Food Research International 115 (2019) 276–282

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

An innovative green extraction and re-use strategy to valorize food supplement by-products: *Castanea sativa* bud preparations as case study

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ARTICLE INFO

Keywords:
Castanea sativa glyceric macerates
Buds-derivatives waste valorisation
Pulsed ultrasound-assisted extraction
UV–visible spectroscopy
Chemometrics
HPLC-phytochemical fingerprint

ABSTRACT

This research takes place in the context of an Alcotra Italy-France trans-frontier project called FINNOVER, which includes among its objectives the "green" innovation of agro-industrial chains. Bud-derivatives are a category of natural products produced macerating meristematic tissues of trees and plants. They are quite expensive compared to other botanicals, since the collection period of their raw materials is extremely limited over the time.

Pulsed Ultrasound-Assisted Extraction has been employed to extract further valuable material from the buds by-products remaining after the production of *Castanea sativa* Glyceric Macerates. UV–Visible spectra coupled with chemometrics were employed, as untargeted phytochemical fingerprints, to quickly screen the best experimental conditions of extraction: a duty cycle of 80%, an extraction time of 15 min and a solvent/ratio of 1/10. Targeted phytochemical fingerprints by HPLC have been used to identify and quantify the main bioactive compounds of the most promising marcs extract comparing it with the corresponding commercial *Castanea sativa* Glyceric Macerate. An innovative extraction and re-use strategy to obtain value-added products from botanicals by-products was developed in alternative to incineration or composting. It was applied to *Castanea sativa* buds production as case study, but it could be analogously applied for other herbal preparations.

1. Introduction

Food waste valorization and re-use strategies, rather than conventional food waste processing (i.e. incineration or composting), are becoming more and more popular and they are commonly named as "2nd generation food waste management" (Lin et al., 2013). Even if these strategies are particularly interesting for food processing companies, nevertheless there are small scale production, i.e. numerous herbal supplements productions, whose waste still represent an important source of botanicals to be valorized.

FINNOVER (Innovative strategies for the development of cross-border green supply chains) is the name of an Interreg ALCOTRA Italy/France trans frontier project started in 2017 with the aim of innovating agro-industrial chains in terms of green circular economy. One of FINNOVER targets is the management of agricultural waste.

Food supplements from botanicals and derived preparations made from plants, algae, fungi or lichens have become widely available on the market. The uses of botanical products have developed differently in the world, depending on specific cultures, nutritional practices, availability of botanical species, company policies, national governmental regulatory, and administrative approaches: these different conditions facilitated the botanical product marketing as food supplements and as medicinal products or viceversa. Due to limited harmonization in the European Community (EU), specific national regulations, adopted at a Member State level, are also applied. Thus, a mutual recognition is the mechanism through which such products can be marketed in EU countries other than those of origin.

In particular, in the most countries of the EU, bud-derivatives, which represent a relatively new category of natural products, are classified as plant food supplements. They are produced starting from the fresh meristematic tissues of both trees and herbaceous plants by maceration using a mixture of solvents (i.e. a mixture of water, ethanol and glycerol) (Hobbs, Malla, & Sogah, 2014; Knoss & Chinou, 2012; Konik, Jungling, & Bauer, 2011; Nicoletti, 2012; Pisanello, 2014;

Abbreviations: UAE, Ultrasound-Assisted Extraction; PUAE, Pulsed Ultrasound-Assisted Extraction; DOE, Design of experiments; CBs, *Castanea sativa* buds; GMs, Glyceric Macerates; HPW, High purity water produced; NA, Normal Atmosphere; PCA, Principal Component Analysis; SNV, Standard Normal Variate; TBCC, Total bioactive compound content; LC–MS/MS, Liquid chromatography - mass/mass spectrometry

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https://doi.org/10.1016/j.foodres.2018.12.018
Received 30 May 2018; Received in revised form 14 September 2018; Accepted 14 December 2018
Available online 17 December 2018

Silano, Coppens, Larranaga-Guetaria, Minghetti, & Roth-Ehrang, 2011).

Nowadays these products are still poorly studied, even if they are widely used for phytotherapy and homeopathy (Donno, Mellano, Cerutti, & Beccaro, 2016; Donno, Beccaro, Cerutti, Mellano, & Bounous, 2015). Their use contributes to the birth of the so-called "Gemmotherapy", a fast-emerging branch of complementary medicine, which is expanding significantly in the market (Fowler, 2006; Gurib-Fakim, 2006).

Bud-derivatives are quite expensive compared to other botanicals, since the phenological stage of buds or young sprouts, necessary to obtain them, extremely limits their collection period over time. Consequently, the valorization of their by-products could have a significant economic impact for the producers and it could be an important innovation in this field.

This research describes a novel tool to enable manufacturers of bud extracts (gemmoderivatives) to evaluate a sustainable waste management option in order to increase their productivity. Nevertheless, the same strategy could be analogously applied also for other herbal preparations, becoming an example of "modus operandi" in a green economy strategy.

CBs were used as case study since this species is one of the most commonly used herbal medicines for its well-known health-promoting properties both against urinary and cardiovascular diseases (Donno, Beccaro, Mellano, Bonvegna, & Bounous, 2014).

Green procedures, namely those with complying with standards set by Environmental Protection Agency of USA (http://www.epa.gov/greenchem-istry/pubs/about_gc.html), are aimed to short processing time, reduce solvents consume, decrease pollution and they are as much as possible energy-saving and cost-saving (Bromberger Soquetta, Marsillac Terra, & Peixoto Bastos, 2009; Giacometti et al., 2018; Putnik et al., 2018). As far as green extractions are concerned, recently, Chemat, Vian, and Cravotto (2012) resumed these concepts in the following definition *"Green Extraction is based on the discovery and design of extraction processes which will reduce energy consumption, allows use of alternative solvents and renewable natural products, and ensure a safe and high-quality extract/product"* (Chemat et al., 2012).

The use of ultrasounds in food industry has recently attracted attention, above all due to the numerous advantages of this technique in food-processing (i.e. filtration, defoaming, degassing, cutting), food-preservation (i.e. enzyme and microorganism inactivations) and natural product-extraction (Chemat, Zill-e-Huma, & Khan, 2011). In particular, UAE is an efficient, green, relatively low-cost and sustainable procedure, used both on a small and large scale, that presents many advantages with respect to conventional extractions (Chemat et al., 2017; Cravotto & Cintas, 2006; Vinatoru, 2015). The ultrasound waves (kHz range), throught cavitation phenomena, are able to mechanically break the wall cells and thus extracting the intracellular liquids using several independent or combined mechanisms such as: fragmentation, erosion, capillarity, detexturation and sonoporation, all linked to the extraction yield increase (Chemat et al., 2017). Cavitation phenomena, a succession of compression and rarefaction phases which generate cavities in the liquid (cavitation bubbles), are the effects of ultrasound propagation in a solid/liquid media. In particular, cavitation bubbles collapse on the surface of the solid material creating microjets, at high temperature and pressure, responsible for breaking cell walls (Kazemi, Karim, Mirhosseini, & Abdul Hamid, 2016).

In particular, high power ultrasonic probes usually operate at around 16–30 kHz and they are generally preferred for extraction applications respect to the ultrasonic bath, due to the direct delivery of ultrasounds in the extraction solvent with minimal ultrasonic energy loss. When UAE is used in pulsed mode (PUAE), the ultrasound processor is turned on (active time) and off (inactive time) intermittently during the extraction process. This mode allows to preserve the heat-sensitive biomolecules from degradation since heat generation is lower respect to continuous sonication (Torres, Talavera, Andrews, Sánchez-Contreras, & Pacheco, 2017).

The solvent utilized in green extractions are bio-grade solvents produced from biomasses such as wood, starch, vegetables and fruits. In particular, ethanol and glycerol are both considered bio-solvents, the first one is produced by the fermentation of sugar-rich materials, the second one is a by-product from the trans-esterification of vegetable oils. Both of them are used on a large scale because they are biodegradable, food grade, cheap and easily available in high purity.

DOE, a well-established concept for planning few informative experiments, has been very useful to extract the maximum amount of information regarding the factors (process variables) affecting the PUAE (Leardi, 2009). It was applied using the whole UV–Vis spectrum of each extract, as multivariate response variable, and coupling chemometrics to quickly screen the best experimental conditions (Granato et al., 2018; Leardi, 2017). This analytical shortcut has been already employed for a rapid untargeted identification of extracts of plants meristematic tissues (Boggia et al., 2017).

Finally, HPLC methods were used to identify and quantify the main bioactive compounds (in particular polyphenols, organic acids, and vitamins), selected as markers for their demonstrated health-promoting activity, obtaining a targeted chromatographic profile, assessing single bioactive class contribution to the phytocomplex and comparing it to the commercial products: synergistic or additive biological effects of several bioactive molecules (phytocomplex), rather than a single active ingredient or a group of compounds, contribute to health promotion (Donno et al., 2014).

2. Materials and methods

2.1. Plant material

CBs were collected from plants spontaneously grown in the valleys of Chisone, Pellice, Germanasca, Bronda, and Varaita (Turin, Italy) and authenticated by a botanist. In particular, the sampling sites were: Bobbio Pellice, Bricherasio, Perrero, Pagno, and Brondello. Chestnut buds were used by an Italian commercial company of food supplements (Geal Pharma, Bricherasio, Turin) for the formulation of the corresponding GMs in the year 2018 according to the European Pharmacopeia 8th edition (2014), following the procedure deriving from the French Pharmacopeia (Ordre_National_des_Pharmaciens, 1965). The collection of the raw materials, in the meristematic phenological stage (buds), was performed over a limited period of time in March 2017, according to the different collection points. The fresh embryonic parts were immediately used in order to preserve their bioactive compounds. In this research original samples belonging to the same production batch were considered for analysis. Waste material obtained from the same herbal medicine production batch was the raw material used for the following extraction steps assisted by pulsed ultrasounds.

2.2. Chemicals

All reagents were obtained from Sigma-Aldrich (Steinheim, Germany) and from VWR International S.r.l (Milan, Italy). HPW with Millipore Milli-Q system was used both in sample preparation and analysis.

2.3. Preparation protocol of CBs extracts (commercial product)

CBs extracts were prepared following the traditional protocol of GMs (Ordre_National_des_Pharmaciens, 1965) using a mixture of water/glycerol/ethanol (50/30/20 *v*/v/v) as extraction solvent and with a 1:20 weight ratio between plant and solvent. About 1 kg of fresh plant was treated. "*Bioactive compounds were extracted through a cold maceration process for 21 days, followed by a first filtration a manual pressing and, after 2 days of decanting, a second filtration (Whatman paper filter, n° 1). The obtained extracts, which represent the commercial products*

were stored in dark bottles at NA, at 4 °C and 95% relative humidity until commercialization" (Donno, Boggia, et al., 2016). At the same time, the wet marcs obtained after the 2nd filtration, which represent the solid by-products, were stored frozen at −20 ± 2 °C until further treatments.

2.4. Waste management: Pulsed Ultrasound-Assisted Extraction

The frozen marcs were carefully homogenized by grinding in a blender (Grindomix GM200, Retsch, Haan, Germany) for 20 s at 5000 rpm and further sieved (150 μm mesh size). Their moisture content (relative humidity) were determined to be 52.0% ± 0.3 by a Sartorius moisture analyzer (Massachusetts, USA). All measurements were made in triplicate and average results were reported.

The extraction operations were carried out directly under the pulsed mode, keeping the temperature under control always below 70 ± 1 °C. The extraction solvent, water/ethanol/glycerol (50/20/30 *v*/v/v), was the same solvent used in the conventional protocol to produce the GMs commercial products.

PUAE was performed directly using a sonicator (Hielscher Ultrasonics UP200 St, Germany) with an operating frequency of 26 kHz, effective output of 200 W, equipped with a titanium (7 mm i.d.) sonotrode suitable for the solvent volumes used (Santos, Lodeiro, & Capelo-Martínez, 2009). The pulse duration and pulse interval refer to "ON" time and "OFF" time of the sonochemical reactor, respectively. The total time of a pulse duration period plus a pulse interval period is the so-called cycle time. The duty cycle (expressed as % and related to a second in steps of 0.1 s) is the proportion of the pulse duration period respect to the cycle time.

2.5. UV–Vis Spectroscopy

All the UV–Vis spectra, obtained by an Agilent 8453 spectrophotometer with 1 nm resolution (Waldbronn, Germany), were recorded in the range 230–500 nm using rectangular quartz cuvettes with 1 cm path length.

Before being analysed, the marcs extracts were filtered under vacuum through filter paper (Macherey-Nagel MN 615 70 mm, Düren, Germany) centrifuged at 3500 rpm for 10 min and properly diluted using a blank mixture of water/ethanol/glycerol (50/20/30 *v*/v/v).

The total spectrum of each analysed sample was collected at room temperature in triplicate, against blank solution, and the results were averaged.

2.6. Experimental design and statistical analysis

Process conditions of the PUAE have been optimized by DOE using a 2^{4-1} fractional factorial design. Four process variables (i.e. factors under study) such as: the amplitude level, the duty cycle, the extraction time and the sample/solvent ratio, at two levels were investigated. The experimental plan illustrated in Table 1 resumes the conditions of the eight planned experiments plus three replicates of central condition (central points). As response variable the score on PC1 of the PCA performed on the UV–Vis spectra was taken into account. In details, a data matrix of 11 rows and 271 columns (the 271 absorbances at different wavelengths in the range 230–500 nm) was prepared using the eleven spectra of the corresponding eleven experiments. This data matrix was used as training set. Analyzing the correlation matrix, it is evident that the variables are highly correlated, as usual handling spectral data. Kaiser-Meyer-Olkin (KMO) and Bartlett's test of sphericity were not applicable since the determinant of the correlation matrix is near to zero. Then PCA was performed by NIPALS algorithm (Wold, Esbensen, & Geladi, 1987) on the column centred data (10 components), after the use of SNV (Barnes, Dhanoa, & Lister, 1989) as preprocessing technique with the goal of removing light scattering or other interfering phenomena (Weeranantanaphan & Downey, 2010). The scores on the first PC (explaining 86.8% of the total variance) were used as response of the DOE.

Table 1
The experimental plan of the 2^{4-1} fractional factorial design and the corresponding response variable (Y).

Experiment	X1	X2	X3	X4	Y (Response variable)
	Amplitude (%)	Duty cycle (%)	Extraction time (min)	Sample/ solvent ratio	PC1_scores
CA01	30	20	5	1/60	0.355196
CA02	50	20	5	1/40	−1.03264
CA03	30	80	5	1/40	1.114245
CA04	50	80	5	1/60	−0.88268
CA05	30	20	15	1/40	1.462808
CA06	50	20	15	1/60	−1.28896
CA07	30	80	15	1/60	−1.48396
CA08	50	80	15	1/40	1.723962
CA09	40	50	10	1/50	−0.35296
CA10	40	50	10	1/50	−0.09684
CA11	40	50	10	1/50	0.481829

Analogously the *C. sativa* GM, formulated by GealPharma using the same raw materials (buds) whose by-products are under study, were tested at two different dilutions in the already mentioned blank mixture (namely commercial product_d80 and commercial product_d100: diluted 1:80 and 1:100, respectively). Their spectra were subsequently used as external test set.

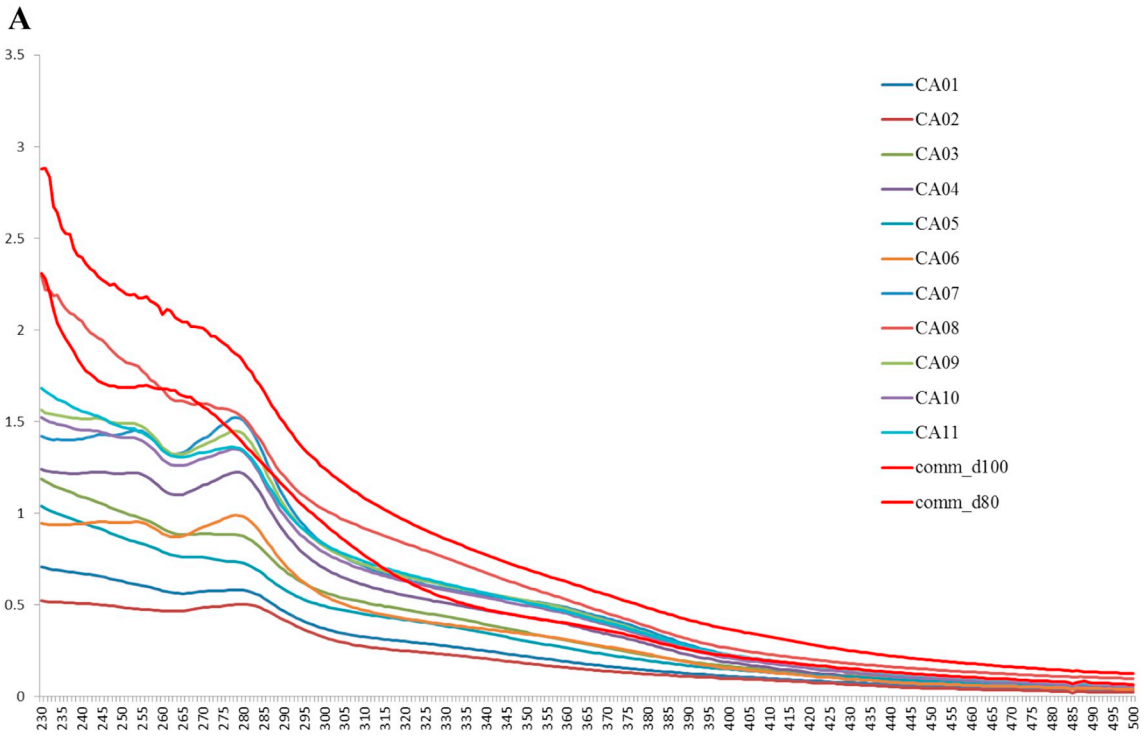
An R-based chemometric software developed by the Group of Chemometrics of the Italian Chemical Society (freely downloadable from gruppochemiometria.it/index.php/software, 2018) was used to perform the multivariate data analysis.

2.7. Chromatographic analysis of bioactive compounds

HPLC–DAD methods were used for phytochemical analysis on bud preparations. They were focused on flavonols, phenolic acids expressed as benzoic and cinnamic acids, catechins, tannins as polyphenolic markers, also providing information on organic acids and vitamin C. In particular, it is known that dehydroascorbic acid has an important biological activity and can be easily converted to ascorbic acid by humans with positive antioxidant effects on human health-status. Thus, the sum of dehydroascorbic acid and ascorbic acid was considered for the evaluation of vitamin C. TBCC was determined as sum of the selected markers with health-promoting activities on humans according to "multimarker approach" (Mok & Chau, 2006). Then phytochemicals were grouped into different bioactive classes in order to evaluate each class contribution to phytocomplex composition.

An Agilent 1200 High-Performance Liquid Chromatograph coupled to an Agilent UV–Vis diode array detector (Agilent Technologies, Santa Clara, CA, USA) was used for the chromatographic analysis. Bioactive molecule separation was achieved on a Kinetex C18 column (4.6 × 150 mm, 5 μm, Phenomenex, Torrance, CA, USA).

Several mobile phases were used for bioactive compound characterization and UV spectra were recorded at different wavelengths, based on HPLC methods previously tested and validated for herbal medicines (Donno, Mellano, et al., 2016): a solution of 10 mM KH_2PO_4/H_3PO_4 and acetonitrile with a flow rate of 1.5 mL·min^{-1} (method A - analysis of cinnamic acids and flavonols); a solution of methanol/water/formic acid (5:95:0.1 *v*/*v*/v) and a mix of methanol/formic acid (100:0.1 *v*/v) with a flow rate of 0.6 mL·min^{-1} (method B - analysis of benzoic acids, catechins, and tannins); a solution of 10 mM KH_2PO_4/H_3PO_4 and acetonitrile with a flow rate of 0.6 mL·min^{-1} (method C - analysis of organic acids); a solution of methanol–water (5:95, v/v) containing 5 mM cetrimide and 50 mM KH_2PO_4/H_3PO_4 with a flow rate of 0.9 mL·min^{-1} (method D - analysis of ascorbic and dehydroascorbic



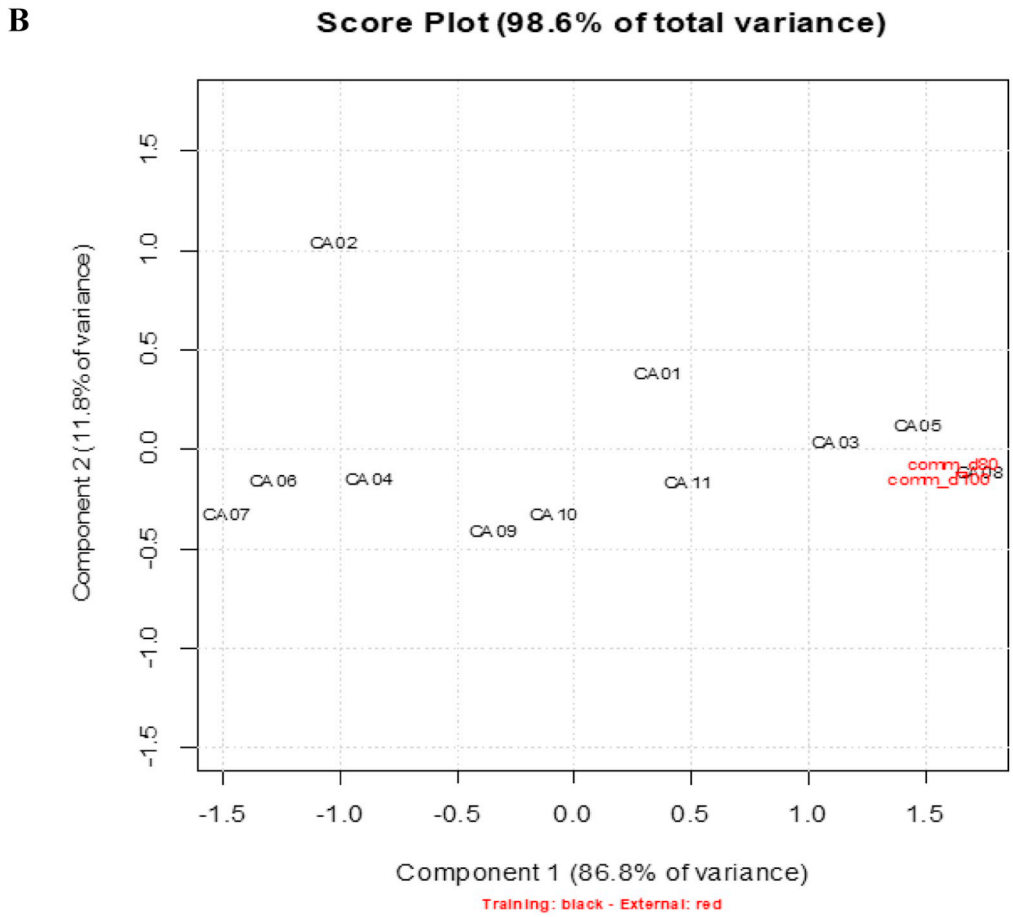


Fig. 1. UV–Vis averaged spectra (230–500 nm) of the eleven experiments selected by the DOE (A) and the score plot on the first two PCs selected by PCA using the vector of UV–Vis absorptions of each extract as multivariate untargeted signal (B). The projections of the two averaged spectra of the *Castanea sativa* Glyceric Macerate diluted 1:100 and 1:80 in the blank solution respectively (commercial product_d80, commercial product_d100), were also reported as external test set in the plot (in red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

acids). UV spectra were recorded at 330 nm (A); 280 nm (B); 214 nm (C); 261 and 348 nm (D).

Biomarker selection was based on compound demonstrated positive health-promoting properties and antioxidant activity by literature. Comparison and combination of retention times and UV spectra with those of authentic standards were used as method for compound identification in samples. All analysis were triplicated; the results were averaged and reported as mg/g of fresh weight marcs. Statistical analysis was carried out using V-PARVUS 2010 (Forina et al., 2010) and the Excel Data Analysis Tool (Microsoft Corporation, Seattle, WA, USA).

3. Results and discussion

Eight extracts (namely from CA01 to CA08), obtained according to the experimental plan described in Table 1, plus three extracts (namely from CA09 to CA11), obtained replicating the experimental condition at the central point of the DOE, were prepared and spectrophotometrically analysed.

At the top of Fig. 1 there are the UV–Vis spectra of these eleven extracts opportunely diluted in the blank solvent, after filtration and centrifugation to clarify them. Since the vector of UV–Vis absorptions of each extract has been proven to be strictly correlated to the whole phytocomplex, it has been used as multivariate non-targeted signal and elaborated as response for each experiment of the DOE. PCA was used to elaborate the multivariate signals. Before chemometric analysis, SNV was used as data pre-treatment.

The corresponding score plot on the first two PCs of $A_{11,271}$ after column centering, whose explained variance is 98.6%, is reported at the bottom of Fig. 1. The projections of the two spectra of the *C. sativa* GM commercial product, diluted 1:100 and 1:80 in the blank solution respectively (as external test set in red), in the score plot were also reported in Fig. 1.

These dilutions of the commercial product were necessary to avoid signal saturation. The first PC, explaining 86.8%, retained all the useful information of the 271 original variables. The corresponding scores were reported in Table 1 and used as "global" response (to be maximized). The other PCA details were reported in the Supplementary material (i.e. score matrix, loading matrix, eigenvalues, explained variance plot). The following model has been obtained by applying Multiple Linear Regression:

$$Y = -9.1\,e-11 - 0.4 \times {}_1 + 0.1 \times {}_2 + 0.1 \times {}_3 + 0.8 \times {}_4{}^{**} + 0.7\,X_1X_2{}^{*} + 0.5\,X_1X_3{}^{*} - 0.1\,X_1X_4.$$

remembering that the following interaction terms X_1X_2, X_1X_3, X_1X_4 are confused with X_3X_4, X_2X_4, X_2X_3 respectively (MacNamara, Leardi, & McGuigan, 2009). The linear term X_4 (** = $p < .01$) and the first two interaction terms (* = $p < .05$) are the only significant coefficients, as highlighted in Fig. 2, and they should be increased. It has anyway to be considered that the linear model is not validated (the predicted value at the center point is significantly different from the experimental values) and therefore is not suitable for predictions.

The projection of spectra obtained from *C. sativa* GM commercial product gave high positive scores on PC1, not far from the "best" samples from the design.

Analyzing Fig. 1, the experimental condition named CA08, whose details are reported in Table 1, seemed to be the most suitable to the aim of the research. In fact, the corresponding extract provides a spectrum having a score on PC1 similar to that of both the commercial products (*commercial product_d80, commercial product_d100*).

Thus, with the aim to obtain something still useful from the buds bagasse, this experimental condition was chosen as the best one among those tested by DOE.

Since X_4 resulted the most important variable in building the model, further experiments were planned setting it to 1/20, 1/15 and 1/10 (experiments: CA_R20, CA_R15, CA_R10) hoping both to improve the extraction yield and to save extraction solvent. The corresponding extracts were prepared, and the corresponding spectra were plotted in Fig. 3 together with CA08 extract all at the same dilution (1:50).

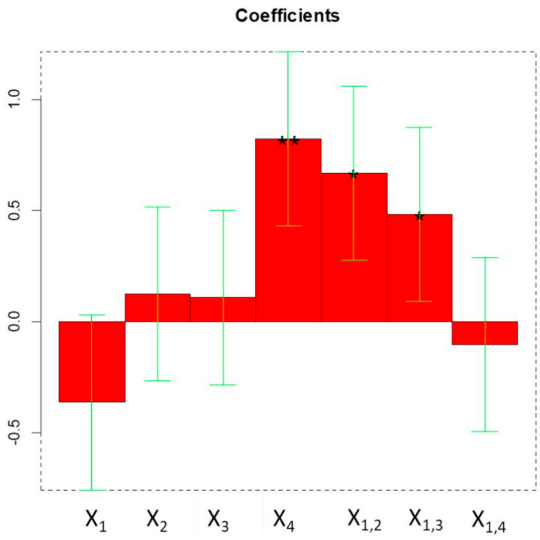


Fig. 2. Coefficients plot of the DOE: the coefficients of the models of Y (PC1_scores) obtained by the DOE (X1: amplitude; X2: cycle; X3: time; X4: sample/solvent ratio) are reported. * = $p < .05$, ** = $p < .01$.

The extract corresponding to CA_R10 resulted the most promising, since it seems more similar to the already mentioned *commercial product_d80* and thus deserving of further HPLC compositional investigation.

Fig. 4 shows the HPLC-fingerprint of the commercial product (*C. sativa* GM), obtained by the fresh buds, and the extract obtained from the corresponding marcs by PUAE, namely CA_R10. Table 2 resumes the content (expressed as mg/100 g of Fresh Weight buds/marcs) in the phytochemical classes both for the commercial products and for the marcs extracts respectively, in order to make a comparison. It is important to point out that about the 12% of the *C. sativa* GM TBCC was preserved in the marc extracts and could be recovered. Particularly, the cinnamic acids (20.18 ± 0.01 mg/100 g_{FW} for commercial product and 14.51 ± 0.01 mg/100 g_{FW} for CA_R10) and the vitamin C (18.08 ± 0.01 mg/100 g_{FW} for commercial product and 11.71 ± 0.01 mg/100 g_{FW} for CA_R10) contents followed by the flavonols (64.22 ± 0.04 mg/100 g_{FW} for commercial product and 18.10 ± 0.02 mg/100 g_{FW} for CA_R10) were more preserved in the marcs extract if compared to the other classes as highlighted in Table 2 and Fig. 4. For several years, biological protective effects of polyphenols and vitamin C have been mainly ascribed to their antioxidant and anti-inflammatory capacities. Studies have showed that phenolics may engage with cellular signalling flow, controlling transcription factor actions and subsequently affecting the expression of those genes involved in cellular metabolism and cellular survival (Donno, Mellano, Prgomet, & Beccaro, 2018). In this research, cinnamic acids, mainly represented by chlorogenic acid and vitamin C in the marcs extract were respectively the 71.92% and 64.76% of the correspondent *C. sativa* GM content. The other bioactive compound classes, as benzoic and organic acids, catechins, and tannins were identified and quantified in the marc extracts, but they showed lower values (about 5–15%) than the relative commercial products.

In this study a preliminary phytochemical fingerprint was obtained: adding other bioactive markers would be an important step for a better chromatographic pattern identification in further fingerprint studies together with a mass spectrometry detection of unknown peaks using LC–MS/MS as a very effective technique for complex herbal preparation analysis.

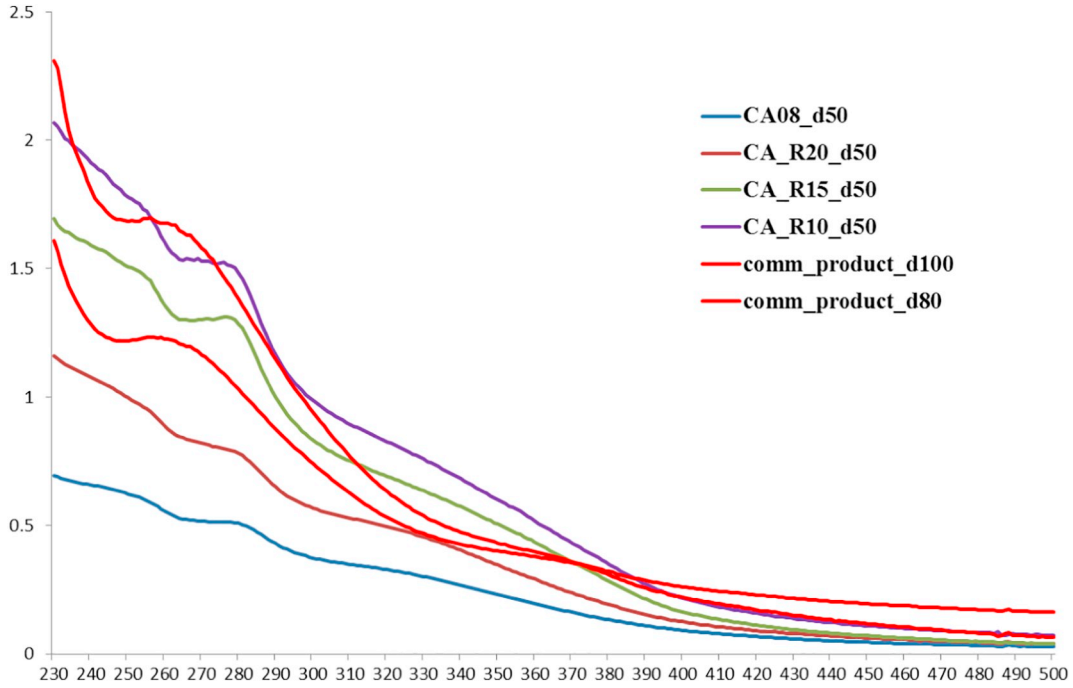


Fig. 3. UV–Vis averaged spectra (230–500 nm) of the following experiments: CA_R20, CA_R15, CA_R10 performed increasing the sample/solvent ratio (X4) are reported together with both CA08 spectrum and the commercial Glyceric Macerate spectra.

4. Conclusions

The valorisation of bud marcs remaining after the production of GMs, in this case study of *C. sativa*, could have a significant economic impact for the commercial producers, representing an important innovation in this sector.

For these reasons, an innovative and eco-compatible strategy to recycle these by-products, which proved to be still rich in bioactive

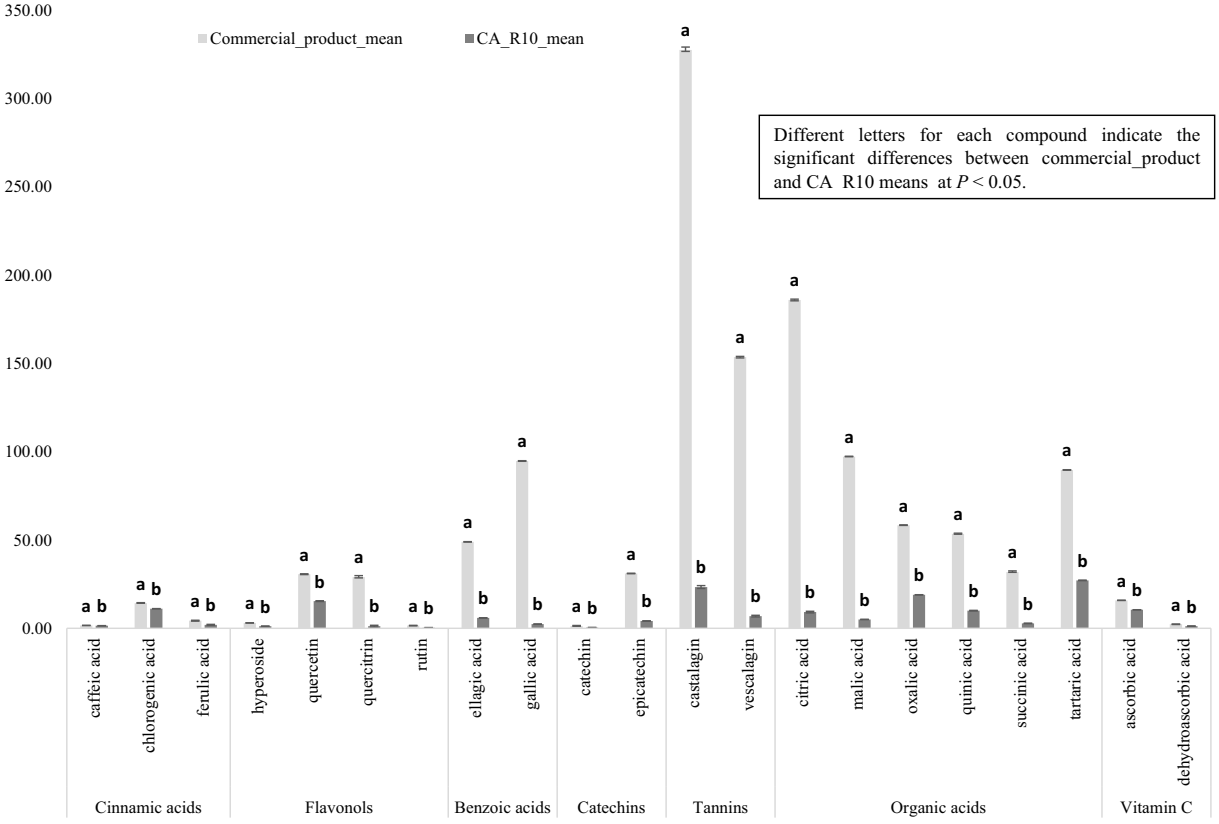


Fig. 4. HPLC-fingerprint of the *Castanea sativa* Glyceric Macerate and the most promising extract obtained by PUAE from the corresponding marcs, namely CA_R10. Results are expressed as mg/100 g of Fresh Weight buds/marcs. Mean values and error bars are reported.

Table 2
HPLC-fingerprint of the *Castanea sativa* commercial GM (Commercial_product) and the most promising extract obtained by PUAE from the corresponding marcs (CA_R10). Results are reported as mg/100 g of Fresh Weight buds/marcs and expressed as mean value ± interval confidence 95%.

		Commercial_product_mean	CA_R10_mean
Cinnamic acids	(mg/100 g_{FW})	20.18 ± 0.01[a]	14.51 ± 0.01[b]
Flavonols		64.22 + 0.04[a]	18.10 ± 0.02[b]
Benzoic acids		143.66 + 0.01[a]	8.23 ± 0.01[b]
Catechins		32.30 ± 0.01[a]	4.53 ± 0.01[b]
Tannins		481.22 ± 0.07[a]	30.27 ± 0.05[b]
Organic acids		516.51 ± 0.01[a]	73.06 ± 0.01[b]
Vitamin C		18.08 ± 0.01[a]	11.71 ± 0.01[b]
TBCC		1276.17 ± 0.13[a]	160.42 ± 0.09[b]

Different letters for each class indicate the significant differences between commercial_product and CA_R10 means at $P < .05$.

compounds, was develop as an alternative to incineration or composting, in order to obtain high added value products. PUAE, using the same solvent of GMs, have allowed to rapidly obtain an extract with a content in secondary metabolites of 160.42 mg/g of fresh weight marcs, which represents about the 12% of the corresponding commercial GM (1276.17 mg/g of fresh weight buds).

The procedure followed in this work could be considered as a promising and rapid tool to manage marcs coming from herbal preparations and it could be applied to other botanical productions.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2018.12.018.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

This work was financially supported by an European Union project called FINNOVER (n° 1198) an Interreg ALCOTRA Italy/France trans frontier project started in 2017 with the aim of innovating agro-industrial chains in terms of green circular economy.

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