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Towards a "dry" bio-refinery without solvents or added water using microwaves and ultrasound for total valorization of fruits and vegetables by-products.

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ABSTRACT: This study aims at total valorization of fruits and vegetables by-products moving towards developing an original concept of "dry" bio-refinery (DBR). Indeed, all valuable products were recovered from food by-products without addition of solvents or water and using green processes. Ginger was chosen as reference matrix since its juice processing generates a large amount of press cake currently considered as waste. Therefore, in this study, after juice processing, ginger press cake (GP) was firstly treated by microwave hydrodiffusion and gravity (MHG) process to recover essential oil (EO) and constituent water present in ginger by-products. Gingerols and 6-shogaol remaining into the ginger presscake residue after MHG (GPMHG) were then extracted by ultrasound assisted extraction (UAE) at different ultrasonic intensities (UI) using constituent water as solvent. The assessment of microwave (MW) power enabled to determine that a power of 1.6 W/g was optimal to recover constituent water and EO, preserving extract quality in a reduced time. The mass extraction yield was enhanced by UAE (16.7 W/cm^2; 0.303 W/cm^3) with an increase of 126 % compared to conventional maceration (CM). Total valorization of ginger by-products was achieved since juice, essential oil, extract rich in phenolics, and solid residue rich in fibers and phenolic acids were obtained from ginger rhizomes (GR) using "dry" bio-refinery without solvent and added water. Finally, the performances of "dry" bio-refinery and conventional bio-refinery (CBR) were compared in term of process time, energy consumption, quantity of waste and quantity of solvent.

1. Introduction

In 2012, the world vegetable and fruit production was 1,106,133,866 and 636,544,883 tons respectively (FAOSTAT-FAO statistical database 2015). Most of this production is destined to food processing industry which generates, after processing, a huge amount of by-products often considered as wastes, since they still constitute a resource for high-value compounds.[1] These high-value compounds provide a large field of application since they can be used for instance as antioxidants, natural chelating agents, or even as bio-solvents or bio-fuels after special treatment.[1,2] Therefore, the production of added products from industrial by-products is considered as a challenge for the current natural product industry and more generally for the extraction field.

Only a few studies have been investigating valorization of by-products. For example, using grape seeds issued from the wine-making industry to recover oil [3] and phenolic compounds,[4] orange peels from the orange juice industry for pectin and flavoring products.[5,6] More recently, the concept of bio-refinery of a plant is increasingly investigated for maximal valorization of natural products from a raw material.[1,7,8] Bio-refinery of natural products intends to value all bioactive compounds from a raw material, which implies to extract those bioactives using different extraction processes. However, an industrial application of bio-refinery would imply extensive use of solvents, high energy costs and extensive extraction duration. In this scope, the use of green extraction is an alternative for well-reasoned processing.[9]

In the general frame of green chemistry, green extraction processes focus on process intensification. The objective of these green extraction processes is to achieve faster extraction rate and more effective energy use, increased mass and heat transfer, reduced equipment size, and reduction of processing steps. For this, innovative technologies can be used such as microwaves,[10,11] ultrasound,[12,13] supercritical fluids,[14,15] electro-technologies [16,17]

or instantaneous controlled decompression DIC.[18,19] Those green extraction processes have proved their efficiency for extraction of natural products [20,21] but more rarely in the case of a bio-refinery.[22] A major interest would be to achieve a bio-refinery without the use of extraction solvents.

The reference matrix chosen for this study is ginger (2.1 million tons in 2012, FAOSTAT-FAO statistical database 2015), due to its composition in valuable natural compounds. It contains products of interest such as essential oil (1 – 4 %), phenolics (gingerols and 6-shogaol, 1 – 2 %), and total carbohydrates (60 – 75 %).[23,24] Ginger, and more specifically rhizomes are variously used as food product or traditional medicine.[25] In food industry, rhizomes are mainly used as spice or condiment (fresh or dried), candy or as juice after cold mechanical pressing. Due to the fact that mechanical pressing does not alter the chemical composition of the pressed product, this process provides huge amounts of press cakes still containing high amounts of bioactive compounds, but currently considered as waste.

Our study aims at total valorization of ginger rhizome press cake generated after juice production moving towards developing an original concept of "dry" bio-refinery (DBR). The novelty of this work relies on extraction of compounds achieved without addition of solvent or water. The only water used in the process was the constituent water extracted from ginger itself. To recover these different fractions, bio-refinery was applied using green extraction processes (microwave hydrodiffusion and gravity followed by ultrasound assisted extraction) and quality of the corresponding extracts was determined. Ultimately, the performances of bio-refinery using green extraction and conventional extraction were compared.

2. Materials and methods

2.1. Plant material and chemicals

Ginger rhizomes (GR) and ginger press cake (GP) were provided by Naturex. GP was obtained after industrial pressing of GR. Initial moisture was 10.7 % and 25.4 % for GR and GP respectively. GR was stored at 4 °C and GP was frozen (-18 °C) before use.

For extraction solvent, only demineralized water and absolute ethanol (Deulep, France) were used. For analysis, water, methanol, acetonitrile, acetone, phosphoric acid 85 %, pentane 98 % and diethyl ether >99 % analytical grade were purchased from Sigma Aldrich.

2.2. Procedures for extraction processes

A bio-refinery concept was developed for total valorization of ginger by-products. The aim of this DBR was recovering at the end of each consecutive step several high valued compounds, without addition of any external solvent or water. The "dry" bio-refinery (DBR) pattern is illustrated in figure 1. As described in the flow sheet, after pressing, GP was firstly submitted to microwave hydrodiffusion and gravity (MHG), followed by ultrasound assisted extraction (UAE). To characterize ginger by-products and to assess the performance of DBR, conventional processes (hydrodistillation and maceration) were performed as reference (figure 1). Microwave (MW) and ultrasound (US) equipments used in this study are presented in figures 2 and 3. Experimental conditions used for each process are described in this section.

2.3.1. Hydrodistillation (HD)

Hydrodistillation was performed as reference process for essential oil (EO) extraction. 1 kg of GP was submitted to HD using a Clevenger-type apparatus.[26] Extraction was performed with 4 L of water for 360 minutes until no more EO was obtained. Then EO was recovered and stored at 4 °C before analysis.

2.3.2. Microwave Hydrodiffusion and Gravity (MHG) apparatus and procedure

For each experiment using MHG, 500 g of GP were treated. Principle and apparatus are described in previous studies.[27,28] Extraction was performed in a microwave laboratory oven (900 W, EOS-GR Microwave Gravity Station, Milestone, Italy) at atmospheric pressure. MW power delivered to GP was varied between 0.6 W/g and 1.8 W/g. MHG process allows the recovery of a juice composed of EO and constituent water. In all extraction experiments, EO was collected and analyzed. Constituent water and ginger presscake residue after MHG (GPMHG) were recovered and stored at 4 °C before use. Each experiment was performed in duplicate.

2.3.3. Ultrasound Assisted Extraction (UAE)

20 g of GPMHG were placed in a double jacket reactor with 500 g of constituent water. The whole was submitted to US (1 kW, UIP 1000 hdT, Hielscher Ultrasonics GmbH, Germany) for 90 minutes. Ultrasonic intensity (UI) in W/cm^2 and power density (PD) in W/cm^3 were both considered to evaluate the ultrasonic power since literature shows that they were both adapted for such type of extraction.[29–32] Moreover, the use of W/cm^3 as unit is more appropriated whether further pilot and industrial up-scaling are envisaged. A range of ultrasonic amplitude was tested: 25 %, 50 %, 75 % and 100 %, corresponding to an UI (and the corresponding PD) of 4.4 W/cm^2 (0.080 W/cm^3), 9.4 W/cm^2 (0.170 W/cm^3), 13.4 W/cm^2 (0.242 W/cm^3) and 16.7 W/cm^2 (0.303 W/cm^3) respectively. UI (W/cm^2) was calculated according to the equation described by Pingret *et al.*.[33] US were applied to the system using a sonotrode immerged in the solvent. Temperature was maintained at 50 ± 5 °C with a cryostat (Alpha RA8, Lauda, Germany) and monitored with an external thermocouple. Plant material was homogenized in the solvent during UAE at 250 rpm with a magnetic stirrer (IKA RCT basic, VWR, France). Liquid samples were collected during the experiment (approximately 2 mL) and filtered on cotton before drying in oven at 100 °C to determine dry matter content.

After extraction, remaining solvent enriched with the extract was separated from the plant material residue by centrifugation at 4000 rpm for 20 minutes (Himac CT6E, VWR by Hitachi Koki Co., Ltd., USA) and filtration under vacuum using a filter paper. Extract was recovered from filtrate by solvent evaporation under vacuum. The extract was stored at 4 °C before analysis. Each experiment was performed in duplicate. For assessment of UAE effect on extraction, a conventional maceration (CM) was performed by mechanical stirring using identical extraction conditions as UAE.

2.3. Characterization and analysis of plant material

GR, GP and GPMHG were submitted to solvent extraction followed by HPLC-DAD analysis to determine the available gingerols and 6-shogaol content in each. For all characterizations, plant materials (GR, GP and GPMHG) were previously freeze-dried and ground below 3 mm. Phenolics extraction was performed according to the procedure described by Mukherjee *et al.* [34] where experimental conditions were optimized. Extraction of gingerols and 6-shogaol from GR, GP and GPMHG obtained at different MW power was performed at 40 °C for 60 minutes under mechanical stirring (IKA Eurostar 20 digital, Germany) in a double jacket reactor. Temperature was maintained at 40 °C using a cryostat (Alpha RA8, Lauda, Germany). Extraction solvent was ethanol/water, 75/25 (v/v). Extraction was performed using a solid/liquid ratio of 1/15 (w/w). After extraction, the liquid phase was separated from the matrix by filtration under vacuum using a 15-35 µm paper filter. Extract was recovered from filtrate by solvent evaporation under vacuum. The extract was stored at 4 °C before analysis.

149 *2.4. High performance liquid chromatography analysis (HPLC-DAD)*

150 Quantification of gingerols (6-gingerol, 8-gingerol, 10-gingerol) and 6-shogaol was
151 done by HPLC (Agilent 1100, France) equipped with diode array detector (DAD). The
152 method described below was developed and validated internally.

153 The column used was a C18 column (5 µm, 4.6 mm x 250 mm, Advanced
154 Chromatography Technologies ACE, Scotland). The mobile phase was composed of two
155 solvents: (A) 100 % acetonitrile and (B) 100 % water with 0.05 % phosphoric acid (v/v). The
156 gradient of solvent was used as follows: 0 minute, 45 % (A), 55 % (B); 5 minutes, 45 % (A),
157 55 % (B); 10 minutes, 50 % (A), 50 % (B); 20 minutes, 55 % (A), 45 % (B); 40 minutes,
158 90 % (A), 10 % (B); 45 minutes, 45 % (A), 55 % (B); 55 minutes, 45 % (A), 55 % (B). The
159 flow rate was set at 1 mL/min. The column oven temperature was 20 °C and the run time was
160 30 minutes. 20 µL were injected. Gingerols and 6-shogaol were detected at a wavelength of
161 282 nm and quantified using external calibration with standards.

162 *2.5. Gas chromatography analysis (GC-FID)*

163 Aromatic profile of ginger EO was done by GC (Agilent 7890, France) equipped with
164 flame ionization detector (FID). The method described below was developed and validated
165 internally. The column used was a VF-5MS column (0.25 µm, 0.25 mm x 30 m, Agilent
166 Technologies, France). The column temperature was 60 ° C for 1 minute, increased at
167 3 °C/min to 240 °C, and was kept at 240 °C for 5 minutes. Split ratio was 1:100 and helium
168 flow rate was 1.1 mL/min with a constant flow. FID detection was performed at 250 °C.
169 Identification was performed by corresponding individual standards retention times and the
170 aromatic profiles were determined by comparison between relative areas on the
171 chromatogram.

172

3. Results and discussion

3.1. Dry extraction of essential oil from ginger press cake by MHG

In the concept of DBR developed in this study (figure 1), MHG was chosen as a "green" process for the recovery of EO and constituent water from GP, as no solvent had to be added for extraction. MHG allows direct extraction of a juice composed of EO and constituent water. Both compounds were therefore extracted at the same time and further separated by gravity due to density difference. Microwave extraction of essential oils using "constituent water" may occur by a mechanism based on the influence of molecules polarity. Essential oils contain organic compounds that strongly absorb microwave energy such as oxygenated monoterpenes. Microwaves interact with organic molecules present in the glands and vascular systems. Thus, such systems undergo a dramatic expansion, with subsequent rupture of the tissue, allowing the essential oil to flow towards the gland layer. Compounds with high and low dipolar moments could be extracted in various proportions by microwave extraction. Organic compounds that have a high dipolar moment will interact more vigorously with microwaves and can be extracted more easily in contrast with aromatic compounds, which have low dipolar moments.

3.1.1. Impact of microwave power on MHG extraction efficiency of essential oil and constituent water

Several powers were assessed in order to evaluate the impact of MW power on extraction efficiency of EO and constituent water from GP. Literature reports that MHG is optimally used with a power of 1 W per gram of plant material.[27] Yet, this aspect can be discussed because of the large variety of plant material which has probably not the same behavior regarding microwaves energy. In this study, an assessment of different MW powers was thus performed to extract as quickly as possible constituent water and EO from GP.

MW power was varied from 0.6 W/g to 1.8 W/g of GP. Global volume of water and EO recovered was measured at different extraction durations (figure 4). The low volume of EO extracted did not allow an accurate measurement of EO extraction kinetics. Extraction was stopped just before thermal degradation of GP. The beginning of thermal degradation was determined performing a temperature monitoring into the press cake. As shown in figure 5, during microwave heating, (i) temperature into the biomass firstly increases linearly -more or less quickly depending on microwave power- until 100 °C (boiling point of water); (ii) then temperature remains constant at 100 °C; (iii) finally temperature presents an inflection point and begins to increase beyond 100 °C. This last stage is considered as the beginning of thermal degradation, which is accompanied by the burn of biomass submitted to microwaves.

The increase in MW power from 0.6 W/g to 1.8 W/g led to the same final volume of constituent water and EO extracted from GP (1 ± 0.1 mL of EO and 300 ± 10 mL of constituent water). As it can be noticed in figure 4, the increase of MW power from 0.6 W/g to 1.8 W/g enabled a considerable reduction of extraction time as well: 83 minutes against 20 minutes for 0.6 W/g and 1.8 W/g respectively. Therefore, the time needed to recover the condensate composed by EO and constituent water was directly dependent on MW power as the former increased with the latter.

Analysis of the different EO and GPMHG recovered after MHG were performed to assess a potential change in their composition according to MW power.

3.1.2. Evaluation of essential oil quality

Table 1 summarizes the results regarding extraction yields and composition of EO and phenolics obtained for GR, GP and GPMHG. The first part of the table refers to the results obtained regarding EO. Extraction yields and aromatic profiles were compared between EO obtained by HD of GR and GP (table 1, first and second columns), and EO obtained by MHG

221 treatment of GP at different powers (table 1, third to ninth columns). First of all, extraction of
222 EO from GP by HD was more efficient than extraction of EO from GR (0.3 g EO/100 g GP
223 and 0.2 g EO/100 g GR). Previous industrial pressing of GR may have caused the de-
224 structuration of rhizomes and therefore may have improved EO availability. It can be noticed
225 as well that MW power had not any effect on EO extraction yields as 0.2 g EO/100 g GP were
226 recovered in all cases. MHG extraction of EO from GP appeared as less efficient than HD of
227 GP, however this result is not very accurate since the design of glassware in the MW
228 laboratory oven provided to recover totally the EO extracted (EO drops remained on reactor
229 walls).

230 Considering composition, zingiberene is generally considered as a characteristic
231 compound in ginger EO. In literature, it is mainly found between 20 % and 30 % in EO.[35,36]
232 GC-FID analysis of EO obtained by HD was in accordance with literature as zingiberene
233 content was 25.2 %. In EO obtained by MHG, zingiberene percentage was constant for
234 powers from 0.8 W/g to 1.6 W/g (medium powers) with a content of 23 to 25 %. However,
235 for extreme powers (0.6 W/g and 1.8 W/g), zingiberene content decreased significantly (both
236 18.4 %).

237 The differences of aromatic profiles between EO obtained by the reference process
238 and MHG indicate that the extraction process impacts EO quality. This result has already been
239 shown and explained in previous works. For example, it is reported that the contact between a
240 plant material and the solvent during the process can lead to EO degradation.[37] From our
241 results, it can be concluded that aromatic profiles of EO were similar for MHG extraction
242 conditions except for 0.6 W/g and 1.8 W/g experiments. For these last powers, long extraction
243 time (90 minutes) and intense MW irradiation (1.8 W/g) respectively could induce a
244 degradation of some compounds in EO.[38]

245

246 *3.1.3. Impact of microwave pretreatment on phenolics extraction efficiency*

247 Constituent water recovered after MHG was analyzed by HPLC to determine its
248 content in gingerols (6-gingerol, 8-gingerol and 10-gingerol) and 6-shogaol. Those
249 compounds are specific phenolics of ginger, 6-shogaol being a degradation product of 6-
250 gingerol by dehydration.[39] They were not detected in the constituent water so we admitted that
251 all phenolics remained into GPMHG. GPMHG obtained after MHG process at different
252 powers and initial GP were characterized as described in section *2.3* in order to show a
253 potential effect of MHG treatment on phenolics content, particularly a potential degradation
254 of these compounds. A characteristic HPLC chromatogram of extracts is illustrated in
255 figure 6. The results are presented in table 1. It can be noticed that generally, MHG treatment
256 did not cause the degradation of gingerols and 6-shogaol when comparing results obtained for
257 GP and GPMHG. They were even better extracted when MHG treatment was performed
258 (0.90 g/100 g of GP and from 1.06 to 1.37 g/100 g of GPMHG). As described by Zill-e-Huma
259 *et al.*,[27,40] MW seem to alter cell walls of ginger, so gingerols and 6-shogaol were more
260 available in GPMHG than in GR and GP for extraction. It can be underlined that 6-shogaol
261 content in GPMHG was higher than in GP (0.08-0.11 % against 0.02 % respectively),
262 certainly due to high temperature associated to MHG process. However these amounts of 6-
263 shogaol were insignificant compared to contents in gingerols recovered (for GPMHG at
264 1.6 W/g: 0.08 % of 6-shogaol and 1.37 % of gingerols in plant material). Extraction at power
265 beyond 1 W/g did not involve the degradation of phenolic compounds in GPMHG as
266 described for onion polyphenols in previous work.[27]

267 The previous results enabled to select a MHG power of 1.6 W/g as optimal for the
268 second step of our "dry" bio-refinery (figure 1) since this power enabled to recover total
269 removable water and EO in 20 minutes, preserving EO quality and without degradation of

phenolics in GPMHG. Therefore the recovery of these preserved phenolics from GPMHG will constitute the third step of the "dry" bio-refinery developed in the study.

3.2. Ultrasound assisted extraction (UAE) of gingerols and 6-shogaol from GPMHG

Phenolic compounds are conventionally extracted from ginger with 75 % ethanol.[34] However, industrials are looking for green processes to extract bioactives from plants without addition of organic solvent. In this work, an alternative process has been investigated to extract phenolics from GPMHG by using only water as solvent and more specifically constituent water previously recovered from GP by MHG (figure 1). Solubilities of 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol were predicted with ACD-Lab software as 0.26 g/L, 0.038 g/L, 0.91 mg/L and 0.0046 g/L respectively, which shows that water could be used as an alternative solvent to solubilize these compounds during UAE.

UAE is a process which is used to increase extraction yield of various phytochemicals.[21,41] US emitted by probe or in bath generate microbubbles which alter vegetal cells by cavitation phenomenon enhancing extraction of targeted compounds.[42] US were applied to GPMHG in water at different UI (with the corresponding PD): 4.4 W/cm^2 (0.080 W/cm^3), 9.4 W/cm^2 (0.170 W/cm^3), 13.4 W/cm^2 (0.242 W/cm^3) and 16.7 W/cm^2 (0.303 W/cm^3). A CM was performed as reference. A monitoring of dry matter content in the liquid phase was carried out to compare the kinetics of solubilization of dry matter according to the UI. Results obtained are presented in figure 7.

Until 25 minutes of extraction, dry matter evolution followed the same trend for each UI assessed. Beyond 25 minutes of UAE, no difference was noticed between CM, UAE (4.4 W/cm^2; 0.080 W/cm^3) and UAE (9.4 W/cm^2; 0.170 W/cm^3) as they all reached 0.20 to 0.24 % of dry mass content in extract after 90 minutes. A significant increase was observed for UAE (13.4 W/cm^2; 0.242 W/cm^3) after 90 minutes with a dry mass content of 0.35 %. The

higher yield was reached with UAE (16.7 W/cm^2; 0.303 W/cm^3) since an extract with a dry mass content of 0.48 % was recovered at the end of experiment. At the end of each experiment, liquid extract was separated from the solid residue by filtration on filter paper and concentrated by water removal. Mass extraction yields were calculated from final dry masses and reported on the figure 8. US with a high UI (13.4 W/cm^2; 0.242 W/cm^3 and 16.7 W/cm^2; 0.303 W/cm^3) had a positive impact on the mass extraction yield with an increase of 126 % from CM to UAE (16.7 W/cm^2; 0.303 W/cm^3). This increase in mass extraction yield could be due to a solubilization of some natural polymers which have been partially disintegrated by US and solubilized into water. Indeed, ultrasonic processes have been reported to impact cell wall polymers such as cellulose, hemicellulose and pectin and non-structural polymers such as starch.[43–46] The degradation results in a modification of macromolecular structures and a decrease of molecular weight which leads to an improvement of the solubilization of polymers. However, US effect has to be assessed on more complex structures since it is not obvious that these simplified models described for US effect on single polymers would be valid for plant materials, which are composed of a large network of various polymers.

On figure 8, quantities of phenolics extracted from plant material are also reported. It can be noticed that phenolics content in extract did not increase as much as the global dry mass: quantity of phenolics extracted was improved by 29 %, by comparing CM to UAE (16.7 W/cm^2; 0.303 W/cm^3). Quantity of gingerols and 6-shogaol available in GPMHG is reported in table 1. This value was determined as 1.37 % of GPMHG. As shown in figure 6, 0.36 % over 1.37 % gingerols and 6-shogaol were recovered from GPMHG by UAE (16.7 W/cm^2; 0.303 W/cm^3), that is only 26 % of available phenolics in GPMHG. From those results, it can be concluded that UAE can increase mass extraction yield, which could be due to a degradation and solubilization of macromolecules such as fibers. However, US did not

appear as the process of choice to extract gingerols and 6-shogaols into water since 74 % of available phenolic compounds remained in the solid residue.

3.3. Large scale microwave and ultrasound assisted extractions

Pilot scale experiments were performed for MHG using the MAC-75 equipment (figure 2b). MAC-75 apparatus is a multimode microwave reactor which contains 4 magnetrons (4 x 1500 W, 2450 MHz) with a maximum power of 6 kW. Contrary to laboratory scale equipment (EOS-GR Microwave Gravity Station), MAC 75 equipment contains a removable and rotating PTFE drum where plant material can be loaded. The rotation ensures a homogeneous microwave distribution to the material treated. The aim of this part was to check whether larger scale experiments could be possible for our study. It is not really an "up-scaling" since the volume of plant material which can be treated and the microwave power were at most 75 L and 6 kW respectively. Approximately 4 kg of press cake were therefore submitted to microwaves during 25 min and condensate (essential oil and constituent water) was recovered at the end of experiment as it was done at laboratory scale. Several food by-products (garlic, onion and ginger press cake) were tested in addition to ginger by-products to validate the method. In all cases, a condensate rich in compounds of interest was recovered, what indicates that MHG process can be considered at pilot scale. For industrial scale, MHG equipment has to be designed totally since no equipment is available for now. However, as a follow-up to that study, an industrial up-scaling is currently studied to use microwave technology for by-products valorization. For UAE, a 30 L extraction tank from REUS company can be used to up-scale laboratory experiments (figure 3b). The reactor is composed of a quadruple output of ultrasound at 25 kHz and a power of 4 x 200 W. Up-scaling using this equipment has already been studied in previous studies and showed that extraction assisted by ultrasound is promising technique that can be considered at industrial scale, especially when water is chosen as solvent.[47]

3.4. Process assessment according to the six principles of eco-extraction

A process assessment of the "dry" bio-refinery (DBR) developed in this work was performed and compared with a conventional bio-refinery (CBR) composed of an HD step for the recovery of EO and an ethanolic extraction step for the extraction of antioxidants from ginger (figure 9). The bio-refineries were evaluated according to the six principles of green extraction developed by Chemat *et al.*.[9] Indeed, extraction methods are designed considering these aspects which aim at recovering a natural and safe extract (principle 6) reducing as much as possible the use of organic solvents (principle 2), the energy consumption (principle 3) and the process time (principle 5). Well-reasoned sourcing (principle 1) and production of by-products with a high added value instead of waste (principle 4) have to be assessed as well. Literature reports that industrials have already developed some tools based on these principles to assess the sustainability of their processes in a context of continuous improvement.[48] In this study, a simplified view of the bio-refineries was assessed. The six parameters considered were defined and calculated as follows:

- **Raw material** (Principle 1): percentage of valorized raw material from food processing industry (in %)
- **Solvent** (Principle 2): (mass of ethanol) / (total mass of solvent used for the bio-refinery) (in %)
- **Energy** (Principle 3): energy consumption for the bio-refinery of 1,150 kg of raw material considering extraction and evaporation steps based on the energy transfer equation [26] (in kWh)
- **Waste** (Principle 4): (mass of waste) / (total mass of solvent + raw material used in the process) (in %)
- **Process** (Principle 5): extraction duration for the bio-refinery (in minutes)

- **Product recovery** (Principle 6): (mass of final product recovered) / (mass of available product in the plant material)

On figure 9, it is important to notice that for each principle, a value close to the center is a positive result whereas a value far from the center corresponds to a negative result. Thus, for "Product recovery", the center corresponds to a recovery of 100 %. Concerning "Energy" and "Process", the maximal values reported on the axis correspond to the values obtained with the CBR.

Compared to HD and ethanolic extraction, MHG and UAE enabled to reduce extraction time from 540 minutes to 110 minutes. Moreover, in the DBR, no waste was generated as illustrated in figure 1, contrary to CBR for which water from HD was considered as waste as it was thrown at the end of extraction. Energy consumption was reduced as well, especially with the replacement of HD by MHG (8.5 kWh and 13.5 kWh for DBR and CBR respectively). Another positive effect of DBR compared to CBR is the absence of organic solvent in the process since none solvent needed to be use for MHG and only constituent water recovered after MHG was employed for UAE. However, DBR was not as efficient as CBR in terms of extraction yields, since a reduction of 55 % for final products recovered was observed for DBR compared to CBR (reduction by 74 % for antioxidants and by 33 % for EO). Yet, DBR was designed to valorize totally ginger by-products with successive and dependent steps whereas in CBR, EO and phenolics were recovered separately and independently by HD and ethanolic extraction respectively. These processes correspond to the processes of reference to recover these compounds that's why better yields were obtained compared to DBR. Finally, the reduced cost of extraction is clearly advantageous for the proposed "dry" bio-refinery method in terms of time and energy.

4. Conclusion

This study aims at total valorization of ginger by-products moving towards developing an original concept of "dry" bio-refinery (DBR). EO was recovered from GP by MHG without solvent and extraction of antioxidants from GPMHG was carried out by UAE using constituent water of GP obtained after MHG as extraction solvent. Larger scale experiments enabled to show that MHG and UAE are promising techniques which can be considered at pilot scale. Although the effect of US was not significant for extraction of gingerols and 6-shogaol from GPMHG compared with a conventional maceration, US considerably improve the mass extraction yield, as a rise of 126 % was noticed between CM and UAE (16.7 W/cm^2; 0.303 W/cm^3). The DBR also appeared as a greener and cleaner process in contrast with a CBR since extraction time, energy consumption, quantity of organic solvent and waste were decreased. Despite that extraction performance was reduced (decrease of extraction yields by 33 % for EO and by 74 % for antioxidants) compared to a CBR, the objective of the study is achieved since a total valorization of ginger by-products into high valued products was performed without addition of any solvent. Indeed, from GR were obtained a juice, an essential oil, an extract rich in phenolics, and a solid residue rich in fibers and phenolic acids, which can be thereafter incorporated in food formulations.

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496 **Table captions :**

497 Table 1: Volatile compounds and antioxidants extracted from ginger plant material.

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499

			GR	GP	GPMHG						
					0.6 W/g	0.8 W/g	1.0 W/g	1.2 W/g	1.4 W/g	1.6 W/g	1.8 W/g
Essential oil	Yield (g/100g fresh plant material)		0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Major compounds (%)	α-pinene	1.2	1.0	2.3	2.6	2.4	2.6	2.3	2.4	2.2
		camphene	4.3	3.8	9.1	10.3	9.2	10.0	9.1	9.4	9.1
		sabinene	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
		sulcatone	0.0	0.8	1.2	2.8	3.3	3.2	3.0	3.2	2.9
		myrcene	0.6	0.6	0.0	1.4	1.4	1.4	1.3	1.3	1.1
		α-phellandrene	0.2	0.1	0.2	0.3	0.3	0.3	0.3	0.3	0.1
		limonene	0.9	0.9	1.7	1.9	1.9	1.9	1.7	1.8	1.7
		β-phellandrene	4.6	4.2	8.7	10.4	10.3	10.2	9.7	10.0	8.6
		terpinolene	0.1	0.1	0.1	0.1	0.3	0.3	0.3	0.3	0.2
		linalol	0.2	0.2	0.3	0.4	0.4	0.4	0.4	0.4	0.4
		borneol	0.5	0.6	0.8	0.9	1.0	0.9	1.0	1.0	1.1
		α-terpineol	0.2	0.3	0.4	0.5	0.5	0.5	0.5	0.5	0.6
		citronellol	0.1	0.3	0.2	0.5	0.4	0.3	0.4	0.4	0.8
		neral	1.7	0.5	0.4	1.3	1.5	1.7	1.5	1.5	1.3
		geraniol	0.1	0.2	0.1	0.3	0.3	0.2	0.2	0.2	0.6
		geranial	3.3	1.0	0.6	1.9	2.2	2.6	2.3	2.5	2.3
		geranyl acetate	0.3	0.1	0.4	0.2	0.2	0.2	0.2	0.2	0.2
		α-curcumene	3.5	13.9	17.0	7.6	7.2	6.6	7.0	6.8	9.9
		germacrene D	1.6	1.3	0.1	1.3	1.4	1.4	1.4	1.4	0.7
		zingiberene	35.7	25.2	18.4	23.2	24.0	24.0	25.1	24.3	18.4
		α-farnesene	6.5	6.5	6.3	5.4	5.5	5.5	5.7	5.5	5.7
		β-bisabolene	5.7	6.8	0.0	4.8	4.7	4.6	4.8	4.7	5.4
		β-sesquiphellandrene	12.1	13.9	12.3	9.9	9.9	9.7	10.2	9.8	10.4
Antioxidants	Total content (g/100 g plant material DW)		1.17	0.90	0.57	1.24	1.06	1.18	1.22	1.37	1.18
	Major compounds (g/100 g plant material DW)	6-gingerol	0.77	0.58	0.31	0.81	0.65	0.79	0.81	0.92	0.79
		8-gingerol	0.15	0.11	0.07	0.14	0.11	0.14	0.14	0.17	0.14
		10-gingerol	0.23	0.19	0.11	0.18	0.19	0.19	0.19	0.21	0.19
		6-shogaol	0.02	0.02	0.08	0.11	0.10	0.08	0.09	0.08	0.08

500
501 DW: Dry weight

502 Table 1: Volatile compounds and antioxidants extracted from ginger plant material.

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Figures captions:

Figure 1: Flow sheet of processes used in the study for total valorization of ginger by-products.

Figure 2: Microwave Hydrodiffusion and Gravity (MHG): from laboratory (a) to pilot scale (b).

Figure 3: Ultrasound assisted extraction (UAE): from laboratory (a) to pilot scale (b).

Figure 4: Effect of MW power on quantity of constituent water recovered by MHG.

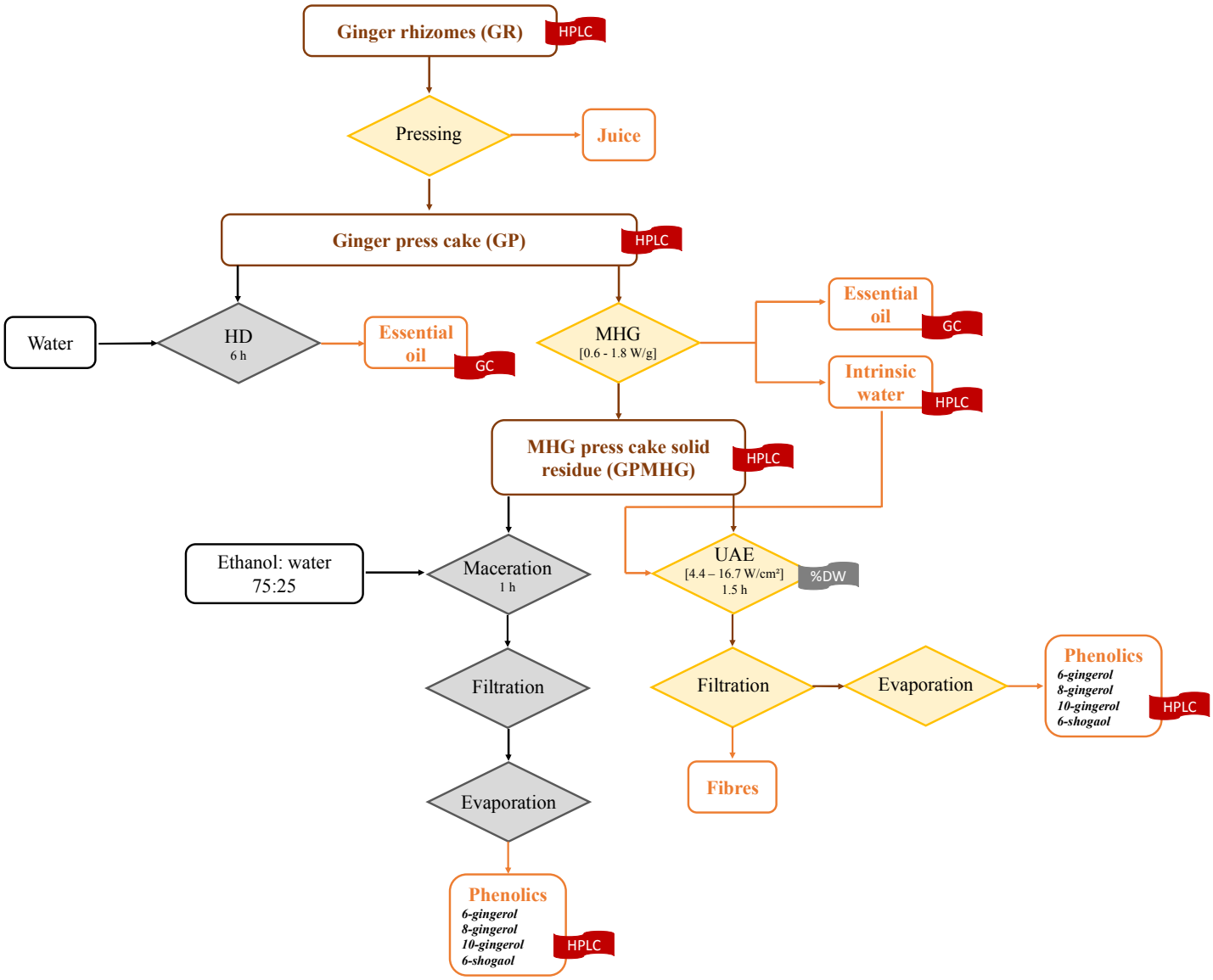
Figure 5: Evolution of temperature in the matrix submitted to microwaves (1.6 W/g).

Figure 6: Characteristic HPLC-DAD chromatogram of a ginger extract at 282 nm.

Figure 7: Evolution of extract's dry weight as a function of ultrasonic intensity (and power density).

Figure 8: Effect of US on extraction yield and gingerols and 6-shogaol content in the extracts.

Figure 9: Process assessment of "dry" bio-refinery and conventional bio-refinery according to the six principles of eco-extraction.



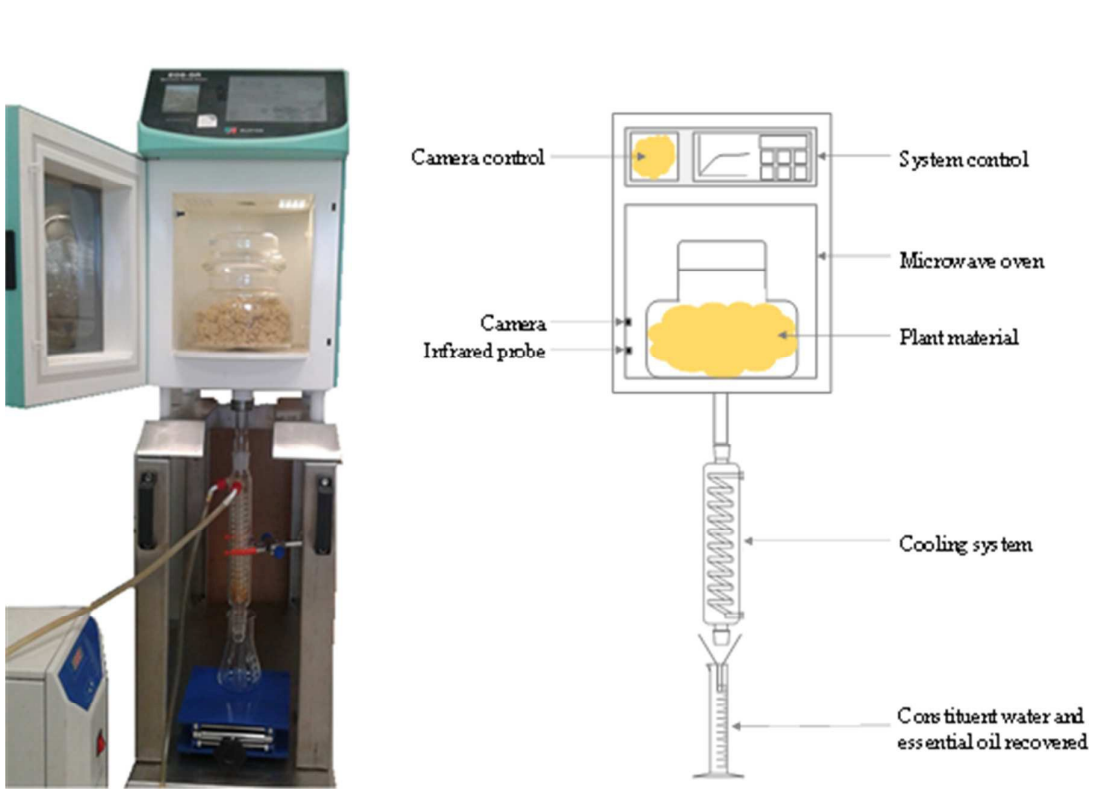
Conventional bio-refinery

"Dry" bio-refinery

Figure 1: Flow sheet of processes used in the study for total valorization of ginger by-products.

MHG: Microwave Hydrodiffusion and Gravity; UAE: Ultrasound assisted extraction; DW: Dry weight.

1



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(a) (b)

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4 Figure 2: Microwave Hydrodiffusion and Gravity (MHG): from laboratory (a) to pilot scale (b).

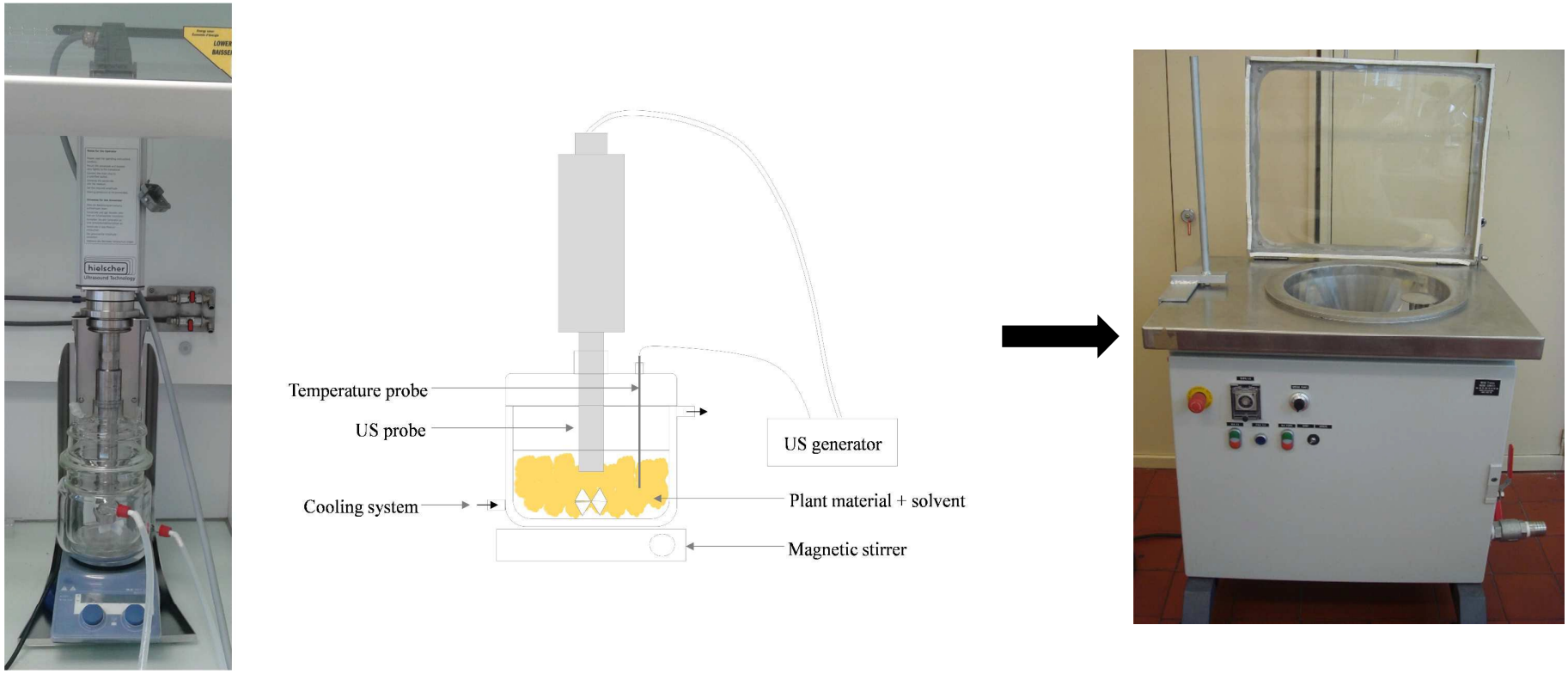

Figure 3: Ultrasound assisted extraction (UAE): from laboratory (a) to pilot scale (b).

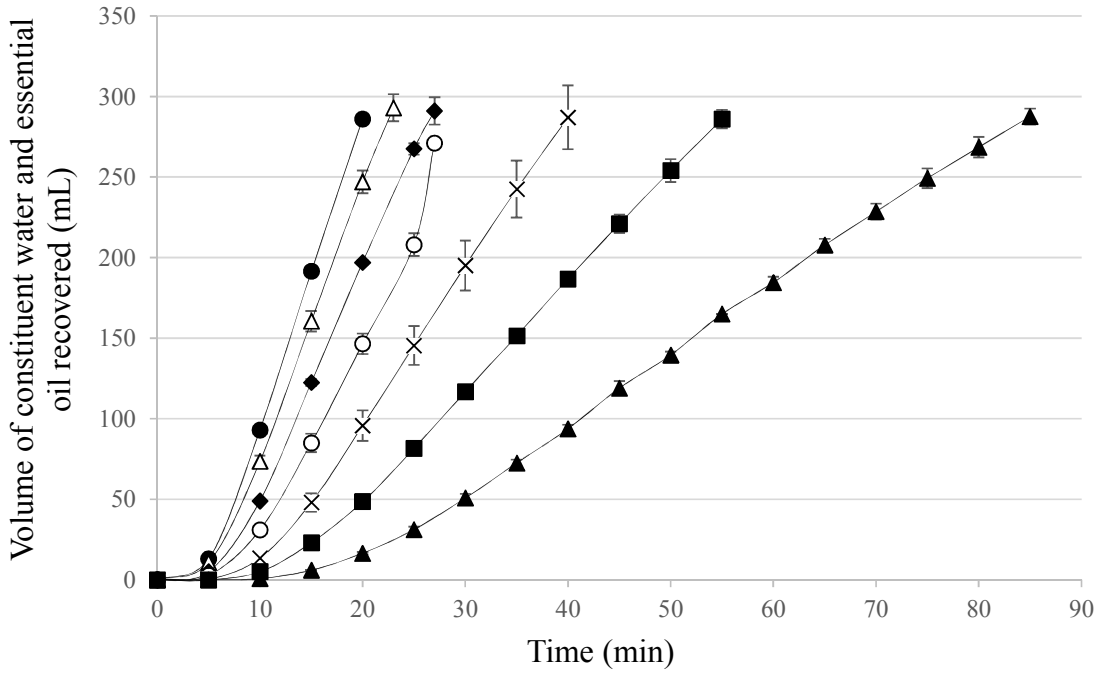

Figure 4: Effect of MW power on quantity of "*in situ*" water recovered by MHG.

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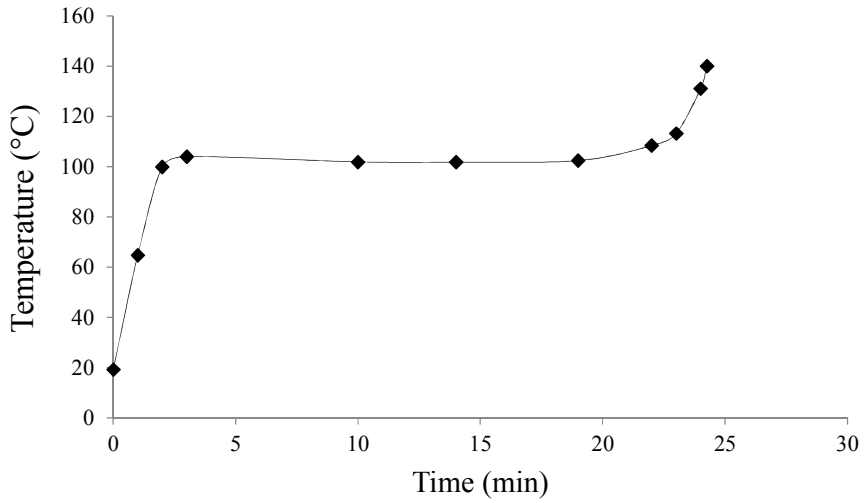

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3 Figure 5: Evolution of temperature in the matrix submitted to microwaves (1.6 W/g).

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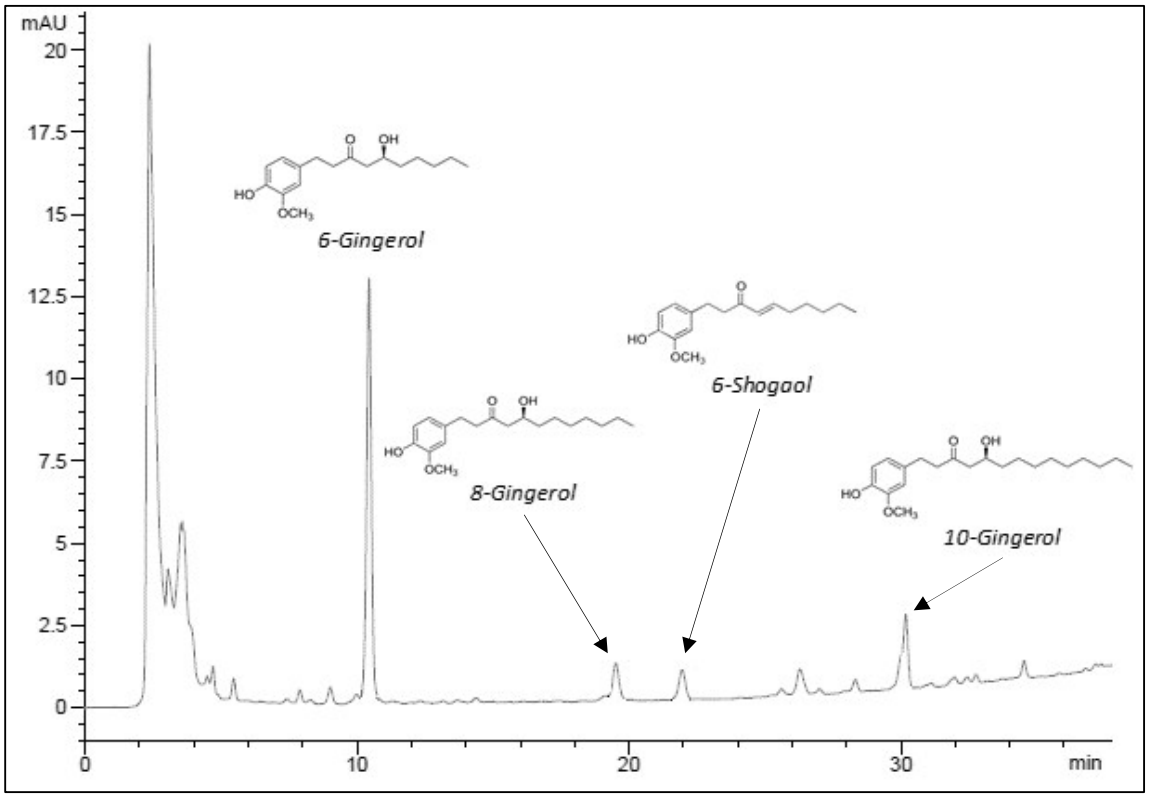

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3 Figure 6: Specific HPLC-DAD chromatogram of a ginger extract at 282 nm.

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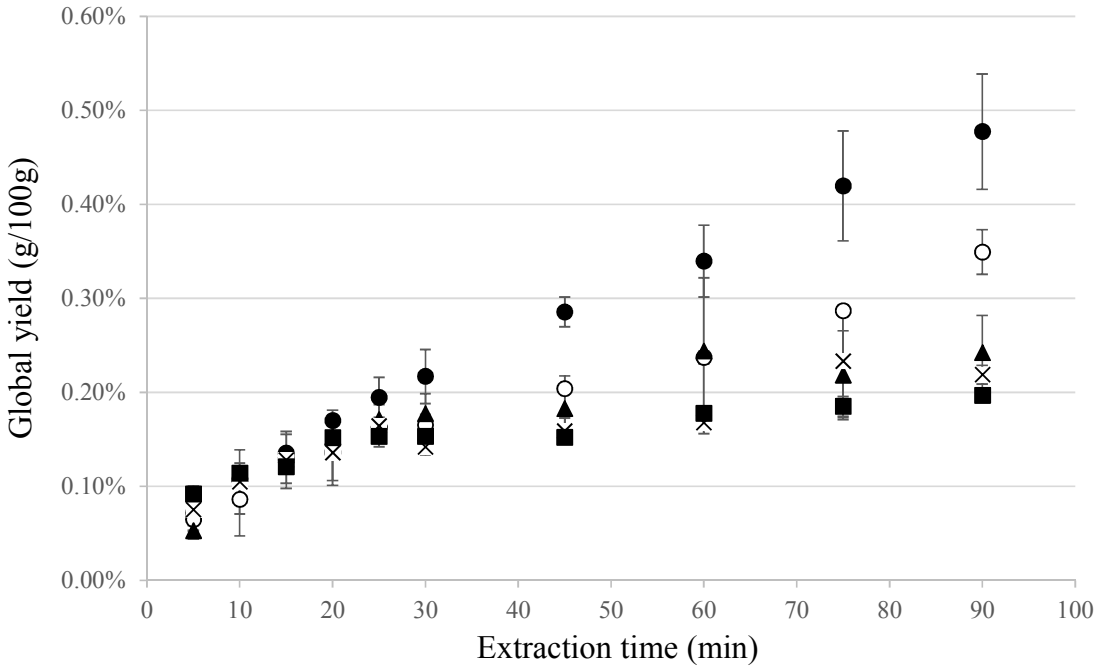

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3 Figure 7: Evolution of extract's dry weight as a function of ultrasonic intensity (and power

4 density).

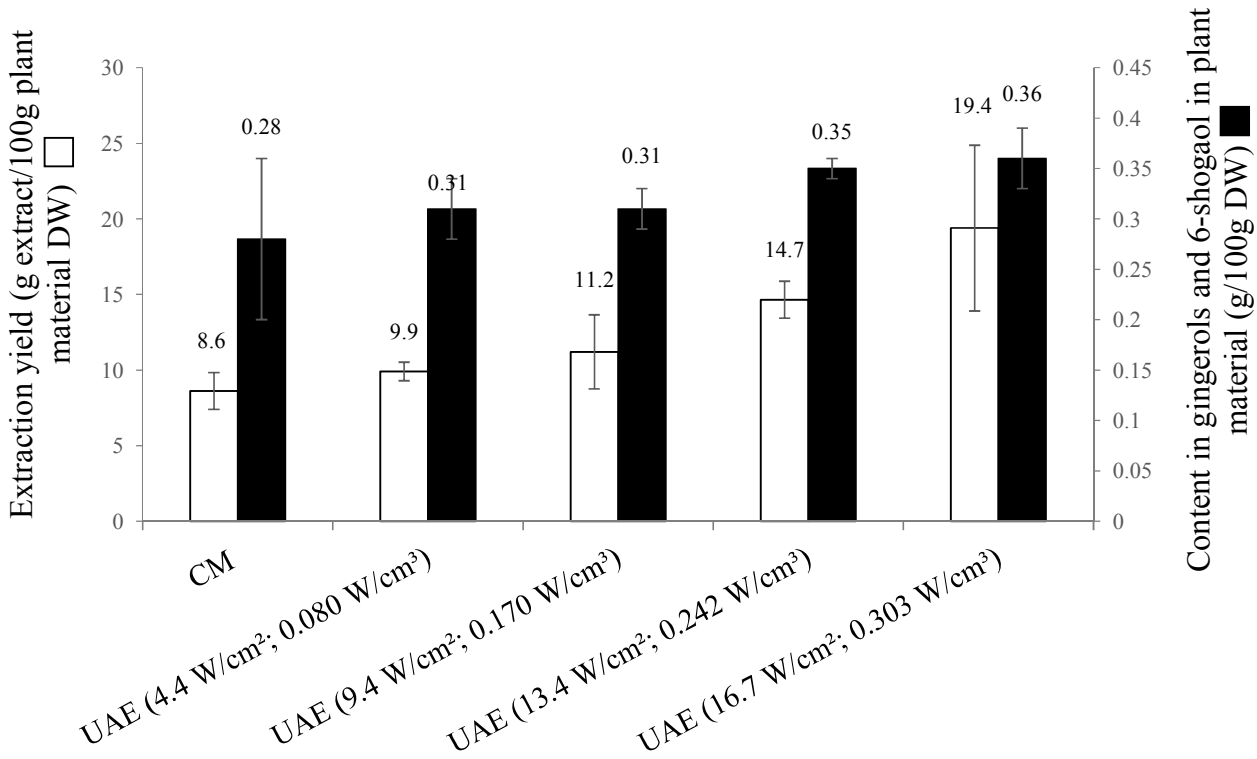
■ CM ×UAE (4.4 W/cm²; 0.080 W/cm³)

▲ UAE (9.4 W/cm²; 0.170 W/cm³) ○ UAE (13.4 W/cm²; 0.242 W/cm³)

● UAE (16.7 W/cm²; 0.303 W/cm³)

5

6



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2 Figure 8: Effect of US on extraction yield and gingerols and 6-shogaol content in the extracts.

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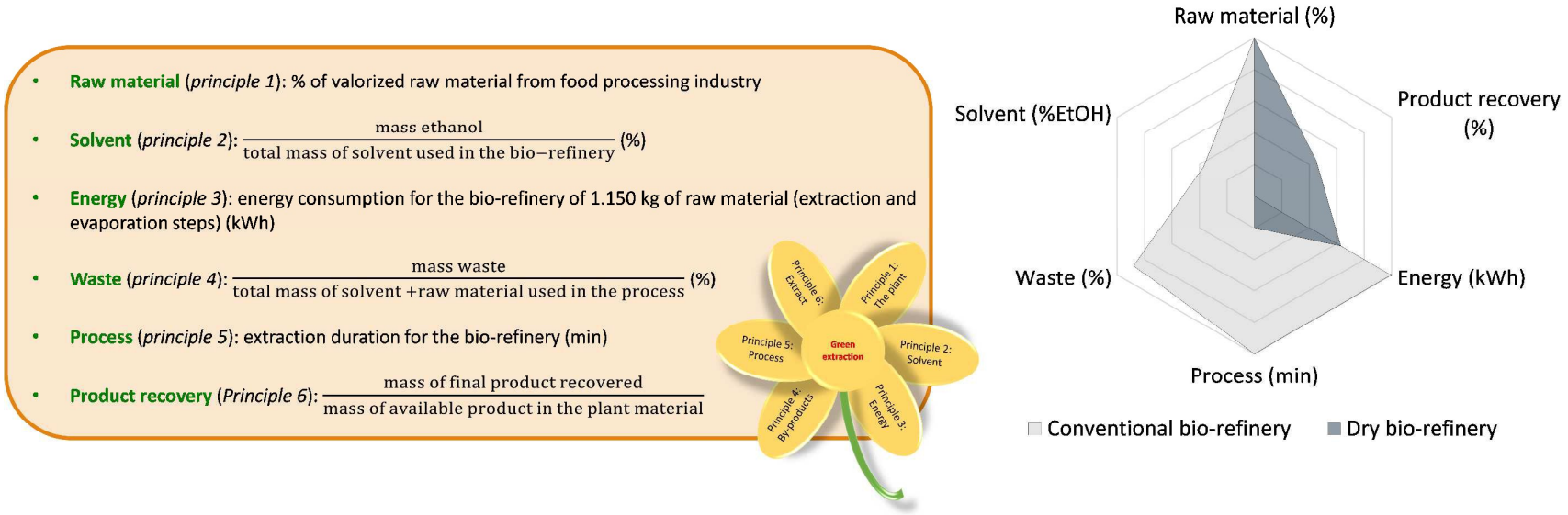

Figure 9: Process assessment of "dry" bio-refinery and conventional bio-refinery according to the six principles of eco-extraction.

Graphical Abstract