\#}$	K-3-F/S-tG-7-dG	79.2 ± 2.7^{b}	55.3 ± 2.7^{c}	137.9 ± 2.5^{a}	29.0 ± 3.5^{d}	10.5 ± 0.6″	17.5 ± 0.1′
24	**12.6**	**1315:** 1153 (100)	243, 269, 330, 368sh	K-3-F/S-tG-7-G	142.5 ± 8.6^{b}	83.0 ± 5.4^{c}	231.1 ± 4.8^{a}	46.1 ± 3.2^{c}	3.1 ± 0.3″	23.8 ± 3.9′
Esters of hydroxycinnamic acids with gentiobiose										
15	**9.8**	**431:** 385 (100)	239, 367, 320, 348sh, 368sh$^{\#}$	Sinp-Glc + formic acid	70.6 ± 2.9^{b}	56.3 ± 1.0^{c}	85.9 ± 1.4^{a}	45.1 ± 1.0^{d}	35.5 ± 0.2″	40.5 ± 0.4′
18	**10.9**	**885:** 867 (100)	243, 275sh, 317, 368sh	SinpCafFer-Gentb	118.8 ± 4.2^{b}	116.4 ± 4.3^{b}	140.2 ± 2.6^{a}	90.1 ± 0.8^{c}	78.5 ± 0.4′	73.8 ± 1.0′
25	**13.7**	**753:** 529 (100)	241, 330, 368sh	diSinp-Gentb	649.9 ± 5.5^{b}	563.5 ± 48.7^{b}	886.2 ± 24.8^{a}	298.8 ± 21.6^{c}	292.7 ± 8.9′	197.2 ± 1.7″
26	**14.1**	**723:** 499 (100)	241, 273sh, 329	SinpFer-Gentb	588.6 ± 3.6^{b}	451.7 ± 18.4^{c}	904.3 ± 25.4^{a}	250.1 ± 21.1^{d}	291.1 ± 12.7′	245.8 ± 3.8′
27	**14.4**	**693:** 469 (100)	246, 326	SinpCoum-Gentb	101.4 ± 4.7^{b}	104.3 ± 4.0^{ba}	133.8 ± 4.2^{a}	68.8 ± 0.8^{c}	80.7 ± 4.7′	74.0 ± 0.6′

28	**14.8**	**959:** 735 (100)	244, 323	triSinp-Gentb	315.6 ± 13.7^{a}	399.8 ± 24.2^{a}	325.9 ± 18.6^{a}	210.7 ± 15.8^{b}	233.5 ± 11.1′	176.8 ± 6.9′
29	**15.2**	**929:** 705 (100)	245, 320	diSinpFer-Gentb	252.2 ± 4.2^{b}	245.8 ± 7.5^{b}	306.2 ± 2.8^{a}	137.8 ± 8.1^{c}	201.5 ± 3.6′	174.5 ± 3.0″
30	**15.5**	**899:** 705 (100)	239, 327	SinpdiFer-Gentb	90.0 ± 1.5^{b}	111.0 ± 2.5^{a}	98.3 ± 1.5^{ab}	tr	89.5 ± 1.1′	98.8 ± 1.6′
Esters of hydroxycinnamic acids with quinic acid										
1	**5.2**	**353:** 191 (100); 179 (45)	200, 263sh, 289	5-CQA	721.6 ± 10.2^{b}	456.2 ± 41.3^{c}	1059.8 ± 3.5^{a}	336.4 ± 22.5^{c}	74.1 ± 2.6′	64.0 ± 2.1′
2	**7.4**	**337:** 163 (100); 191 (6);	228, 266, 316, 343sh, 368sh$^{\#}$	4-p-CoQA	405.3 ± 16.2^{b}	311.1 ± 12.3^{b}	592.3 ± 8.8^{a}	182.7 ± 12.5^{c}	120.3 ± 2.5′	134.9 ± 6.8′
6	**8.3**	**367:** 193 (100); 134 (4)	240, 327	4-FQA	111.7 ± 2.5^{b}	88.9 ± 3.6^{b}	222.8 ± 6.6^{a}	78.5 ± 4.8^{b}	33.5 ± 0.2″	41.1 ± 0.3′
16	**10.4**	**367:** 191 (100); 173 (64)	241, 277sh, 332, 369sh	5-FQA	56.5 ± 2.0^{b}	47.8 ± 1.5^{bc}	78.8 ± 0.6^{a}	41.3 ± 1.5^{c}	tr	34.1 ± 0.4
				Total	**10605.5 ± 148.6^{b}**	**7419.7 ± 378.0^{c}**	**15986.7 ± 270.3^{a}**	**4588.7 ± 319.8^{d}**	**2203.0 ± 50.9″**	**2765.5 ± 2.0′**

P – peak number; RT – retention time (min); $[M-H]^-$: Main MS^2 $[M-H]^-$ – molecular ion and main product ions observed with percentage in brackets (*m/z*), other ions were found although not discriminated; λ_{max} - maximum wavelengths (nm); $^{\#}\lambda_{max}$ of co-eluting compounds; sh –indicate a shoulder in λ_{max}; tr – traces were detected. BB_fd1 – batch 1 broccoli by-products submitted to freeze-dried; BB_d40 – batch 1 broccoli by-products submitted to air-drying at 40ºC; bBB_fd – Batch 1 broccoli by-products submitted to blanching, followed by freeze-drying; bBB_d40 - Batch 1 broccoli by-products submitted to blanching, followed by air-drying at 40ºC; BB_fd2 - Batch 2 broccoli by-products submitted to freeze-drying; BB_MHG - Batch 2 broccoli by-products submitted to microwave hydrodiffusion and gravity (MHG);CQA: caffeoylquinic acid; *p*-CoQA: *p*-coumaroylquinic acid; FQA: feruloylquinic acid. Q: quercetin; dG: sophoroside; G: glucose; K: kaempferol; MC: methoxycaffeoyl; C:Caf: caffeoyl; tG: sophorotrioside; S: Sinp: sinapoyl; F: Fer: Feruloyl; Co:Coum: Coumaroyl; Gentb: gentiobiose. Values are expressed as µg/g of by-products dry weight. In each line different letters (a,b,c, and d) or different symbols (′ and ″) mean significant differences ($p < 0.001$).

Table 4

Processing technique (equipment)	**Time of operation (h)**	**Energy consumption (kJ)**
Freeze-drying (Freezer, freeze-drier, and vacuum pump)	168	131 328
Air-drying (Oven)	64	69 120
Freeze-drying with a pre-blanching step (Microwave, freezer, freeze-drier, and vacuum pump)	168	131 616
Air-drying with a pre-blanching step (Microwave and oven)	40	43 560
MHG dehydration (MHG apparatus)	0.75	1 350

Credit author statement

- **Sónia S. Ferreira**: Conceptualization, Investigation, data curation, Writing - Original Draft. designed and performed the dehydration experiments, analysed the data, and wrote the paper.
- **Filipa Monteiro**: Investigation, data curation.
- Cláudia P. Passos: Conceptualization, Investigation, data curation, Writing - Review & Editing.
- **Artur M. S. Silva**: data curation (NMR), Writing - Review & Editing.
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