Intensification of natural products extraction processes : innovative processes and alternative solvents

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- M. Jacotet-Navarro, N. Rombaut, S. Deslis, A.-S. Fabiano-Tixier, F.-X. Pierre, A. Bily, F. Chemat, Towards a "dry" bio-refinery without solvents or added water using microwaves and ultrasound for total valorization of fruit and vegetable by-products, Green Chem. 18 (2016) 3106–3115.

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- M. Jacotet-Navarro, A-S. Fabiano-Tixier, A. Bily, F. Chemat, *Life Cycle Assessment (LCA) as a tool for green extraction of natural products*, GENP2016 - Green Extraction of Natural Products - II Edition, May 31st - June 2nd 2016, Turin – Italy.

- M. Jacotet-Navarro, N. Rombaut, S. Deslis, A.-S. Fabiano-Tixier, F.-X. Pierre, A. Bily, F. Chemat, *Bioraffinerie sans solvant et sans eau pour la valorisation complète des co-*

produits issus de fruits et légumes, Valorisation des biomasses locales pour une économie circulaire, November 25th 2016, Arles – France.

Poster communications

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- M. Jacotet-Navarro, S. Deslis, N. Rombaut, A.-S. Fabiano-Tixier, F.-X. Pierre, A. Bily, F. Chemat, *Development of a "dry" bio-refinery for total valorization of fruits and vegetables by-products*, Fruits & Veg Processing, 4-6 April 2016, Avignon – France.

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AAPH	2,2'-azobis(2-methylpropionamidine) dihydrochloride
AUC	Area under the curve
CA	Carnosic acid
CBR	Conventional bio-refinery
CM	Conventional maceration
CMC	Critical micellar concentration
CMR	Carcinogenic, Mutagenic, Reprotoxic
CO	Carnosol
COSMO-RS	Conductor-like screening model for real solvents
CPME	Cyclopentyl methyl ether
CSE	Conventional solvent extraction
DAD	Diode-array detector
DBR	Dry bio-refinery
DFT	Density functional theory
DIC	Deodorization by instant controlled pressure drop
DMC	Dimethylcarbonate
DPPH-	Anion 2,2-diphényl-1-picrylhydrazyl
DPPH•	Radical 2,2-diphényl-1-picrylhydrazyl
DPPH-H	2,2-diphényl-1-picrylhydrazyne
DW	Dry weight
EFSA	European Food Safety Authority
EO	Essential oil
ESI	Electrospray ionization

EtOH	Ethanol
FID	Flame ionization detector
FLH	Fluorescein sodium salt
GC	Gas chromatography
GMO	Genetically modified organism
GP	Ginger press cake
GPMHG	Ginger press cake residue after MHG
GR	Ginger rhizomes
GRAS	Generally recognized as safe
H_3PO_4	Phosphoric acid
HB	Hydrogen bond
HD	Hydrodistillation
HPLC	High performance liquid chromatography
HPTLC	High performance thin layer chromatography
HRE	Heat reflux extraction
HSP	Hansen solubility parameters
IL	Ionic liquid
LC	Liquid chromatography
LCA	Life cycle assessment
LCI	Life cycle inventory
MAD	Microwave accelerated distillation
MAE	Microwave assisted extraction
MAHD	Microwave assisted hydrodistillation
MASD	Microwave assisted steam distillation

MeTHF	2-methyltetrahydrofuran
$MgCl_2$	Magnesium chloride
MHC	Minimum hydrotropic concentration
MHG	Microwave hydrodiffusion and gravity
MS	Mass spectrometry
MW	Microwave
MWHD	Microwave hydrodistillation
Na_2CO_3	Sodium carbonate
NaDES	Natural deep eutectic solvents
ORAC	Oxygen radical absorbance capacity
PAH	Polycyclic aromatic hydrocarbons
PBS	Phosphate-buffered saline
PD	Power density
PEF	Pulsed electric fields
PLE	Pressurized liquid extraction
RA	Rosmarinic acid
REACH	Registration, Evaluation and Authorization of CHemicals
RED	Relative energy difference
RMCD	Randomly methylated β-cyclodextrin
ROO•	Peroxyradicals
SFE	Supercritical fluid extraction
SFME	Solvent-free microwave extraction
SWE	Subcritical water extraction
TFA	Trifluoroacetic acid

TLC	Thin layer chromatography
TOF	Time of flight
TPC	Total phenolic content
TZVP	Triple zeta valence polarized basis set
UA	Ursolic acid
UAE	Ultrasound assisted extraction
UI	Ultrasonic intensity
US	Ultrasound
USAE	Ultrasound assisted extraction
UV	Ultraviolet
VOC	Volatile organic compounds

INTRODUCTION GENERALE

La recherche et développement dans le domaine de l'extraction végétale s'est largement intensifiée ces dernières années en raison de l'intérêt croissant pour les ingrédients naturels. Que ce soit pour des applications agroalimentaires, cosmétiques ou pharmaceutiques, les plantes offrent un large panel de molécules d'intérêt qu'il est possible d'extraire par différents procédés. Avec les préoccupations environnementales et sociétales inhérentes au secteur industriel d'aujourd'hui, il est désormais nécessaire d'inventer et développer de nouveaux procédés d'extraction œuvrant pour une chimie verte plus propre vis-à-vis de l'environnement et plus sûre vis-à-vis des utilisateurs et consommateurs. Il est donc question de réduire les temps de procédé, la consommation énergétique, la toxicité et la quantité de solvant utilisé, la quantité de déchets générés, et d'augmenter les rendements et la sélectivité d'extraction. Pour cela, il est possible d'intégrer dans les procédés des innovations technologiques, qu'elles soient relatives au solvant ou au type de procédé lui-même.

Les travaux de cette thèse intitulée « Intensification des procédés d'extraction du végétal. Procédés innovants et solvants alternatifs » s'intègrent complétement dans ces problématiques. Cette thèse industrielle est pilotée par l'équipe GREEN au sein de l'UMR408 INRA-UAPV et se déroule en collaboration avec l'entreprise Naturex, leader mondial de l'extraction végétale. Les principaux objectifs de ce travail sont d'une part d'intégrer des innovations technologiques dans les procédés d'extraction et de les confronter avec les techniques communément utilisées en industrie ; et d'autre part de mieux appréhender leur apport à l'échelle industrielle pour la production d'actifs végétaux. Plus particulièrement, il s'agit de :

- mettre en œuvre des procédés alternatifs pour améliorer le taux d'extraction, le rendement en actif, les interactions solutés/solvants des procédés industriels existants, tout en diminuant la durée totale du procédé, le nombre d'opérations unitaires, la consommation énergétique et l'impact global du procédé sur l'environnement ;
- développer de nouveaux extraits notamment à partir de co-produits d'extraction au vu des quantités conséquentes générées ;
- remplacer les solvants pétroliers et les solvants pouvant avoir un impact sur l'environnement, l'utilisateur ou le consommateur par des solvants alternatifs plus « verts » ;
- accompagner scientifiquement l'implémentation industrielle des améliorations de procédés.

Afin de répondre à ces problématiques, ce manuscrit se décline en cinq chapitres se référant à l'intensification de procédés par des technologies innovantes, la substitution de solvants nocifs par des alternatives plus vertes et la valorisation de co-produits d'extraction en produits à haute valeur ajoutée.

Le chapitre I présentera une étude bibliographique sur l'éco-extraction des produits naturels en général. Le terme d'intensification de procédés et les principes de l'éco-extraction seront définis et mis en relation. Puis, pour chaque principe (relatifs à la matière première, au solvant, à l'énergie, au procédé, aux co-produits et à l'extrait final) seront présentés un constat de la situation actuelle, les bonnes pratiques à mettre en œuvre pour s'inscrire dans la démarche d'éco-extraction et enfin des exemples de « success stories » initiés par des académiques ou des industriels. Les exemples ne se limiteront pas à un type de plante en particulier, cependant des exemples relatifs au romarin et au gingembre seront donnés dans la mesure du possible puisqu'il s'agit des matrices d'intérêt dans cette thèse.

Le chapitre II décrira les différentes méthodologies expérimentales utilisées dans cette thèse. Les protocoles et techniques d'extraction, ainsi que les méthodes d'analyse qualitative et quantitative seront présentés.

Le chapitre III proposera d'intensifier le procédé d'extraction des acides rosmarinique, carnosique et ursolique à partir du romarin par l'utilisation d'ultrasons et de micro-ondes. Trois équipements ultrasons et trois équipements micro-ondes seront étudiés. Les technologies alternatives utilisées seront comparées aux procédés conventionnels. L'efficacité et la sélectivité d'extraction, ainsi que la consommation énergétique, seront évaluées en fonction du procédé utilisé **(Figure 1)**.

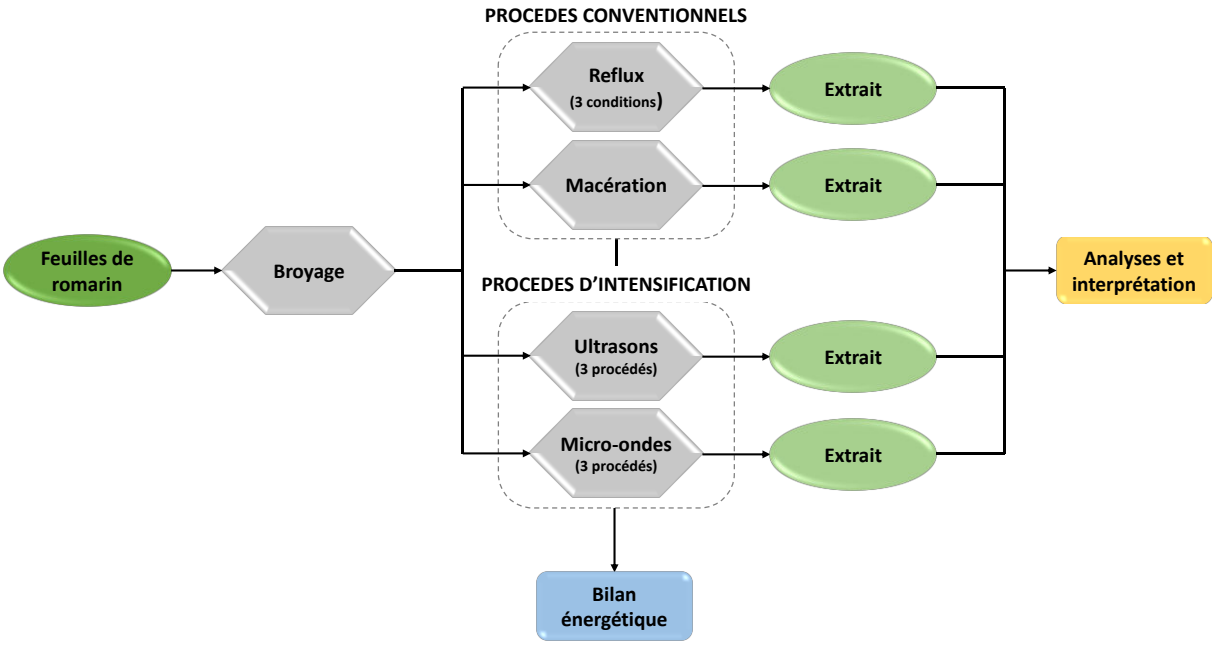


Figure 1. Schéma expérimental suivi dans le chapitre III.

Le chapitre IV s'attachera à évaluer l'effet du solvant (et plus particulièrement celui du ratio en éthanol dans des mélanges hydro-alcooliques) sur la composition et l'activité d'extraits de romarin. Plus particulièrement, les capacités réductrice, anti-radicalaire et antioxydante seront étudiées. Dans cette partie, il s'agira d'observer si teneur en composés d'intérêt et activité de l'extrait sont corrélées ou non. De plus, afin de mieux comprendre le phénomène de solubilisation qui intervient lors de l'extraction, les solubilités des deux principaux antioxydants du romarin (acides rosmarinique et carnosique) dans les différents ratios éthanol:eau seront évaluées avec un outil prédictif (COSMO-RS) et comparées aux solubilités expérimentales **(Figure 2)**. Il sera donc ensuite possible de discuter et conclure sur les différences entre « solubilisation » et « extraction ». Enfin, au vu des résultats obtenus, un procédé inspiré de la bio-raffinerie sera proposé pour extraire successivement l'acide carnosique et l'acide rosmarinique en faisant uniquement varier le pourcentage d'éthanol dans le solvant d'extraction.

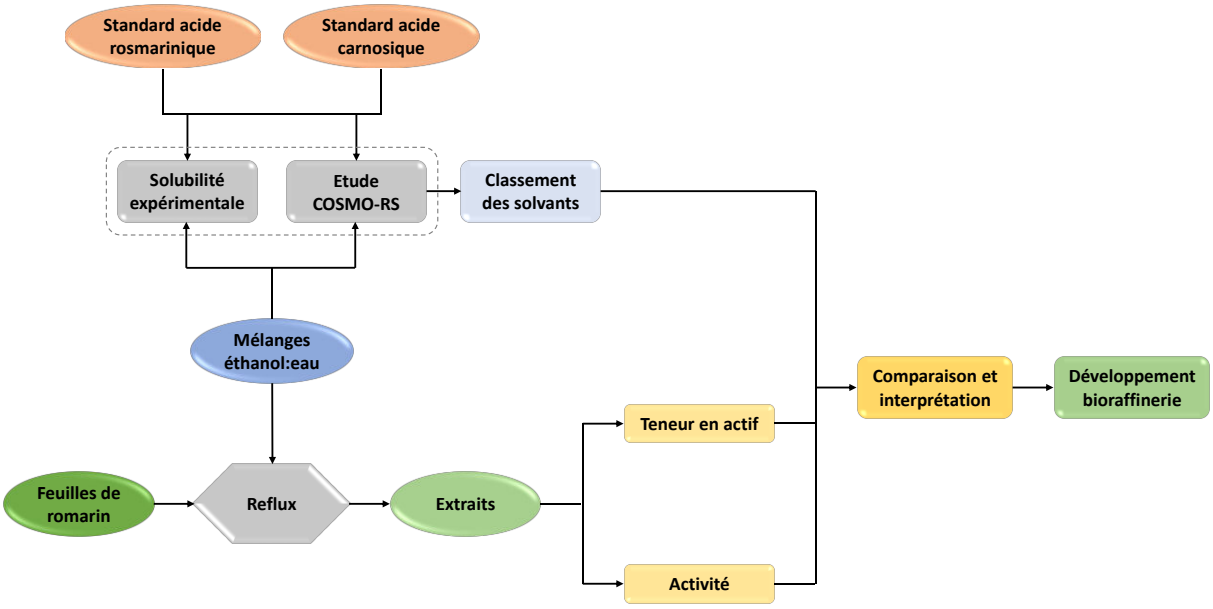


Figure 2. Schéma expérimental suivi dans le chapitre IV.

Enfin, le chapitre V proposera un procédé inspiré de la bio-raffinerie pour valoriser les co-produits du gingembre. Il s'agira d'une bio-raffinerie « sèche » sans solvant ni eau ajoutés utilisant des technologies vertes. En effet, le press cake de gingembre récupéré après extraction du jus sera d'abord traité par micro-ondes (MHG : Microwave hydrodiffusion and gravity) pour obtenir une huile essentielle, puis par ultrasons dans son eau de constitution pour récupérer un extrait riche en composés phénoliques et un résidu riche en fibres et antioxydants. Les paramètres de chaque procédé micro-ondes et ultrasons seront optimisés pour maximiser les rendements d'extraction. Enfin, un bilan environnemental sera dressé et comparé à celui d'une bio-raffinerie mettant en œuvre des procédés conventionnels **(Figure 3)**.

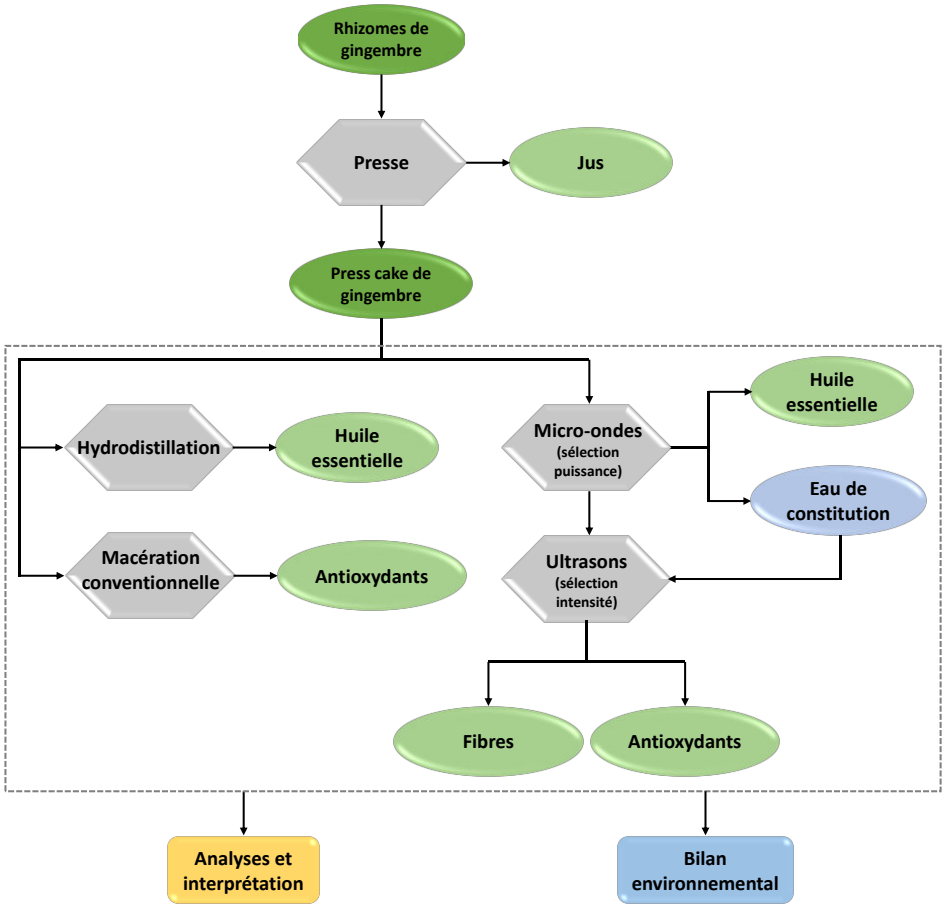


Figure 3. Schéma expérimental suivi dans le chapitre V.

En résumé, les objectifs de ce travail de thèse sont précisément :

- D'intensifier le procédé d'extraction de composés d'intérêt à partir du romarin par l'utilisation de technologies innovantes ;
- D'évaluer l'effet du ratio en éthanol dans des mélanges hydro-alcooliques sur la composition et l'activité d'extraits de romarin dans le but de développer un procédé inspiré de la bio-raffinerie ;
- De valoriser les co-produits de fruits et légumes en produits à haute valeur ajoutée par un procédé inspiré de la bio-raffinerie.

Pour terminer, les principales conclusions de ce travail seront dégagées et des perspectives d'études futures seront proposées.

CHAPTER I . GREEN EXTRACTION OF NATURAL PRODUCTS

DEFINITION, PRINCIPLES, GOOD PRACTICES GUIDELINES AND SUCCESS STORIES.

I.1 INTRODUCTION

The aversion towards synthetic additives generated a great interest and a growing demand on natural extracts in the last decades. For example, in 2014, the overall market of antioxidants was about 1700 million euros of whom natural antioxidants accounted for 550 million euros.

Natural extracts can be sourced in huge number of plants materials and includes, as illustrated in **Figure I-1**, primary and secondary metabolites as antioxidants, essential oils (EO), proteins, fats, dietary fibers, dyes or even saponines [1–4]. They are largely used as ingredients in the food processing industry for their texturing, preservative or coloring properties [5–7], and as active compounds in cosmetics or pharmaceuticals [8,9]. Conventionally, such extracts are obtained using solid-liquid extraction. Besides the only step of extraction, pre-treatment of plant material (drying, grinding…) and post-treatment of liquid extract (filtration, concentration, purification…) are also necessary to ensure global process efficiency. Such processes, particularly when they are not optimized, are often time and energy consuming, induce the use of huge amount of water or petroleum solvents harmful for environment and users and generate large quantity of waste. Moreover, resulting extract is not always safe as it may contain residual solvents, contaminants from raw material, or denatured compounds due to drastic extraction conditions.

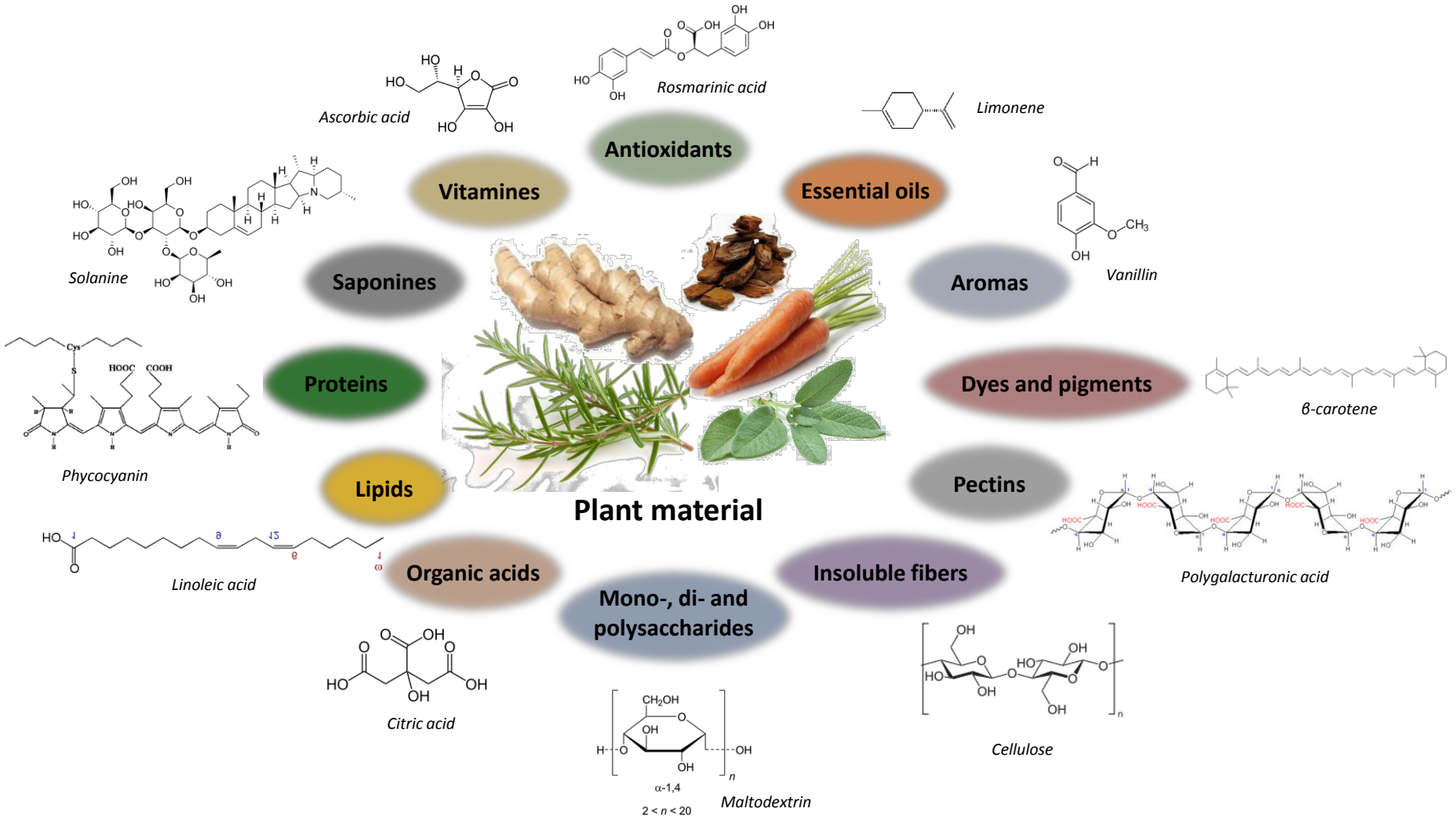


Figure I-1. Types of compounds extracted from plant materials.

In this context, plant extraction specialists firstly aimed at intensifying their processes. Principle of intensification is presented in **Figure I-2**. The objective is to obtain higher extraction efficiency and higher quality extract while reducing extraction time, number of unit operations, global energy consumption, quantity of solvent in the process, environmental impact, economical costs and quantity of waste generated [10].

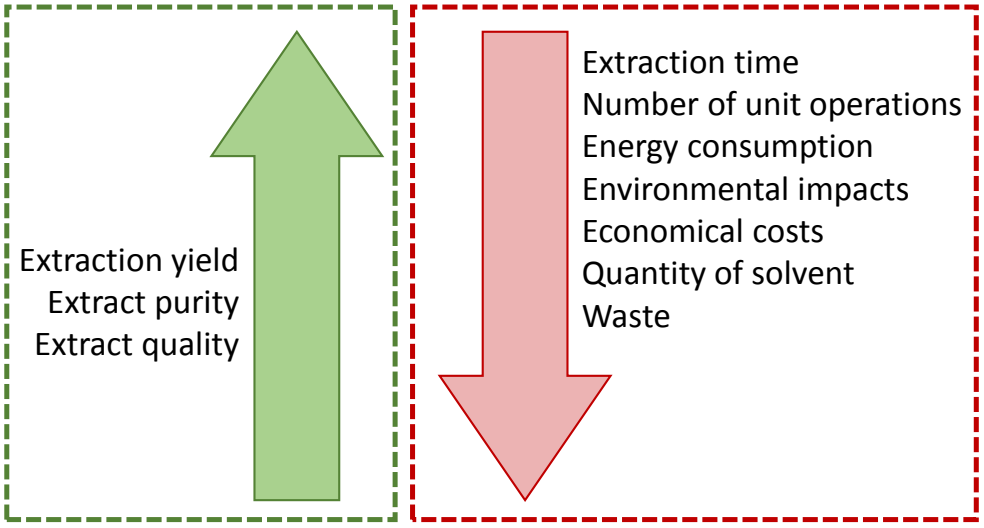


Figure I-2. Principle of intensification.

In the last decades, with the increasing interest to environmental, economic and safety considerations, innovative alternatives with durable and green values have been hugely implemented in food processing, cosmetic and pharmaceutical industries. It was particularly initiated by the publication of the twelve principles of green chemistry [11] and the twelve principles of green engineering [12] which define the good practices to be adopted in order to engage in this demarche. Following this, plant extraction researchers and industrials met on January 2010 to define the term of "green extraction" and establish, in the same spirit of Anastas *et al.*, the six principles of green extraction [13]. These principles intend to act on the main inputs and outputs regarding a global extraction process: the raw material, the solvent, the energy, the process itself, the waste and by-products (organic or not) and the final extract **(Figure I-3)**.

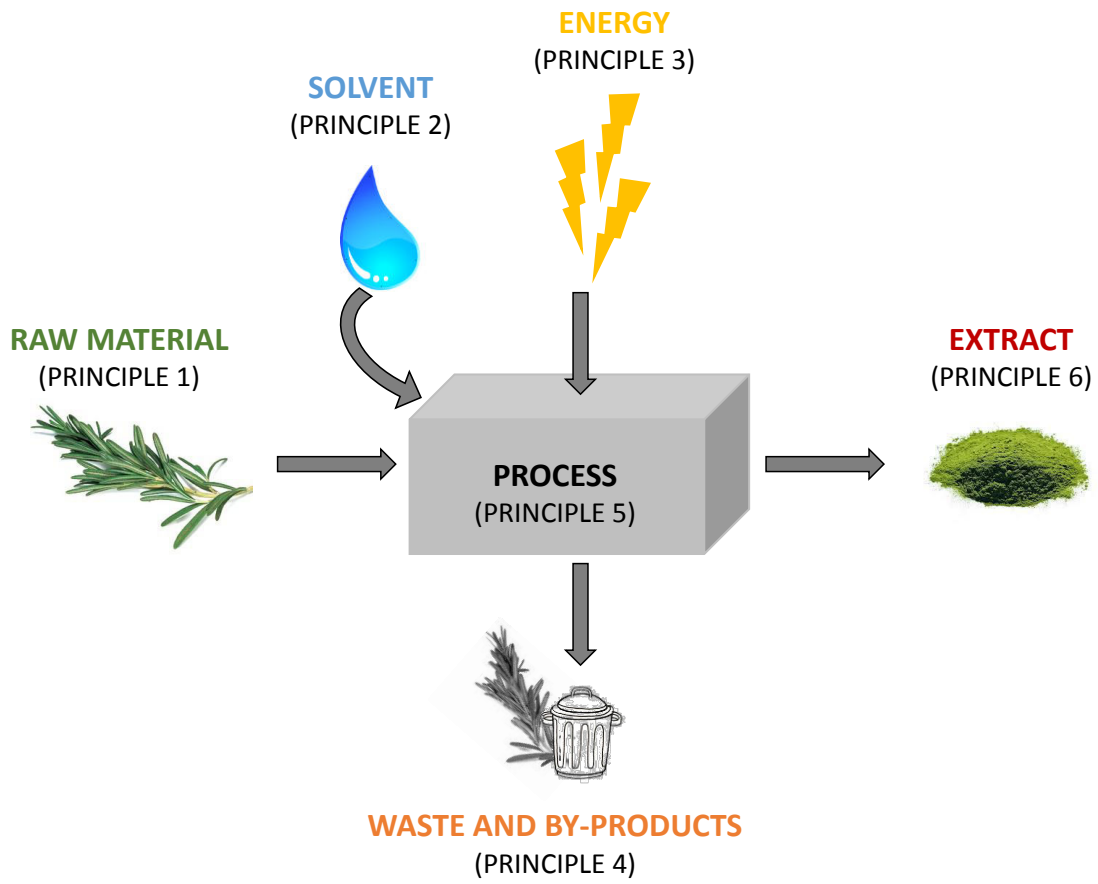


Figure I-3. Essential inputs and outputs on an extraction process related to the six principles of green extraction.

By 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*". In this definition, the main ideas of intensification principle are identified. The listing of green extraction principles was established to guide scientist and industrials in their demarche towards green innovation, not only regarding the process, but in all aspects of solid-liquid extraction [14].

- Principle 1: Innovation by selection of varieties and use of renewable plant resources.
- Principle 2: Use of alternative solvents and principally water or agro-solvents.
- Principle 3: Reduce energy consumption by energy recovery and using innovative technologies.

- Principle 4: Production of by-products instead of waste to include the bio- and agro-refining industry.
- Principle 5: Reduce unit operations and favor safe, robust and controlled processes.
- Principle 6: Aim for a non-denatured and biodegradable extract without contaminants.

Hence, in this chapter, good practices guidelines regarding each principle are developed. Plus, examples of success stories are presented in order to show the potential and the feasibility of such practices at different scales. The examples given are not limited to one kind of plant material, however particular applications regarding rosemary and ginger are raised since they are the two matrices of interest in this thesis.

I.2 PRINCIPLE 1: INNOVATION BY SELECTION OF VARIETIES AND USE OF RENEWABLE PLANT RESOURCES.

I.2.1 Context

The increasing demand of natural products and extracts to answer the needs of food, cosmetic and pharmaceutic industries is leading to the over-exploitation of natural plant resources. This production of natural extracts has to take into account a number of issues, such as respect for the environment and human health or production with high yields, which are key issues for industrials. History reports several examples of plant extinction because of overutilization. More particularly, the large-scale uncontrolled harvesting of natural resources may carry the risk of making the species rare or even extinct. The preservation of biodiversity is therefore mandatory in the respect of future generations. In this context, in green extraction, fully renewable resources have to be favored either with intensive cultivation or in vitro growth of plant cells or organisms. Moreover, there is a need to integrate the raw material in an industrial vision regarding environment, social and economic aspects, in order to preserve natural resources.

I.2.2 Good practices guidelines

To limit the impact on the environment of processes and to produce extracts that meet the values of green extraction, a number of good practices should be implemented [15]:

- Integrate the raw material to an ecosystem vision including a social/ societal, environmental and economic diagnosis.
- Rely on standards such as ISO 26000 or relating to production monitoring.
- Implement protocols to promote the performance of the resource by varietal selection. The aim of varietal selection is to increase yields, produce varieties containing certain target compounds or select varieties adapted to region and climate.
- Support treatment technologies that (i) enable efficient production of crops and crop protection products (ii) meet the demands of an agriculture more friendly to environment and men with varieties resistant to pests and pathogens.
- Avoid cultures generating pollution, threatening biodiversity and competing with local vegetation or local food crops.
- Better manage and develop the inputs during cultivation (water, pesticides).

- Value health benefits of the extracts and enhance the diversity of products that can be obtained (to expand the plant resources).

I.2.3 Success stories

The integration of principle 1 in raw material sourcing can be performed by using renewable resources, by selecting some varieties of plant (high content in targeted compounds or absence of undesirable molecules) and by using new technologies. This section aims at presenting some success stories regarding such demarches.

I.2.3.1 Use of renewable resources

Currently, most medicines are extracted from plants. The anti-cancer paclitaxel (Taxol®) extracted from the bark of the western yew (*Taxus brevifolia*) is the best known example [13]. The molecule of paclitaxel is presented in **Figure I-4**. During the 1970's, no less than 30 tons of bark were collected for clinical trials, considering that 10 kg of dry bark produce only 1 g of taxol after extraction and purification. These low yields may result in an overexploitation of trees; however, due to the complexity of the molecule of interest, a synthetic way is not economically viable. In this context, a large number of research projects have been aimed at finding alternatives to felling trees of this threatened species. Since 1980, paclitaxel and docetaxol (Taxotere®) are prepared by semi-synthesis from the natural precursor, 10-deacetylbaccatine III, extracted from needles and branches (renewable resource) of different yew tree species.

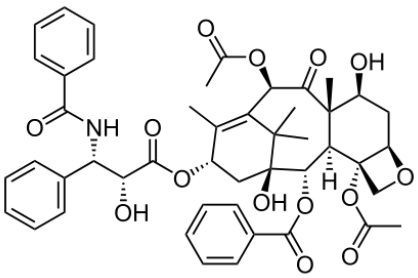


Figure I-4. Paclitaxel.

Another example concerns (−)-α-bisabolol, a sesquiterpene with marked anti-inflammatory, antibacterial and antifungal properties **(Figure I-5)** [14]. Its natural occurrence is mainly by steam distillation of candeia plant (*Eremanthus erythropappus* (DC) MacLeish)

which grows in the Atlantic Brazilian rainforest, in the south of Minas Gerias State [16]. This area is in precarious ecological situation, making the sustainable supply of candeia oil seriously under threat. Symrise, Revlon and Rossman are amongst the 187 companies which launched cosmetic products containing α-bisabolol in the last two decades. In order to integrate sustainability into its corporate social responsibility strategy, Symrise recently decided to stop harvesting natural α-bisabolol from the candeia tree, thus preserving threatened areas.

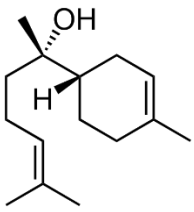


Figure I-5. (−)-α-bisabolol.

I.2.3.2 Varietal selection

Plant composition varies widely among varieties. This variation is caused by several factors such as environmental factors (climate, soil and fertilization), but the most important factor determining the composition and the content of active compounds is the genetic variation. Thus, breeders have the opportunity and challenge to produce new varieties with modified compositions and improved contents of active compounds.

I.2.3.2.1 Improvement of Artemisinin content in Artemisia annua L. [14]

A big effort is running in the natural selection of varieties with much higher concentrations of active ingredients as the example of the production of artemisinin **(Figure I-6)**. Artemisinin is known as an anti-malarial substance isolated from wormwood, *Artemisia annua* L., originating from Asia. The active principle of this plant is a sesquiterpene lactone with a peroxide bridge, which is present in the aerial parts of the plants at a concentration ranging from 0.01 to 0.05 %. Extracting this active substance turns out to be hardly cost effective because of the low concentrations in the plant. Much experimental work has been

done to produce varieties of *Artemisia annua* L. with higher concentrations of artemisinin. Currently some varieties present contents greater than 1 %.

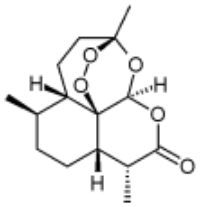


Figure I-6. Artemisinin.

I.2.3.2.2 Cassava with low cyanogenic glycosides content [17]

Cassava is one of the most important root crop in the world (241 million tons, FAO 2010). It is also the second most important staple crop in Africa and is used extensively for starch production in South East Asia. Most of cassava production is used for human consumption. However, the natural presence of cyanogenic glycosides, especially linamarin, is of concern in terms of food safety as it may release free cyanide (HCN). HCN is very toxic and lethal in high amounts. To reduce health problems associated with the ingestion of cassava products, the development of varieties with low cyanide content has been undertaken. Traditional plant breeding has generated cultivars with high and also others with low levels of cyanogenic glycosides. The use of genetic modification has led to the development of varieties free from these compounds.

I.2.3.2.3 Claryssime project: sage rich in sclareol

So far, the market of sage has been driven by that of EO considering the rest of the plant as a by-product. However, this situation is changing and market requirements are evolving. Needs in EO (for fine perfumery mostly) are remaining stable contrary to the strongly developing demand for sclareol, another compound of interest that can be found in clary sage **(Figure I-7)**.

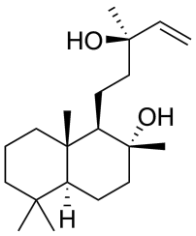


Figure I-7. Sclareol.

Sclareol is source of a hemi-synthesis molecule commonly called Ambrox, highly used for its olfactory power and high biodegradability. For regulatory reasons, functional perfumery is increasingly using Ambrox®, thus increasing the need for sclareol. Indeed, this molecule is used instead of gray amber of cachalots, formerly very prized and which completely disappeared. One of the aim of the project was to improve the agricultural production of sclareol by identifying the plant's genes involved in the synthesis and secretion of sclareol. These genes involved in the metabolism of sclareol were identified as well as the location of sclareol in the plant. More precisely, it was discovered that sclareol was stored in the cuticle of the calyx of flowers. Botanical varieties of grown clary sage were adapted to produce more sclareol and harvest period was correlated with optimal stage of maturity of plant for sclareol [18].

I.2.3.3 Development of new technologies: Example of Plant milking [14]

A new technology known as "plant milking" was developed for the production and extraction of substances of interest, preventing the destruction of plant material. These plants are grown in a greenhouse in liquid medium. Actually, secretion and exuding of targeted substances through the roots in the culture medium are triggered by physical, chemical or biological stimulation **(Figure I-8)**. These substances are then collected by standard extraction and purification methods. This process is particularly performed to produce active principles from rare plants, whose chemical synthesis is difficult and costly. Therefore, "plant milking" appears as an extraction way that respects biodiversity. For example, such process enabled the production of, among others, tropane alkaloids of pharmaceutical interest from *Datura innoxia*. In this case, harvesting yields of three times more secondary metabolites were obtained in one year compared to an equal area of field-grown plants. Good results were also obtained with garden rue (*Ruta graveolens*) which contains furocoumarins (substances used to treat eczema and psoriasis), and with edelweiss, rich in antioxidants. Regarding yew, "plant milking" yields much larger quantities of paclitaxel than traditional harvesting

methods. Finally, considering a possible implementation of such technique at larger scale, it was calculated that a few hundred greenhouses would be sufficient to satisfy world demand in paclitaxel for one year.

Figure I-8. Plant milking technology. (PAT plant milking©).

I.3 PRINCIPLE 2: USE OF ALTERNATIVE SOLVENTS AND PRINCIPALLY WATER OR AGRO-SOLVENTS.

I.3.1 Context

In solid-liquid extraction, it can be admitted that extraction efficiency strongly depends on the solvent. Indeed, even if process is optimized, if the solvent does not solubilize the solute after its release from raw material, it will be difficult to reach acceptable yields. Moreover, beyond its high dissolving power, solvent has to be a good "extractant", meaning that it must penetrate sufficiently into raw material to "recover" molecules of interest from their location in the plant [19]. Dissolving phase actually occurs only in a second stage. Therefore, a good compromise between extractant and dissolving properties must be achieved, which makes solvent selection even more complicated.

Conventional solvents generally used in plant extraction are presented in **Table I-1**. They have the important advantage to present relatively low boiling point and low enthalpy of vaporization, which facilitates their removal after extraction and limits the energy consumption related to this step. Moreover, some of them are very selective towards targeted molecules, such as hexane for the extraction of lipophilic molecules. However, besides their obvious advantages, these solvents also present negative aspects. In most cases, they are petroleum-sourced (therefore obtained from non-renewable resources), VOC (Volatile Organic Compounds) emitter and harmful to human health and environment. Indeed, as shown by the pictograms in **Table I-1**, they may be carcinogenic or even CMR (Carcinogenic, Mutagenic, and Reprotoxic) like hexane.

In this context, a growing aversion towards such solvents emerged. The increase of drastic legislation regarding their use in food, cosmetic or pharmaceutical fields (for example Directive n° 2010/59/UE or European Pharmacopeia) forced industrials and academics to find potential replacement solvents. Ideally, these alternative solvents may be comparable/improved in terms of efficiency, more environmentally friendly, safer for users and rather cheap. Finally, last but not least, they may present an acceptable boiling point in order to be easily removed and recycled after extraction without degrading final extract.

	Molecular formula	Density	Boiling point	Specific heat capacity	Enthalpy of vaporization	logP	Dielectric constant (20 °C)	Safety
	/	/	° C	J/(mol.K)	kJ/mol	/	/	/
Acetone	C_3H_6O	0.791	56.1	125.5	31.3	-0.02	20.7	
Chloroform	$CHCl_3$	1.49	61.3	114.3	31.4	1.92	4.81	
Dichloromethane	CH_2Cl_2	1.33	40	102.3	28.6	1.38	8.93	
Diethyl ether	$C_4H_{10}O$	0.713	34.6	172.5	27.2	1.13	4.33	
Ethanol	C_2H_6O	0.789	78.5	112.4	38.6	-0.31	24.5	
Ethyl acetate	$C_4H_8O_2$	0.897	77	170	31.9	0.89	6.3	
Hexane	C_6H_{14}	0.675	68.7	197.7	28.9	3.46	1.89	
Isopropanol	C_3H_8O	0.786	82.4	160.8	44.0	0.23	17.9	
Methanol	CH_4O	0.792	64.5	79.5	38.3	-0.57	32.7	
Water	H_2O	1.000	100	75.3	40.7	-1.38	80.1	

Table I-1. Relevant properties of most common conventional extraction solvents.

I.3.2 Good practices guidelines

The choice of the solvent is crucial in the demarche of green extraction. It must particularly ensure the durability of global process. To this end, good practices guidelines must be respected as far as possible [15]:

- Evaluation of a potential "solvent-free" alternative
- Use of 100 % natural, natural origin, renewable or agro-sourced solvent (on the condition of having good knowledge, evaluation and control of potential related risks)
- Avoid the use of solvent which might affect the safety and health of production operators and consumers: it must not be a CMR or toxic (or present low toxicity); it must not induce allergenic effect and not belong to endocrine disruptors.
- Use of solvent suitable with industrial facilities
- Prefer a solvent with high rate of recyclability (high bio-degradability and no bio-accumulation) to limit global process impact on environment
- Use of solvent with low VOC emissions related
- Use of solvent which limit energy consumption and cost of global process (solvent with low boiling point, low specific heat capacity and low enthalpy of vaporization).
- Ensure a maximal solvent recovery at the end of the process using various available techniques.

I.3.3 Success stories

One of the main challenges of current extraction field is the elimination or replacement with greener alternatives of harmful solvents (for users and environment). This part relates some success stories regarding this challenge. First of all, a solvent-free extraction process will be presented, followed by examples of new generation solvents with green values ("green solvents") and finally decision-making tools to assist this demarche of solvent substitution.

I.3.3.1 Solvent-free microwave extraction (SFME)

As indicated by principle 2's specifications, the ideal solvent in a green extraction process is actually the absence of solvent. In some cases, it is possible to develop solvent-free processes, particularly with water-rich materials. Among the available solvent-free extraction processes [20], SFME have been particularly investigated in last decade [21,22].

First of all, SFME can be used as an alternative process to conventional hydrodistillation (HD), of which general concerns are long extraction time, high water consumption, and compounds degradation. Actually, microwave (MW) heating is added to existing process **(Figure I-9)**: since it occurs from the inside to the outside of plant material, it is no longer necessary to use solvent as heating media. The biomass can be directly placed in MW reactor without any water or solvent. Under MW heating, constituent water of plant material reach its boiling point (100 °C) and vaporize. Resulting vapors rich in EO rise and are condensed in a Clevenger-type apparatus. Finally, EO is recovered by gravity difference. Most examples of EO SFME are related in current literature: from oregano [23], from flowers [24], from cardamom [25], from rosemary [26], from salvia [27] and more generally from aromatic herbs [28–30] and spices [31].

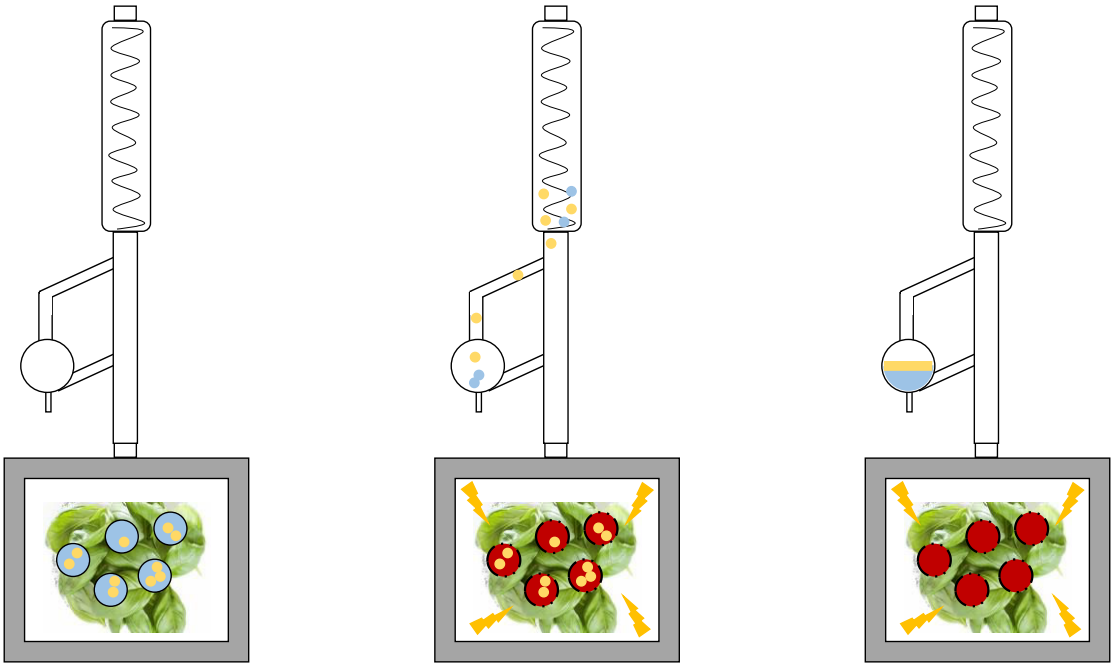


Figure I-9. Scheme of microwave assisted hydrodistillation processing.

In 2008, Chemat *et al*. developed a new process of SFME to extract compounds other than EO from various plant material [32]. This process combines MW and gravity, which explains the name of Microwave Hydrodiffusion and Gravity (MHG). MHG procedure is performed at atmospheric pressure or under pressure in the case of thermosensitive compounds. Plant material is directly placed in the MW reactor without addition of any

solvent and is MW-heated. MW focus on molecules having a dipolar moment (rather polar), particularly the biological water contained in plant cells. The increase of temperature causes the explosion of biological cells and consequently the release of water and polar solutes out of plant material. Under gravity, this juice moves naturally along the reactor and downwards a condenser outside MW cavity **(Figure I-10)**. MHG has been hugely used to extract various compounds from various plant materials, particularly EO from rosemary and citrus [33,34], dyes from grape [35], fruit juices rich in phenolics and sugars from apricot and plums [36,37], antioxidants [38] and phenolics from algae [39].

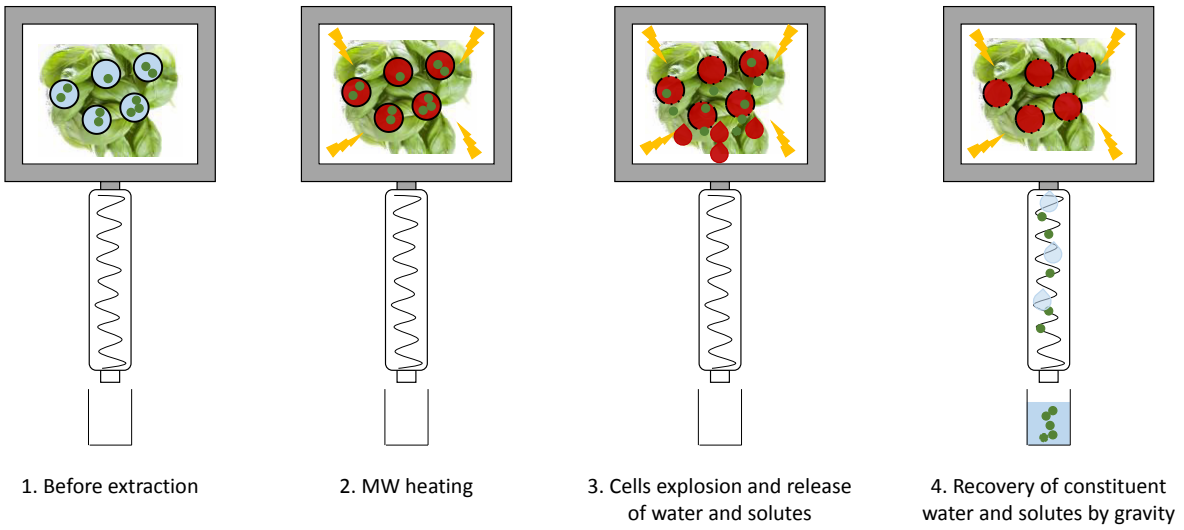


Figure I-10. MHG extraction processing.

In all cases, SFME enables to reduce drastically extraction time, from several hours with conventional HD to few minutes. Moreover, resulting extracts present improved organoleptic properties and are completely safe since no solvent is used in the procedure. The only requirement with such process concerns initial plant material moisture: to avoid any problem of burning during the process, initial raw material must be rich in water or previously humidified.

I.3.3.2 Use of greener solvents

The definition of solvent greenness is quite difficult. Currently, there is no systematic method to determine how “green” a solvent is. Several factors must be assessed, such as

solvent sourcing, availability, environmental footprint, recycling, health hazards or boiling point. Databases of solvents regarding these different information are existing, such as GSK classification [40], hence being a guide for "green" solvent selection [41].

Seven categories of green solvents have been studied and developed in the last few years **(Figure I-11)**: bio-based solvents, obtained from biomass including energy crops or forest products; eco-friendly solvents (renewable and bio-degradable); fluorinated solvents; liquid polymers; ionic liquids (IL) and natural deep eutectic solvents (NaDES); supercritical fluids; and water. A focus on water and bio-based solvents is done in following sections.

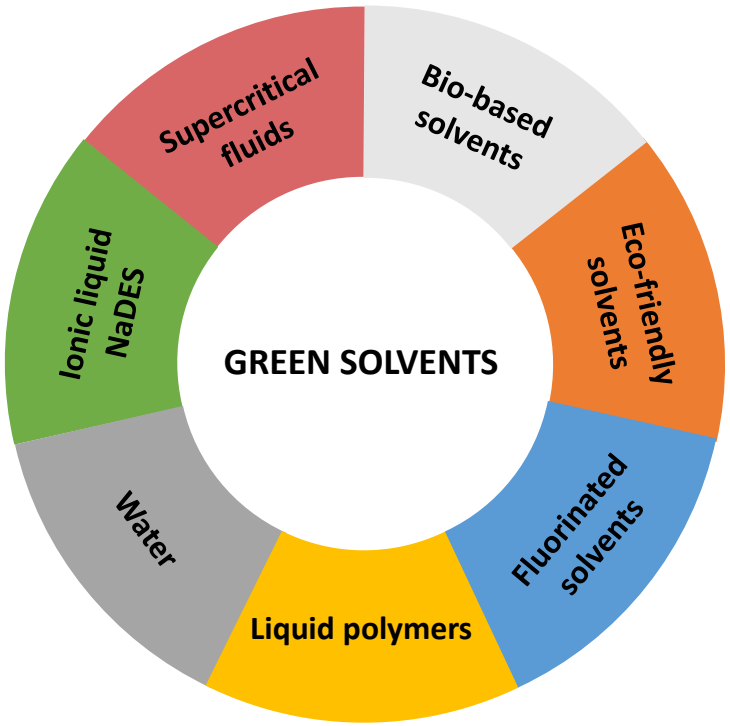


Figure I-11. Main categories of green solvents (adapted from [19,42])

I.3.3.2.1 Water

Among the different solvents which can be used for natural products extraction, water is recognized as the cheapest and safest for users. Its physico-chemical properties are presented in **Table I-1**. It can be noticed that its dielectric constant is very high and logP very low compared to other solvents. Therefore, water is very polar at ambient temperature and its use is limited to hydrophilic molecules. However, this solvent offers a great possibility to

modulate its polarity, which considerably increases the scope for the extraction of less polar molecules.

The first mean to decrease water polarity is to increase temperature and pressure. It results in the rupture of the hydrogen bonding network and causes an important decrease of the dielectric constant. As illustrated in **Figure I-12**, it is possible to reach near-zero values, corresponding to very less polar solvents. When water temperature exceeds 100 °C under pressure, we talk about *subcritical water*.

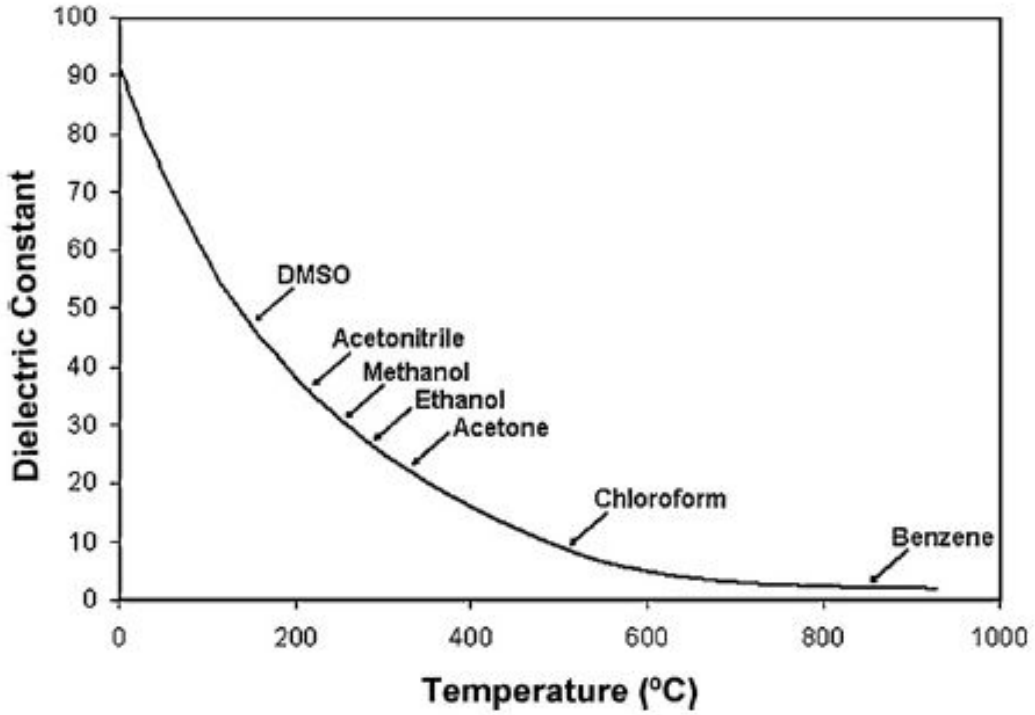


Figure I-12. Evolution of water dielectric constant regarding temperature and comparison with conventional solvents (from [43]).

Subcritical water extraction (SWE) is also known under the terms of superheated water, hot water or pressurized low polarity water extraction. In all cases, it corresponds to a temperature range between 100 °C and 374 °C (beyond this temperature we reach the supercritical state). It is important to keep in mind that water remains at liquid state, that's why it is necessary to be under pressure. The minimum pressure range required to keep water in a liquid state at selected temperature are presented in **Table I-2**.

Temperature (°C)	Pressure (bar)
100	1.0
120	2.0
140	3.6
160	6.1
180	10.0
200	15.5
300	85.8
374	219.1

Table I-2. Temperature-pressure couples to keep water at liquid state (from [44])

Subcritical water have been used in various cases to extract lipophilic compounds, such as antioxidants and phenolics from rosemary, ginger or potato peel [43,45–50]. Globally, SWE appears as a fast, reliable, clean, green and environmentally friendly technique to extract polar, mid-polar and non-polar compounds from plant material. However, its use is limited in the case of thermosensitive compounds because of too high processing temperature.

Another way to decrease water polarity is the addition of surfactant or hydrotropes in the solvent [51,52]. Such products, by their very structure **(Figure I-13)**, are able to solubilize low polarity compounds in water.

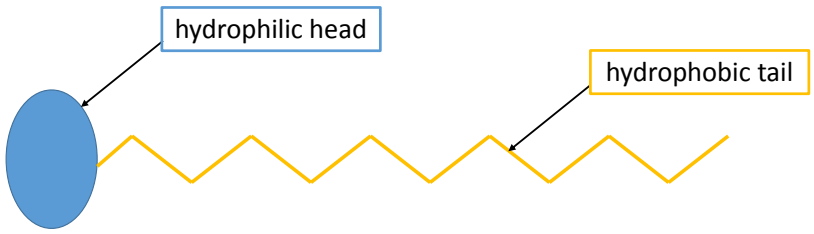


Figure I-13. Surfactant/hydrotrope structure.

However, surfactants and hydrotropes do not present the same chemical characteristics. Surfactants are characterized by a critical micellar concentration (CMC) beyond which they form micelles in solutions, thus solubilizing low polarity molecules in water **(Figure I-14)**.

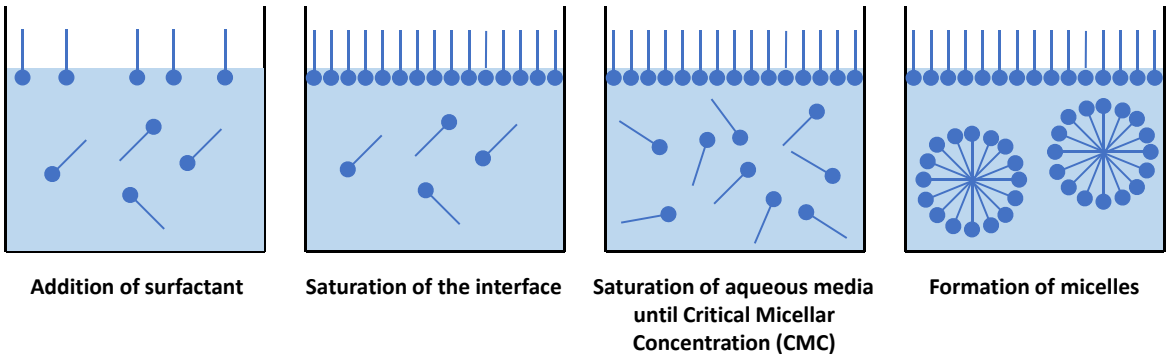


Figure I-14. Scheme illustrating the Critical Micellar Concentration.

Regarding hydrotropes, the hydrophobic tail is shorter. In solution, they don't form micelles (they don't have a CMC), but they self-aggregate gradually. However, they have a concentration threshold at which the solubility of hydrophobic compounds increases significantly: this threshold is called the minimum hydrotropic concentration (MHC) [53]. **Figure I-15** illustrates the difference in behavior of surfactants and hydrotropes in aqueous solution.

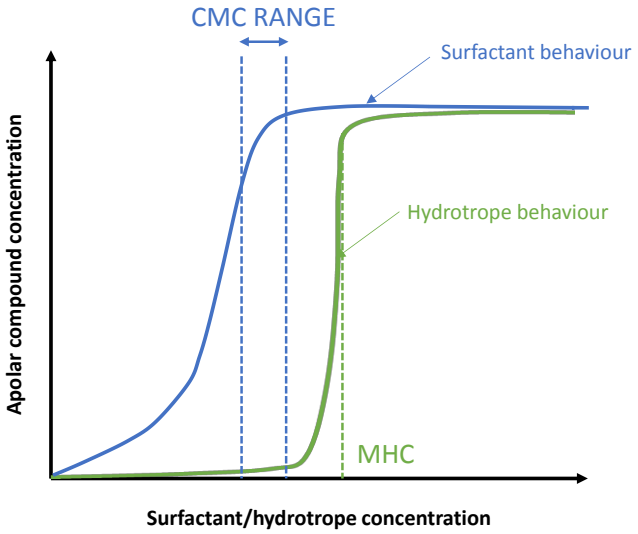


Figure I-15. Surfactant and hydrotrope behavior according to their concentration in aqueous solution.

Various commercial surfactants/hydrotropes are available, they are classified in three categories: anionic, cationic, zwitterionic or non-ionic. Depending on raw material and

targeted compounds, one or other category must be selected. **Table I-3** illustrates some example of surfactant/hydrotrope assisted extraction.

	Matrix	Analyte	Surfactant/ hydrotrope	Experimental conditions	Ref
SURFACTANT	Sage *Salvia miltiorrhiza* Bunge	Cryptotanshinone, tanshinone	Triton X-100 (non-ionic)	0.8 mol/L surfactant in water 65 °C – 10 min	[54]
	Olive mill wastewater	Antioxidants	Genapol-X080 (non-ionic)	5 % surfactant in water NaCl 5 % pH 2.5-3.5 50 °C - 20 min	[55]
	Red flesh orange juice	Phenols, carotenoids	Tween-80 (non-ionic)	5 % surfactant in water NaCl 20 % pH 2.5-3.5 55 °C - 30 min	[56]
HYDROTROPE	Black pepper *Piper nigrum*	Piperine	Sodium *n*-butyl benzene sulfonate	0.05 – 3.4 mol/L hydrotrope in water 30 °C – 2 h	[57]
	Sour orange *Citrus aurantium* L. seeds	Limonin	Sodium salicylate or sodium cumene sulfonate	2 mol/L hydrotrope in water 60 °C – 6 h	[58]
	Coleus Forskohlii roots	Forskolin	Sodium cumene sulfonate or *p*-toluene sulfonate	2 mol/L hydrotrope in water 20 - 90 °C – 3 h	[59]

Table I-3. Examples of surfactant and hydrotropic extraction (adapted from [53]).

I.3.3.2.2 Bio-based solvents

By definition, bio-solvents are obtained completely or partially from renewable raw materials. As presented in **Table I-4**, they are derived from cereal crops (corn, wheat…),

wood or generally from lignocellulosic material using fermentation processes or by synthesis in accordance with the principles of green chemistry. They can be as well integrated in bio-refinery processes since cereal and wood by-products still remain high quality lignocellulosic materials. Bio-solvents are biodegradable, non-toxic and generate very low amount of VOC compared to petroleum solvents. Hence, in extraction field, they are good candidates to replace harmful solvents such as hexane. The replacement of hexane with bio-based solvents have been hugely investigated in current literature. Some of the most studied alternative are presented in **Table I-4**. Among them, Me-THF is the most promising since it enables to reach similar or improved yields regarding the extraction of fats from rapeseed, carotenoids from carrots and aromas from buds black current, in comparison with hexane [60]. Moreover, it is of great interest as it presents a low boiling point compared to other bio-based solvents, hence limiting the energy consumption related to its removal from final extract.

		ORGANIC ACID ESTERS		TERPENES		SYNTHETIC	ALCOHOLS
		Ethyl acetate	**Ethyl lactate**	**D-limonene**	**α-pinene**	**Me-THF**	**Bio-ethanol**
	Source	Esterification of ethanol and acetic acid Dehydrogenation of bio-ethanol	Corn fermentation Lactic acid esterification	Extraction of citrus by-products	Hydrodistillation of wood rich in turpentine oil	Hydrogenation of furfural from lignocellulosic feedstock	Fermentation of sugars (sugar beet) Enzymatic hydrolysis of starch From lignocellulosic raw material
Properties	Boiling point (°C)	77	154	175	158	80.2	78.5
	Log P	0.86	-0.19	4.45	4.37	0.82	-0.31
	Dielectric constant (20 °C)	6.3		2.44	2.58	6.97	24.5
	Specific heat capacity (J/(mol.K))	170					112.4
	Enthalpy of vaporization (kJ/mol)	31.9	45.0	39.5	37.8	32.3	38.6

Table I-4. Source and properties of common bio-solvents (adapted from [61]).

I.3.3.3 Decision-making tools for solvent selection

When we have to develop a new process from scratch, it is often difficult to determine directly the best extraction solvent. Depending on the type of raw material and molecules of interest, a large range of potential candidates is available and it is impossible to test experimentally each of them without a preliminary selection. Number of experiment and time required to screen every solvent would be too much important. To assist the selection, it is possible to use computational methods based on chemical and thermochemical aspects. In extraction field, two methods have been particularly used in the last decade: Hansen and COSMO-RS approaches, which will be described in the following parts.

I.3.3.3.1 Hansen Solubility Parameters (HSP)

HSP enable to characterize efficiently solute-solvent interactions according to the well-known "like dissolve like" principle. They are derived from the solubility parameter δ_H using **Eq. I-1**. They actually correspond to the three main molecular interactions which may take place between molecules, namely dispersion forces (δ_d), attraction forces/polarity (δ_p) and hydrogen bonding (δ_h) [62]. These three parameters define a 3D space where all solvents and solutes can be placed. To screen which solvent might potentially solubilize a given solute, the approach presented in **Figure I-16** must be adopted. Firstly, the SMILES of solutes and solvents have to be generated and implemented in the software to calculate the HSP. Then, the solubility sphere of solute is determined. Any solvent located in this sphere is likely to solubilize the solute. Indeed, the closer the solute and solvent parameters are, the better the solubility is [63]. Solute-solvent distance D is defined according to **Eq. I-2**.

Eq. I-1 $$\delta_H = (\delta_d^2 + \delta_p^2 + \delta_h^2)^{1/2}$$

Eq. I-2 $$D = (4x(\delta_{d(solvent)}-\delta_{d(solute)})^2+(\delta_{p(solvent)}-\delta_{p(solute)})^2+(\delta_{h(solvent)}-\delta_{h(solute)})^2)^{1/2}$$

Potential miscibility between a solute and a solvent can also be quickly determined calculating the "Relative Energy Difference" (RED). It corresponds to the ratio between the distance D and the radius R of the solubility sphere **(Eq. I-3)**. $0 < RED < 1$ indicates that a

molecule is in the solubility sphere and is likely to solubilize the solute. However, RED > 1 corresponds to a poor solubility.

Eq. I-3. $$RED = \frac{D}{R}$$

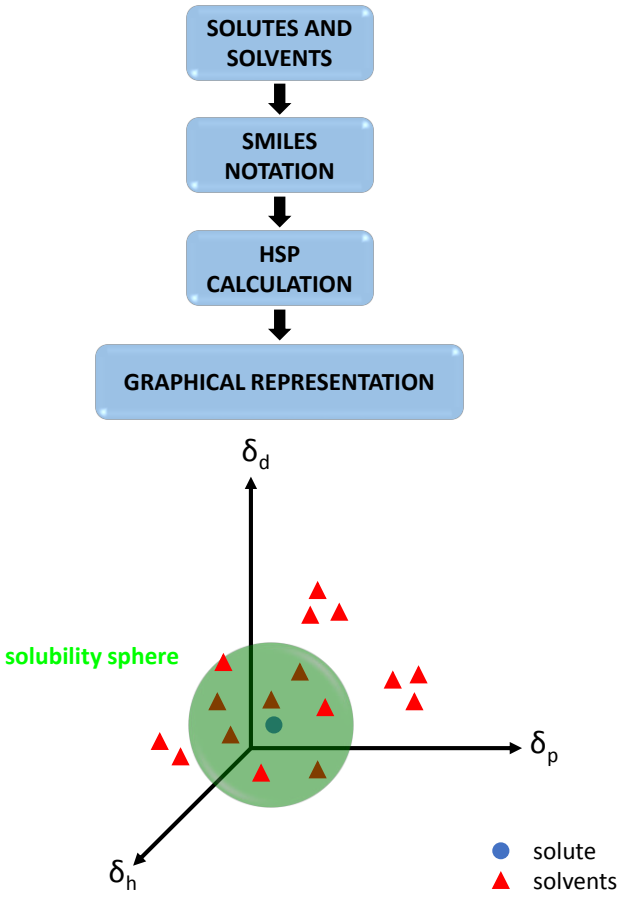


Figure I-16. Determination of solubility sphere using Hansen Solubility Parameters.

Therefore, the use of HSP could be a helpful tool to perform a first selection of potential solubilizing solvents regarding a solute. However this qualitative approach does not consider Van-der-Waals bonds and acid-base effects, that's why solvent selection can be completed with complementary software as COSMO-RS.

I.3.3.3.2 COSMO-RS calculations [61,64]

The Conductor-like Screening Model for Real Solvents (COSMO-RS) is a method for molecular description and solvent screening based on a combination of quantum chemistry (COSMO) and statistical thermodynamics (RS) to determine and predict thermodynamic properties without experimental data [65].

COSMO-RS is a two-step procedure: microscopic and macroscopic steps.

First, the COSMO model is applied to simulate a virtual conductor environment for the molecule of interest which is then embedded into a virtual conductor **(Figure I-17 (a))**. In such an environment, a surface is built on the molecule and it generates an important number of electrostatic charges. The structure and the charge distribution are then optimized to find the minimal energy of the system (molecule in its most stable state) thanks to algorithm-based calculations (Density Functional Theory). This charge density is called σ-surface **(Figure I-17 (b))**. This optimal distribution is afterwards segmented and reduced to a histogram called σ-profile **(Figure I-17 (c))**. The color code gives information about the charge density in each point of the molecule. The green color corresponds to a zero charge density (generally ascribed to carbon skeleton, $\sigma = 0$ on the histogram), the blue color to a positive charge density δ^+ (generally ascribed to hydrogen atoms, $\sigma < 0$ on the histogram) and the red color to a negative charge density δ^- (generally ascribed to oxygenated and nitrogenized groups , $\sigma > 0$ on the histogram). Actually, the σ-profile traduces in 2D the information provided in 3D by the σ-surface. At this stage, the molecule of interest is isolated and does not take into account the molecules in its neighborhood. In order to consider a molecule as a solvent (or as a solute in a solvent) and quantify the interaction energy associated, an additional step based on statistical thermodynamic calculations is required. Solvent interactions are reduced to local interactions by pair of surface portions represented by charge density σ and σ'. All interactions of surfaces are assumed to be in close contact. These contacts can be ideals, non-complementary or can highlight hydrogen bonding which generate energies of interactions. The sum of these energies for a defined area is the interaction energy functional E_{int} **(Eq. I-4)**.

Eq. I-4. $$E_{int} = E_{misfit} + E_{hb} + E_{vdw}$$

If a contact on a surface area a_{eff} (effective contact area) is considered in the particular situation of complementary molecule (where $\sigma = \sigma'$), the interaction energy, also called misfit energy E_{misfit}, is equal to zero and the contact is "ideal." In the general case, there is a mismatch between the partners, $\sigma \neq \sigma'$, therefore E_{misfit} is not zero. In the case where two opposite polarity surfaces are in contact, additional energy appears, hydrogen bonding (HB) energy E_{hb}. HB donors have a strongly negative screening charge density whereas HB acceptors have strongly positive screening charge densities. HB interaction can generally be expected if two sufficiently polar pieces of surface of opposite polarity are in contact. This energy is added to the electrostatic Coulomb energy from a σ_{hb} threshold. In addition to electrostatic misfit and HB interaction, Van der Waals interactions between surface segments are taken into account. The contribution of Van der Waals interactions is not dependent of its vicinity, this is not an energy of interaction but rather a contribution to the energy of the reference state.

After having established the concept of interaction energies on a given molecule surface, it is required to obtain a coherent model of molecules in solution. The σ-profile, $P_s(\sigma)$, is actually the sum of σ-profiles of P_{xi} components, weighted by their molar fraction X_i **(Eq. I-5)**.

Eq. I-5. $$P_s(\sigma) = \Sigma X_i \, . \, P_{xi}(\sigma)$$

The chemical potential of a surface segment with screening charge densities in an ensemble described by normalized distribution function $p_s(\sigma)$ is noted $\mu_s(\sigma)$. This approach can first allow estimating the affinity of a molecule depending on the contacting charge density σ. The molecule is considered as a solvent and this affinity is represented as a σ-potential curve $\mu s(\sigma)$. Secondly, the estimation of the affinity of a molecule i in a solvent S is translated by computing its chemical potential μi^S ($Kcal.mol^{-1}.A^2$). This chemical potential is obtained by integration of the σ-potential over the surface of i **(Figure I-17 (d))**. The σ-potential is calculated from the statistical thermodynamics of molecular interaction based on the obtained σ-profile. This chemical potential μiS, given in the equation below, is the standard chemical potential minus $RTln(x_i)$ which allows the prediction of almost all thermodynamic properties of compounds or mixtures including solubility.

Eq. I-6. $$\mu i^S = \mu_i^{C,S} + \int P_{xi}(\sigma).\mu_S(\sigma).d\sigma$$

With $\mu_i^{C,S}$ the combinatorial contribution to the chemical potential resulting from the derivation of the combinatorial free energy expression.

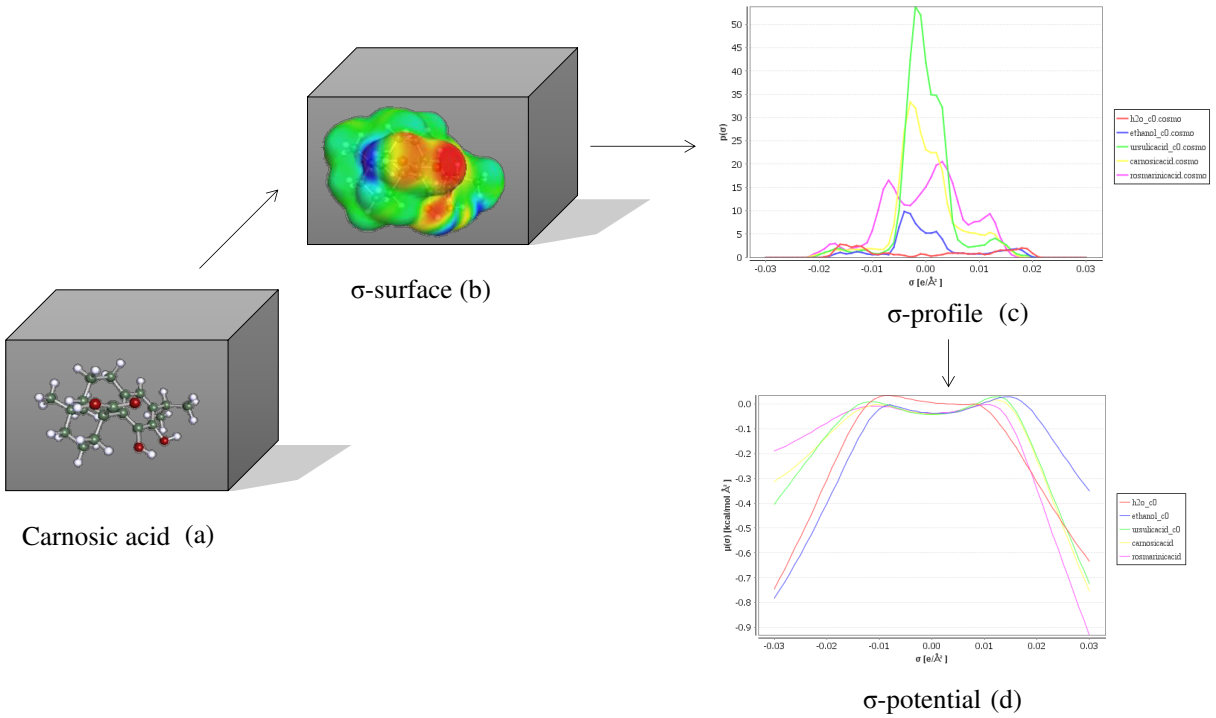


Figure I-17. Step calculation with COSMO-RS: (a) molecule emerged; (b) molecular surface; (c) energies of local surface interactions between σ-profiles of targeted molecules and two solvents (water, ethanol); (d) σ-potentials of targeted molecules and solvent.

In practice, the relative solubility of various solutes in various solvents is calculated from the following equation [66]:

Eq. I-7. $$\log_{10}(x_i) = \log_{10}\left[\frac{\exp(\mu_i^{pure} - \mu_i^{solvent} - \Delta G_{i,fusion})}{RT}\right]$$

With xi = solubility of *i*

μ_i^{pure} = chemical potential of pure compound *i*

$\mu_i^{solvent}$ = chemical potential of *i* at infinite dilution

$\Delta G_{i,fusion}$ = free energy of fusion of *i*

The logarithm of the best solubility is set to 0 and all other solvents are given relatively to the best solvent. A solvent with a log10 (x-solub) value of −1.00 yields a solubility which is decreased by a factor 10 compared to the best solvent.

I.3.3.3.3 Examples

Replacement of hexane or more generally petroleum solvents is one of the major challenges in current extraction field. HSP and COSMO-RS computational methods were particularly used for this purpose as exemplified in **Table I-5**. They enabled to determine potential greener alternatives. Therefore, they appear as very efficient tools to screen quickly an unlimited number of solutes and solvents in terms of potential miscibility and solubility. It is however necessary to confirm theory by experiment since such computational methods only simulate solubilization properties of solvents and not “extractant” power.

Solute	Computational method	Tested solvents	Reference solvent	Potential solvents for replacement	Reference
Free fatty acids Diacylglycerol Triglycerides Phospholipids	HSP COSMO-RS	CPME, D-limonene, DMC, ethanol, ethyl acetate, ethyl lactate, isopropanol, MeTHF, p-cymene, α-pinene	n-hexane	CPME, ethyl acetate, MeTHF	[67]
Sterols Triglycerides Phospholipids Waxes	HSP COSMO-RS	D-limonene , ethanol, ethyl acetate, ethyl lactate, isopropanol, methyl acetate, MeTHF, p-cymene	n-hexane	MeTHF	[68]
Aromas from blackcurrant buds	HSP	Butanol, ethanol, ethyl acetate, ethyl lactate, isopropanol, methyl acetate, MeTHF, supercritical CO_2, α-pinene	n-hexane	MeTHF	[69]
Aromas from caraway seeds	COSMO-RS	Butanol, DMC, ethanol, ethyl acetate, ethyl lactate, isopropanol, MeTHF, α-pinene	n-hexane	DMC, ethyl acetate	[70]
Aromas from basil	HSP COSMO-RS	Vegetables oils	dichloromethane	Sunflower oil	[71]
α-mangostin	HSP COSMO-RS	D-limonene, DMC, ethanol, ethyl acetate, ethyl lactate, MeTHF	dichloromethane	DMC, ethanol, ethyl lactate, MeTHF	[72]

CPME : Cyclopentyl methyl ether ; DMC : Dimethylcarbonate ; MeTHF: 2-methyltetrahydrofuran.

Table I-5. Examples of alternative solvents investigation using HSP and COSMO-RS approaches.

I.4 PRINCIPLE 3: REDUCTION OF ENERGY CONSUMPTION BY ENERGY RECOVERY AND USING INNOVATIVE TECHNOLOGIES.

I.4.1 Context

Energy consumption is one of the major concerns of current industries, whatever sector of activity. It can be related to environmental issues, increased production costs and consequently loss of profitability. In this context, the new challenge of most industrials is to best understand energy requirements of their processes, in order to minimize energetic input and favor recycling.

Among the most energy-intensive industries, 26 % belong to chemistry and plastics manufacturing and 16 % to food processing sector **(Figure I-18)**. Plant extraction field can be included in these two categories; there is therefore a direct interest to optimize extraction processes in terms of energy efficiency. Moreover, traditional extraction processes such as HD are well-known to be very energy-consuming, which opens up many energy optimization possibilities.

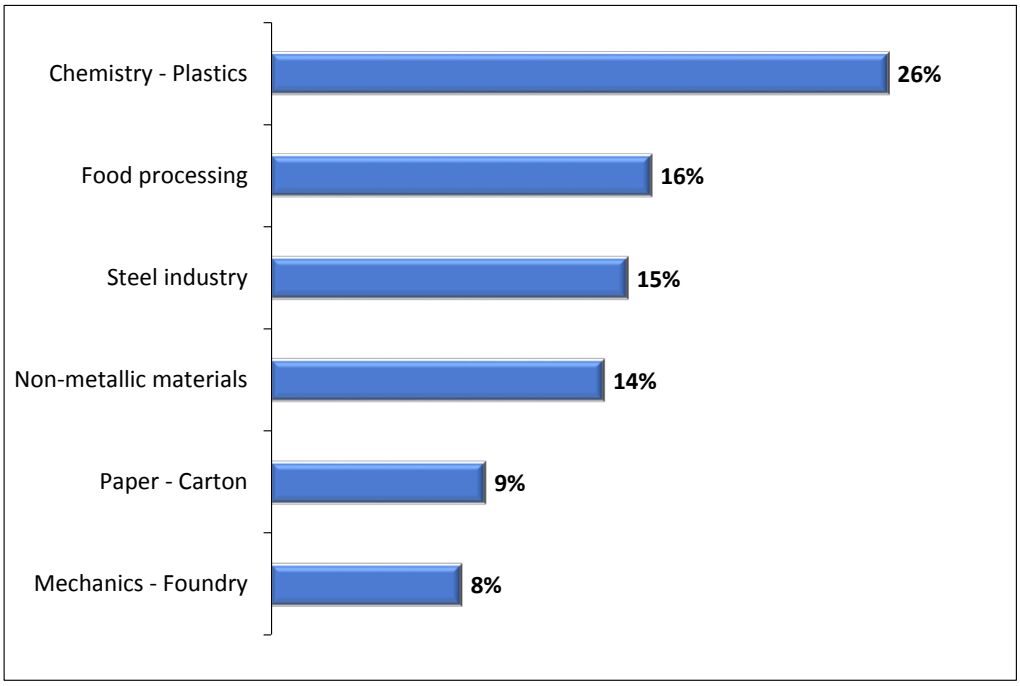


Figure I-18. Assessment of the most energy-intensive industries (from [73]).

Generally, energy consumption related to extraction processes can be reduced adopting four main approaches: optimizing existing process; recovering the energy released during the process; assisting existing processes, particularly with innovative technologies; and using innovative processes [13].

I.4.2 Good practices guidelines

In the demarche of green extraction, the decrease of energy consumption can be achieved using different strategies [13,15]:

- Focus on "low-energy" processes:
    - Conception of processes minimizing as far as possible energy consumption: extraction at ambient temperature, concentration of final extract under pressure, good dimensioning of facilities…
    - Optimization of existing processes, particularly extraction time (which aims at being reduced)
    - Development of innovative processes with high energetic efficiency
    - Assistance of existing processes with new technologies
    - Development of intensified continuous processes
    - Optimization of energetic resources limiting energy waste and favoring energy recovery and reuse
    - Use of Life Cycle Assessment (LCA) demarche as decision tool to compare different processes in terms of energy and improve energy performance ("eco-conception" demarche)
- Optimized management of:
    - Water (recycling, recovery of rainwater)
    - Solvent (recycling)
    - The whole process, from raw material to final product, through the choice of facilities and cleaning agents.

I.4.3 Success stories

In this section, various examples illustrate the four main approaches to reduce energy consumption related to extraction processes: optimization of existing processes, energy recovery, assistance to existing processes and innovative processes.

I.4.3.1 Optimization of existing processes

Process optimization might be the first step to be performed in order to save energy. General optimization procedure is presented in **Figure I-19**. By definition, optimization is "*the act of obtaining the best result possible or the effort for achieving the optimal solution under a given set of circumstances. In design, development, processing operation, and maintenance of engineering systems, common goals are either to minimize the cost or maximize the desired profits as product quality and operation yield*" [74]. Thus, in this demarche, energy consumption must be decrease as far as possible since it is related to high costs. In most scientific studies, optimization of process is performed regarding only one kind of parameter which is the quality of final extract (yield and activity)[75–78]. Operating parameters (temperature, duration, pressure, pH…) are thus selected to maximize this criterion, despite they might be related to high energy consumption. Therefore, with the increasing interest in energy saving, more and more processes or equipment are designed considering this aspect.

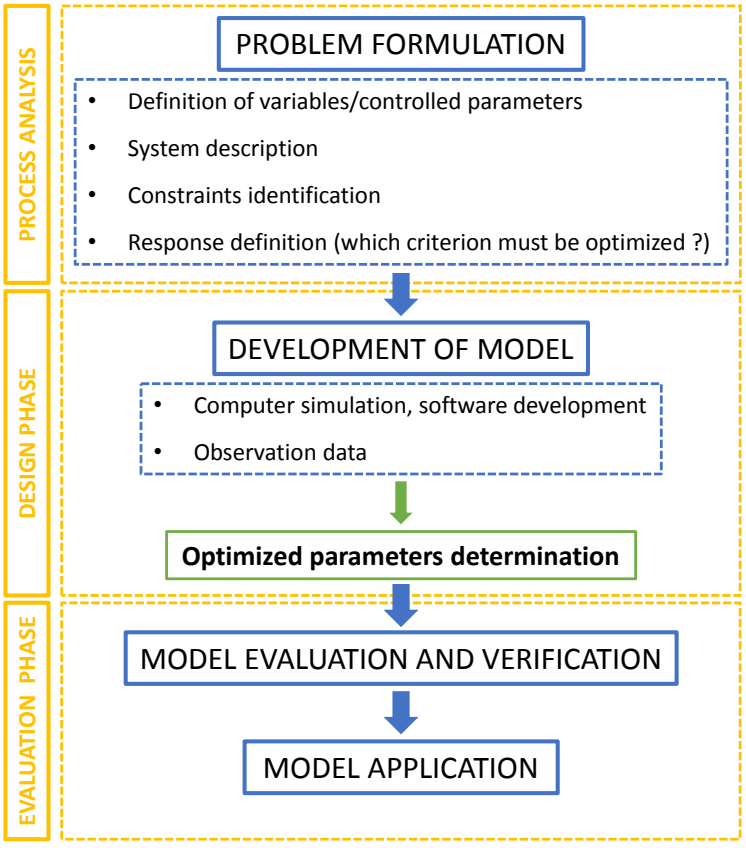


Figure I-19. Process optimization procedure (adapted from [74]).

As an example, Müller *et al.* published some work about the optimization of mechanical oil extraction from *Jatropha curcas* L. seeds [79]. In this study, the objective was to optimize the process increasing oil recovery efficiency and decreasing oil residues in remaining press cake. Various variables were investigated, including specific energy input applied to the mechanical cylinder screw press used for extraction. The first results showed that the higher oil recovery was obtained with a seed material throughput of 4.00 kg/h (around 90 %), however specific energy input related was too high compared to other throughputs. A correlation of specific energy input and oil recovery efficiency versus material throughput was demonstrated, and it enabled to determine the optimal material throughput to maximize both oil recovery and energy efficiency (11 kg/h).

I.4.3.2 Energy recovery

Energy recovery during any process is a new challenge for industrials. Indeed, a process uses only a part (more or less important) of provided heat (called useful heat). The remaining part is said to be “fatal”, and if it is not recovered and recycled, it is wasted (with related costs). To meet this challenge of heat valorization, different ways are available as illustrated in **Figure I-20**.

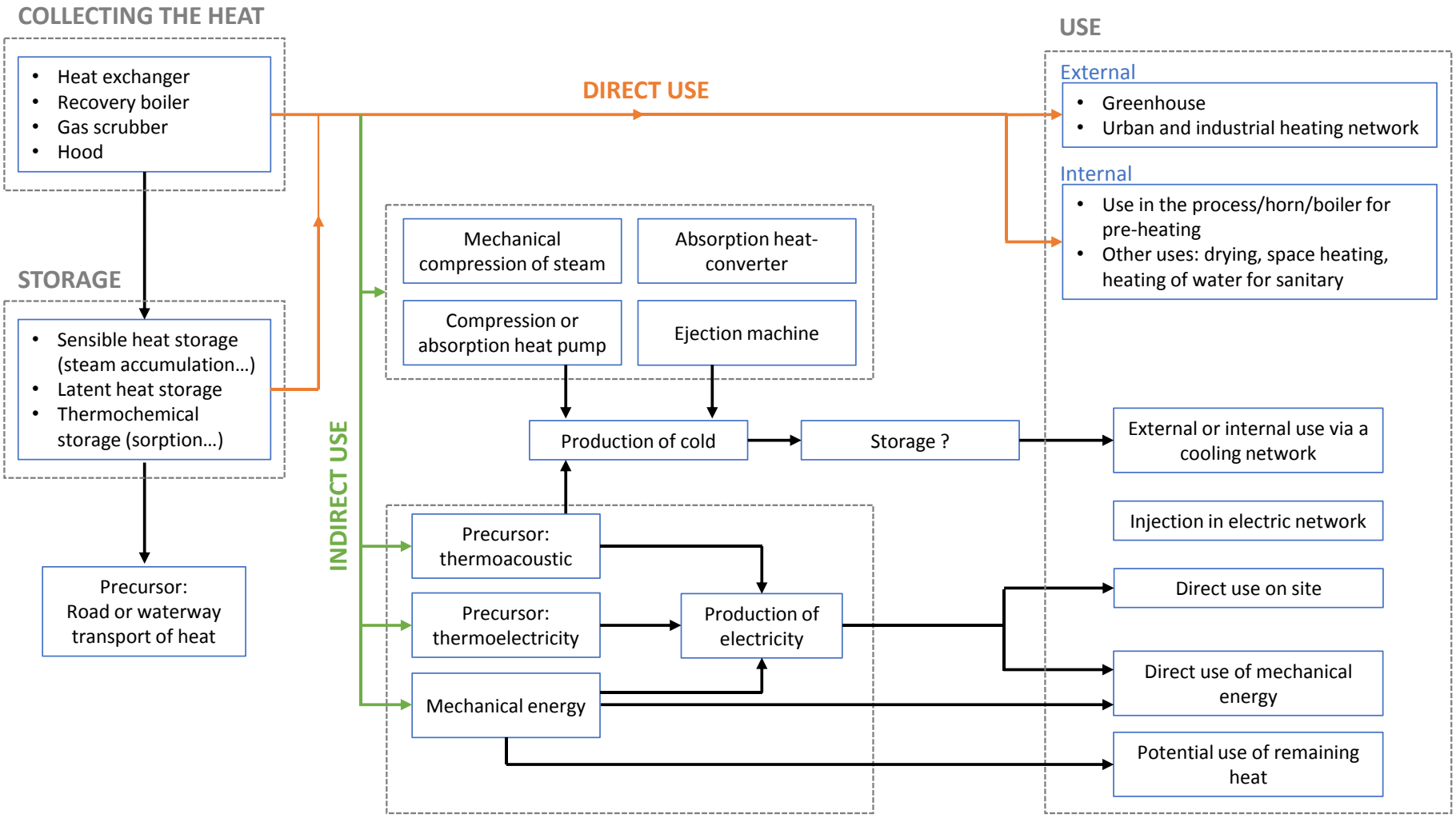


Figure I-20. Ways to valorize fatal heat from industrial processes (adapted from [73]).

Some societies have already developed equipment to ensure the recycling of fatal heat, such as Belfort area engineers which developed a motor able to generate mechanic or electric from an external heat source (over than 150 °C) [80].

In extraction field, most processes are performed at solvent boiling point and therefore need an external heat source to reach wanted temperature (generally with boiler at industrial scale). Depending on the solvent, energy input might be more or less important. Basil *et al.* published a work at laboratory scale about extraction of flavor compounds from rosemary by superheated water [45]. This process was compared with conventional steam distillation in terms of extraction efficiency and energy and water costs. Water is one of the solvents which need the highest energy to be heated and converted at gaseous state. Working under pressure enables to decrease the energetic input. Indeed, whereas 2550 kJ/kg are needed to convert water at 30 °C to steam at 100 °C, only 505 kJ/kg are necessary to heat water from 30 °C to 150 °C under 15 bars. Plus, the study also claimed that much more heat could be recycled in a superheated water extraction, depending, however, on heat exchangers size. Heat advantage of superheated water extraction per kg of water is announced to be about 20 compared to steam distillation, which makes it viable for up-scaling purpose. Therefore, when designing a new process or optimizing an existing one, energy advantage related and potential to recover heat must be taken into consideration as far as possible.

I.4.3.3 Assistance to existing processes

When conventional extraction processes are not enough efficient to recover acceptable yields of specific compounds of interest from raw material, it is possible to intensify and assist the process with innovative technologies. Ultrasound (US) or MW can be used for this purpose. They have been hugely employed to intensify extraction of various compounds from various plant materials [81–89]. Generally, it results in the decrease of extraction duration and energy consumption compared to conventional process, for similar or enhanced extraction yields. In the case of rosemary, the application of US or MW to extraction media enabled to increase by three times the yield of total phenols extracted compared to corresponding conventional extraction [90]. US technology was also employed by Paniwnyk *et al.* to intensify antioxidants extraction from rosemary, and it resulted once again in improved extraction yields [91]. As another example, MW were used to assist ginger solvent extraction, and as with US, greater yields were obtained in a drastically reduced extraction time

(compared to Soxhlet) [92]. Fundamentals of US and MW are described in the following parts.

I.4.3.3.1 Principle of US

US are mechanical waves that can propagate in an elastic medium and that have a higher wave frequency (20 kHz to 10 MHz) than sounds audible to humans (10 Hz to 20 kHz). Ultrasonic wave is characterized by the frequency f (in Hz), the wavelength λ (in m), the celerity c (in m/s), and the power P (in W) that quantify the ultrasonic energy provided by US to the system. This power per unit of US emitting surface defines the ultrasonic intensity (UI) (W/cm^2). Power can as well be related to the volume submitted to US, corresponding in this case to the power density (PD) (W/cm^3) [165].

As illustrated in **Figure I-21**, US can be classified in two main categories [166,167]:

- Power US (20 kHz – 1 MHz, high-intensity sonication I > 1 W/cm^2) employed for extraction and processing applications
- Diagnostic US (1 MHz – 10 MHz, low-intensity sonication I < 1 W/cm^2) typically used as a non-destructive analytical technique (quality assurance, process control…), providing more particularly physico-chemical properties of matter.

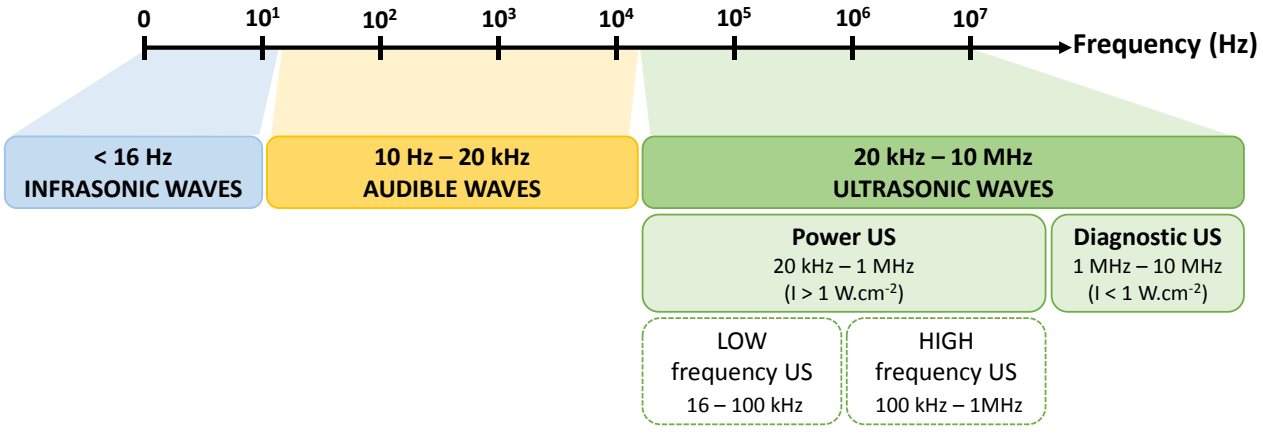


Figure I-21. Ultrasound frequency range.

Generally, ultrasonic effect is characterized by various physical and chemical phenomena, including agitation, vibration, pressure, shock waves, shear forces, microjets, compression and rarefaction, acoustic streaming, cavitation and radical formation [167].

Power US are the most used in extraction field. They are two such categories **(Figure I-21)**: low frequency US (16 – 100 kHz) for which physical effects (agitation, vibration, cleaning, degassing, emulsification) dominate and high frequency US (100 kHz – 1 MHz) for which chemical effects (reactional mechanism change, generation of free radicals…) are more important [167].

Extraction effects of sonication are mainly driven by the force of acoustic cavitation. Indeed, the propagation of US through a medium induces cycles of compressions and rarefactions in the molecules of the medium **(Figure I-22)**. Such series of compressions-rarefactions induce an acoustic pressure P_a in the medium defined by **Eq. I-8** and **Eq. I-9** [61].

Eq. I-8. $$P_a = P_A \sin 2\pi f t$$

Eq. I-9 $$P_A = (2 I \rho c)^{1/2}$$

Where P_A: maximal amplitude of the pressure of the wavelength

f: frequency

t: time

I: ultrasonic intensity

ρ: density of the medium

c: velocity of the wave in the medium

Compression phase corresponds to a positive pressure whereas rarefaction phase corresponds to a negative pressure. Such alternating pressures causes distention and connection between molecular groups in the medium **(Figure I-22)**. According to the medium, negative pressure is more or less extended. During rarefaction, beyond a certain distance between molecules, local pressure falls below the vapor pressure of the liquid, generating voids in the medium. These voids correspond to cavitation bubbles formed from dissolved gases. Depending on their or ultrasonic conditions, these bubbles will be stable or

will implode in the medium. This phenomenon of creation, expansion and implosive collapse is called the acoustic cavitation [61,165–167].

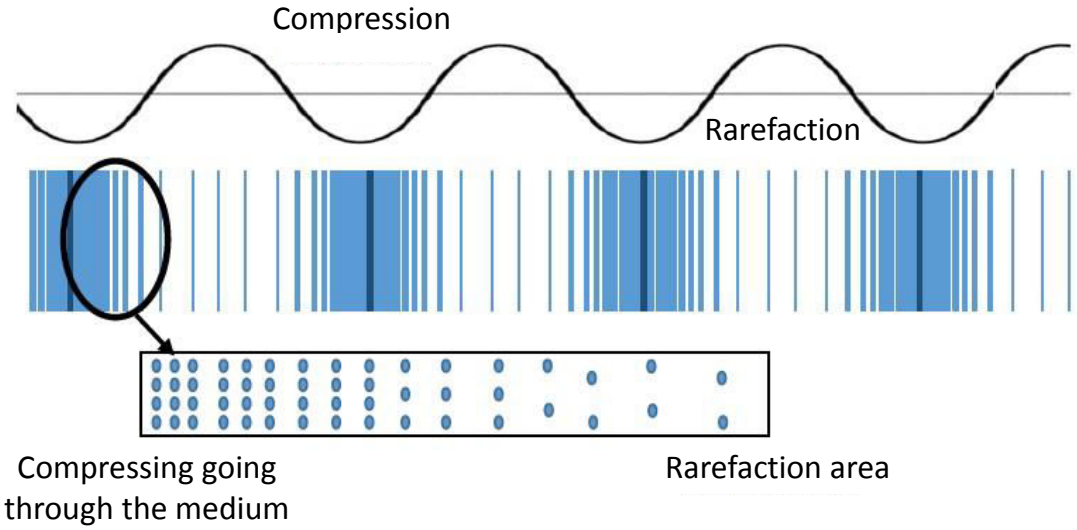


Figure I-22. Compression and rarefaction cycles induced by an ultrasound wave (from [61]).

Cavitation bubbles are divided in two categories, corresponding namely to transient cavitation and stable cavitation. Stable bubbles exist for several cycles of compression and rarefaction, therefore their lifetime is relatively long. In contrast, transient bubbles exist for a very short period of time, and collapse violently. This collapse corresponds actually to an implosion which generates temperatures and pressures superior to 2000 °C and 500 bar respectively locally in the medium. During UAE, when this phenomenon occurs on plant material surface, microjets and shock waves are generated directly towards the surface **(Figure I-23)**. It results in cell disruption, and consequently in a better solvent penetration in vegetable cell and a better mass transfer of solutes to the solvent [167,168].

As mentioned in previous lines, ultrasonic frequency determines US applications (power US for extraction/processing and diagnostic US for non-destructive analytical technique). Regarding power US, besides UI the main parameters influencing ultrasonic cavitation and more generally UAE are the plant material, the solvent, the temperature and the presence of dissolved gases. Most of these factors can have influence over each other, particularly solvent properties which are strongly temperature-dependent. **Table I-6** relates

the effects of solvent type, extraction temperature and the presence of dissolved gases on ultrasonic cavitation and on extraction efficiency [61,167].

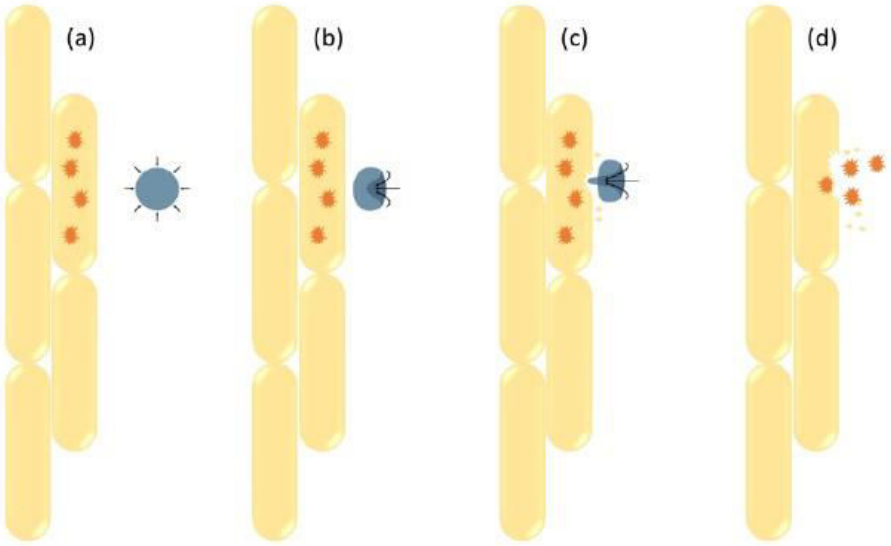


Figure I-23. Collapse of cavitation bubble and release of targeted compounds from plant material. (a) Bubble close to plant surface, (b) Collapse during compression phase, (c) Microjet toward cells, (d) Release of cell content (from [61]).

External factors		Effect on cavitation and extraction efficiency
Increase of temperature		• Increase of extraction rates • Enhancement of diffusion rate • Increase of solute-matrix interaction disruption • Decrease of cavitation phenomenon (voids formed during rarefaction phase are filled with solvent vapour resulting in a less violent collapse)
Solvent	Increase of viscosity	• Decrease of cavitation phenomenon
	Increase of vapour pressure	
	Increase of tension surface	
Presence of dissolved gases		• Increase of cavitation phenomenon

Table I-6. Factors influencing ultrasonic cavitation and extraction efficiency.

I.4.3.3.2 Principle of MW

MW are electromagnetic waves with a frequency range comprised between 300 MHz and 300 GHz (corresponding to wavelengths ranging from 1 mm to 1 m). On the electromagnetic spectrum illustrated in **Figure I-24**, they are located between radio and infrared waves. The most common frequency used in domestic oven or in laboratory and industrial equipment is 2450 MHz, generally with a power comprised between 100 W and 1000 W [169].

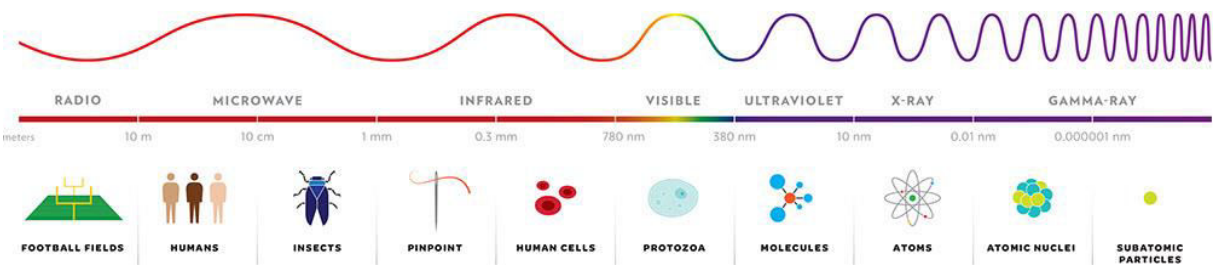


Figure I-24. Electromagnetic spectrum (from [170]).

MW, by the very definition of an electromagnetic wave, result from the application of an electric field combined with a magnetic field that are self-propagating in space. They are perpendicular to each other and to the direction of propagation. Amplitudes of fields vary sinusoidal with time and propagation **(Figure I-25)**.

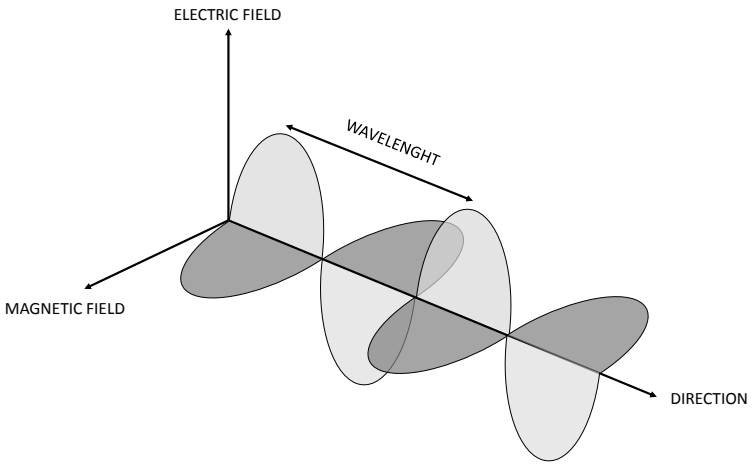


Figure I-25. Illustration of the electromagnetic wave.

The interaction between an electromagnetic field and a material (plant, solvent) depends on its dielectric properties. Such properties are characterized by the dielectric constant (or permittivity) Ɛ' and the dipole moment (or dielectric loss factor) Ɛ''. By definition, the dielectric constant of a molecule corresponds to its capacity to be polarized by an electric field and the dipole moment to its ability to convert MW energy into heat [169,171]. From Ɛ' and Ɛ'' can be calculated the dissipation factor (tan δ) using **Eq. I-10**.

Eq. I-10. $$\tan \delta = Ɛ'' / Ɛ'$$

MW will interact preferentially with molecules presenting a high dielectric constant. Generally, they are polar or have a dipolar moment. Under MW, these molecules attempt to align with the electric field which switches approximately 2.5 billion times per second. Since there are internal interactions between molecules (hydrogen bonds or Van der Waals forces), this alignment is done with resistance, causing molecular friction. The sum of dipolar rotation, molecular friction and molecular collisions results in heat generation in the medium. Hence, heating occurs from the inside to the outside of material, unlike conventional heating when heating is done by conduction and convection, from the outside to the inside **(Figure I-26)**. Moreover, there is a heating selectivity towards polar molecules. In plant extraction, depending on the aim of the experiment, it is possible to focus MW heating on solvent (in this case a polar solvent must be selected), directly on plant material (in this case an apolar solvent rather transparent to MW must be chosen) and even to perform extraction without solvent (MW will focus on constituent water of plant material).

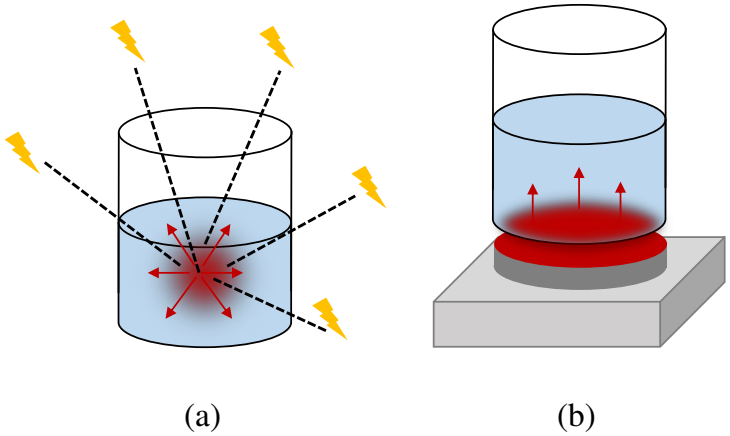


Figure I-26. Comparison between MW heating (a) and conventional heating (b).

Domestic, laboratory scale and industrial scale MW ovens are all composed of the same parts. They are presented in **Figure I-27**. The magnetron is the generator of MW. It enables to transform electric energy into electromagnetic waves. Afterwards, generated waves are directed by the waveguide into MW cavity. This cavity is actually a Faraday cage able to trap MW. In this MW cavity is placed the reactor (in Teflon to be transparent to MW) containing the sample which will be submitted to MW. Finally, in laboratory and industrial equipment, a monitoring system is generally added to the system in order to control MW power, time, temperature and pressure.

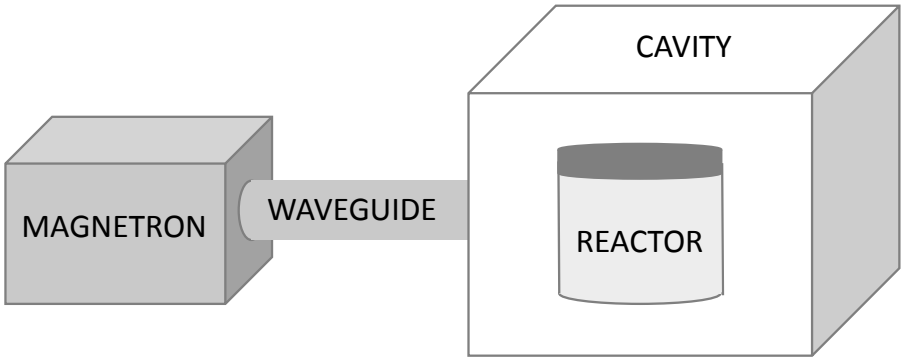


Figure I-27. Microwave oven components.

MW can be applied either in monomode or multimode. In monomode, the application of MW is performed thanks to a cavity that maintains a single mode of propagation of the

wave, which is directed by the waveguide. It enables precise control of electric field but it is reserved for the treatment of small volume experiments.

In a multimode cavity, electromagnetic waves are reflected from the walls and scattered randomly. This type of cavity allows to perform experiments with larger amounts of product, but the control of field distribution is quite difficult and high temperature gradients can be observed in the product. Wave brewers or rotating plates can be used to overcome this problem.

I.4.3.4 Innovative processes

Contrary to previous section where innovative technologies were only used to assist conventional solid-liquid extraction, in this part "innovative processes" refer to completely different processes than conventional ones, which request specific facilities or solvents.

When optimization of existing process or addition of external technologies during the process are not viable energetically, it is sometimes necessary to reconsider completely global procedure. Innovative process may be adopted for pre-treatment phase upstream from extraction process, or may be the process itself. The main innovative technologies which can be used in current extraction field at industrial level are summarized in **Figure I-28**.

Figure I-28. Assessment of available innovative processes for green extraction.

In much cases, these innovative technologies appear as energy efficient since they enable to reach maximal yields in reduced extraction time. Performed upstream from the main process, they permit an optimal pre-treatment of raw material. For example, US pre-treatment was performed for 30 min prior to HD of *Carum carvi* L. seeds. It resulted in rapid release of EO during HD, that is 80 % of EO recovery in 30 min against 90 min without pre-treatment [93]. In another case, deodorization by instant controlled pressure drop (DIC) was performed on rosemary leaves before ethanolic extraction. It permitted to improve significantly rosmarinic acid (RA) extraction yield since that of DIC-treated leaves was twice as much as untreated leaves. Moreover, DIC-treatment step enabled to recover EO and consequently deodorize plant material. EO extraction was also intensified as DIC-treatment lasted 3 min against minimum 4 h for conventional HD [94]. As last example of successful plant material pre-treatment with innovative process, Kubra *et al*. compared different drying method to dehydrate ginger. MW drying resulted in reduced drying time and maximal volatiles retain in specific conditions [95].

Innovative technologies also offers efficiency opportunities as main extraction process. Pressurized liquid extraction (PLE) appears particularly as an efficient and sustainable approach in various studies. Indeed, Hu *et al*. used this process to extract phenolics from ginger with ethanol or water as solvent [96]. It resulted in reduced extraction time and solvent consumption, and consequently in a decrease of energy consumption and costs. Extraction solvent 70 % ethanol, pressure 1500 psi, temperature 100 °C and extraction time 20 min were determined as the optimal conditions to maximize extraction efficiency. Under these conditions, actives recovery was 106.8 %, 109.3 % and 108.0 % for 6-gingerol, 8-gingerol and 10-gingerol respectively, compared to corresponding Soxhlet extraction. PLE was also performed to extract antioxidant from rosemary leaves with ethanol [97]. Good results were obtained in terms of extraction yields, extract activity, and both polarities antioxidants extraction (RA and carnosic acid (CA)).

I.5 PRINCIPLE 4: PRODUCTION OF CO-PRODUCTS INSTEAD OF WASTE TO INCLUDE THE BIO- AND AGRO-REFINING INDUSTRY.

I.5.1 Context

Industrial extraction of natural products mainly includes the juice production from fresh fruits and vegetables, the production of vegetable oil from various seeds and the active compounds extraction from diverse biomasses using liquid solvents. It often focuses on the extraction of one single product of interest from an initial biomass and consequently generates, each year, huge amounts of waste and by-products (about 220 million tons). Depending on the type of raw material processed and the technologies employed, the quantity of waste produced can vary considerably. As presented in **Table I-7**, in some cases, more than 50 % of processed raw material is considered as waste and by-products at the end of process. Juice (from fruits and vegetables) and vegetable oil (from oleaginous) productions are particularly concerned since 30-50 % and 40-70 % of processed raw material respectively are regarded as waste and by-products at the end of process.

Production processes	Percentage of waste and by-products (%)
Corn starch production	41 - 43
Fruits and vegetables juice production	30 - 50
Fruits and vegetables processing and preservation	5 - 30
Potato starch production	80
Sugar production from sugar beet	86
Vegetable oil production	40 - 70
Wheat starch production	50
White and red wine productions	20 - 30

Table I-7. Percentage of food waste and by-products generated by different processes (from [98]).

The type of waste is very diverse since it is directly raw material- and process-dependent. **Table I-8** illustrates the large variety of residues which can be obtained from only one kind of raw material. Therefore, in a context of population growth, decline in agricultural productivity, increase in the productivity costs and underutilization of available resources, a way to manage at best this waste is urgently required [99].

Food Type	**Type of residues**
Cereals, grains rice, wheat, corn	Straw, stem, leaves, germ, husk, bran, fibres
Edible oils	Press solids, oil cakes, oil and water emulsions, shell of seeds
Fruits and vegetables	Stem, pits, seeds, peel, pulp, pomace, non-nutritive fibres

Table I-8. Type of residues from different foodstuffs after processing (from [98]).

Beyond the current waste and by-products management options, the production of food ingredients is the most cost-profitable for industrials, followed by the animal feed, the energy recovery, the land spreading and finally the land filling which relates to a negative added value **(Figure I-29)** [98].

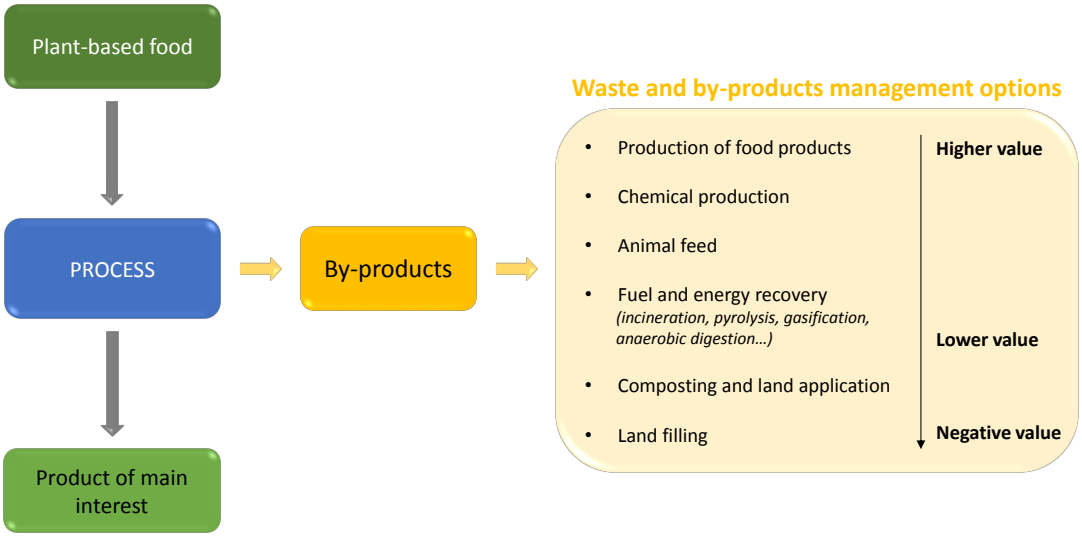


Figure I-29. Relative classification of food waste and by-products management options.

In major cases, despite they are considered as waste, food processing and extraction by-products still contain organic molecules with high nutritional value (such as antioxidants, proteins, dyes, vitamins or oils) or high energetic potential [100–102]. Plus, they constitute a low cost raw material and large volumes are available, which present specific economic and environmental interest for industrials. **Figure I-30** relates some examples of bio-based product applications in current lines of business.

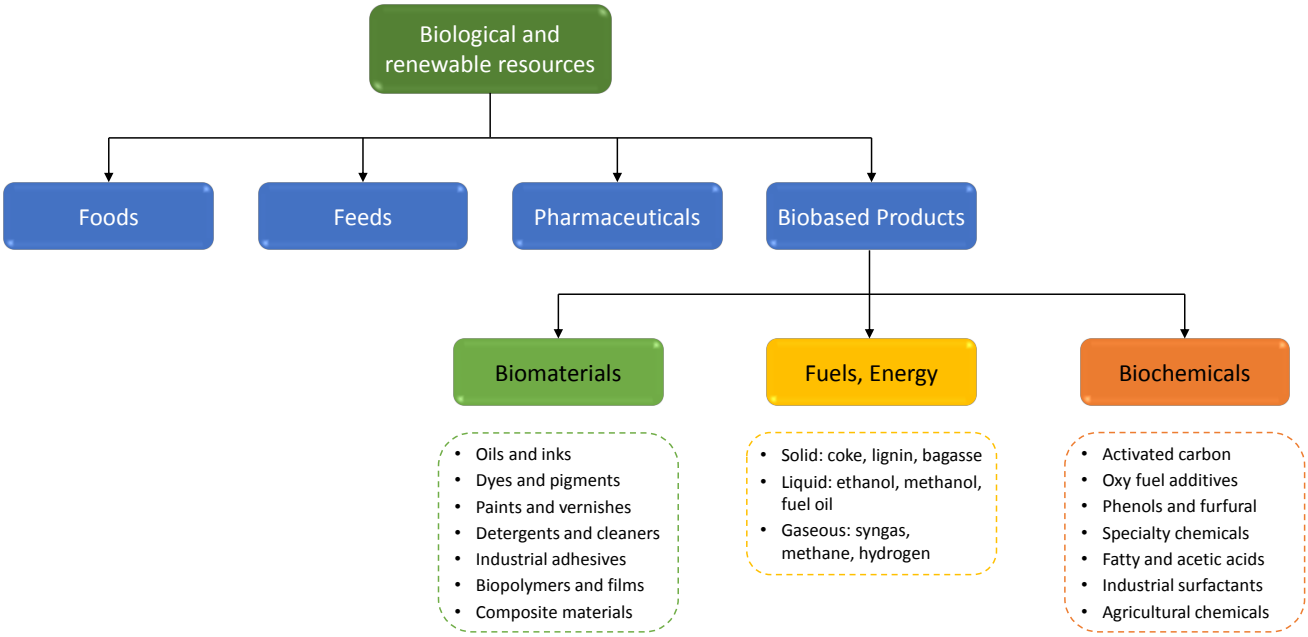


Figure I-30. Potential products and bio-products from biological biomass (adapted from [103]).

In this context, food processing and extraction by-products appear as good candidates for valorization. This demarche fits perfectly with the emerging bio-refinery concept which aims at a complete exploitation of a biomass and its different building blocks. The different parts can be transformed afterwards into bio-energy (heat, fuel and electricity) and bio-based products (foodstuffs, chemicals and biomaterials), using extraction, fractionation and conversion processes with particular facilities or network of facilities [100].

I.5.2 Good practices guidelines

Good practices guidelines regarding the management of extraction waste and by-products have two main objectives [15]:

- The development of valorization pathways for a better use of raw material and complete exploitation of by-products
    - Performing a second extraction/process to generate other high value products
    - Using residual raw material to feed livestock
    - Converting the residual biomass into energy
- The reduction of effluents and waste in terms of quantity and harmfulness with:
    - The well-reasoned conception of processes to anticipate and reduce waste production
    - The limitation of liquid and gaseous effluents, particularly greenhouse gases
    - The choice of low environmental impact recycling processes.

I.5.3 Success stories

This section aims at presenting some achievements in terms of food and extraction by-products valorization. These examples illustrate previous good practices guidelines regarding principle 4. Animal feed application, high value compounds recovery and conception of bio-refinery are more particularly developed.

I.5.3.1 Animal feed

Currently, food and extraction by-products are hugely used for livestock feed because of their richness in various nutrients. This is the case for edible oil cakes which present, after processing, a protein content ranging from 15 % to 50 %, making them ideal candidates to supplement animal diet and enhance production efficiency. As an example, the diet of laying hens was supplemented with 10-20 % of processed flaxseed and it resulted in the production of high quality eggs (“omega eggs”) valuable for their balance of unsaturated fatty acids [104]. Flaxseed by-products were also added to beef cattle diet and engendered an improvement of animal performance and carcass value [105,106]. In another case, distillated rosemary leaves (by-product resulting from EO extraction) were transformed into pellets and

incorporated in pregnant sheep diet in 0, 10 % and 20 % proportions. Effect on cooked lamb fillets was assessed and it resulted in decline of lipid oxidation and rancidity incipient due to the presence of antioxidant compounds. Thus, shelf life of lamb-based dishes can be extended by feeding ewes with such pellets [107]. It was also showed that sheep diet supplementation with distillated rosemary leaves improved significantly fatty acids profile of lamb meat, with an increase in polyunsaturated and unsaturated fatty acids content and a decrease in saturated fatty acids rate [108].

Beyond the various by-products, some of them have the ability to replace more traditional feed dedicated to livestock because of their specific composition. It is the case of ginger waste meal resulting from oleoresin extraction process: it can substitute maize in animal diet thanks to its high concentration in carbohydrates. A study carried out by Omage *et al.* also showed that rabbit diet supplemented with such ginger by-product induced hypocholesterolemic and hypolipidemic effects without affecting growth performance [109].

Finally, some by-products cannot be used in their native form to feed livestock since their composition is not compatible with their diet. As an example, coffee pulp (by-product of coffee processing) must be free of tannins, caffeine and other polyphenols to avoid any anti-nutritional issue in the final feedstock. However, the compounds which must be removed from the by-products might have potential interest in other markets. Plus, others by-products such as sugarcane tops (representing 5 % to 10 % of total cane production) offer very low content in essential nutrient (as proteins or minerals) and don't fulfill animal nutritional requirement. To increase their nutritive value, they may be transformed into silage using a specific process. As positive point, the generated product is particularly palatable and attractive to livestock [110].

I.5.3.2 Extraction of high value compounds

Food and extraction by-products offer a large variety of valuable compounds, as illustrated in **Figure I-31** for fruits and vegetables residues. Beyond the potential products, we can cite antioxidants, proteins, EO, or dietary fibers. They can be recovered using various pre-treatment and extraction techniques, from the most conventional to the most innovative [111].

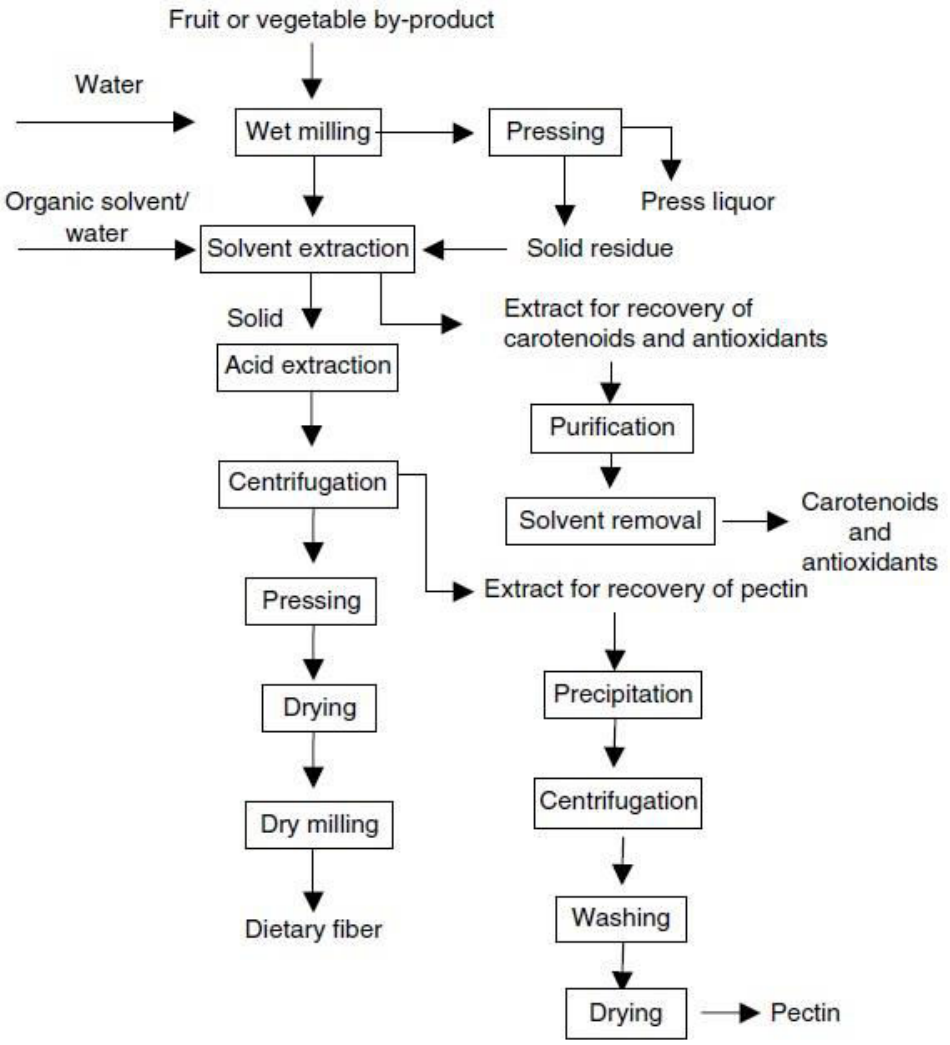


Figure I-31. Flowsheet regarding the valorization of fruits and vegetables by-products (from [112]).

I.5.3.2.1 Antioxidants

Antioxidants can be extracted from a very large range of extraction and food by-products. There are generally recovered by conventional solvent extraction (CSE). The polarity of solvent used hugely influences the type of extracted compounds and thus the final antioxidant activity. Low-medium polarity solvents such as hexane or acetone can be used to extract low-medium polarity antioxidants such as tocopherols or some phenolic terpenes. On the other hand, higher polarity solvents such as ethanol or water can be used to extract flavonoids glycosides or phenols. Plus, temperature, extraction time and pH in the case of aqueous solvent are the main parameters affecting the extraction efficiency [112]. Beyond the wide number of examples about antioxidant valorization from extraction and food by-

products, many of them deal with fruit and vegetables residues. Indeed, as examples, polyphenols were extracted from ginja cherry [113], grape [114,115], apple [115,116] or citrus by-products [117,118] using different techniques. Rosemary by-products from EO industry were also valorized to recover specific powerful antioxidants: RA, CA, carnosol (CO), caffeic acid and chlorogenic acid. Rosemary waste was submitted to solid-liquid extraction with 96 % ethanol for 4 h. Authors suggested that the previous EO extraction would have modified the plant structure, favoring consequently antioxidants extraction in the next steps. However, some degradation phenomenon were testified by higher amounts of CO compared to CA in the final extract [119].

I.5.3.2.2 Proteins

Oilseeds by-products generally contain between 15 and 50 % proteins and are consequently a common source of protein products. The treatment of oilseeds by-products in acidic or neutral medium leads to a protein concentrate (almost 70 % proteins) after elimination of non-protein compounds by precipitation. Oilseeds by-products can also be treated in alkaline and neutral medium to solubilize proteins and carbohydrates and generate a protein isolate (more than 95 % proteins) after further purification steps. Alternative methods can also be performed in order to enhance recovery rate and to avoid denaturation of proteins. As an example, in the case of de-oiled rice bran, conventional alkali hydrolysis was replaced by hydrolysis in subcritical water at 200 °C for 30 min. It resulted in improved extraction yields and a final product exhibiting high antioxidant activity [120].

I.5.3.2.3 Dietary fibers

Dietary fibers can be valorized for their properties of texture and their health benefits in human diet. They are divided into soluble (pectin, gum) and insoluble (cellulose, most hemicellulose and lignin). Their isolation from by-products can be performed as a last process after the extraction of other valuable compounds (antioxidants, dyes or proteins). The most current by-products for dietary fibers production are citrus peels, and apple pomace [115,118,121,122]. Regarding pectin extraction, dried by-products are generally extracted at high temperature in acidic medium (pH 2.5) and pectin is afterwards precipitated by the addition of alcohol [123]. The further purification steps consist in washing the previous precipitate with acidified and alkalinewater, and finally neutral alcohol. Insoluble fibers

extraction, on the other hand, includes microbial fermentation, chemical and mechanical treatments.

I.5.3.2.4 Essential oils and aromas

Beyond the various food and extraction residues, the major sources of EO are the citrus by-products and more precisely the peels. Depending on the variety, essential content ranges between 0.05 and 5 %. Citrus EO (and more particularly those of lemon and orange) are mainly composed of limonene (until 95 %) [124] which can be valorize as agro-sourced solvent and be used for plant extraction to replace hexane [125]. Various techniques are available to extract EO from citrus residues, from traditional processes as HD to the most intensified with supercritical fluids, MHG or DIC [28,118,126].

Another ways to generate aroma from by-products are the microbial synthesis or the bioconversion. Indeed, some by-products such as apple pomace or cassava bagasse can be used as substrate to produce fruity aroma [127].

I.5.3.3 Development of bio-refinery

Bio-refinery concept **(Figure I-32)** aims at total valorization of a biomass into its different building blocks to generate valuable bio-chemicals or energy, using chemical, physical and thermal processes. The development of bio-refinery is increasing since it is not selective with respect to the type of biomass, and a large range of very various products can be generated. Plus, the major part of available by-products contain various compounds of interest (EO, pectin, proteins…) which could be recovered in a single integrated process. With definitive economic and environmental advantage, it is a universal tool that should be more developed at industrial level.

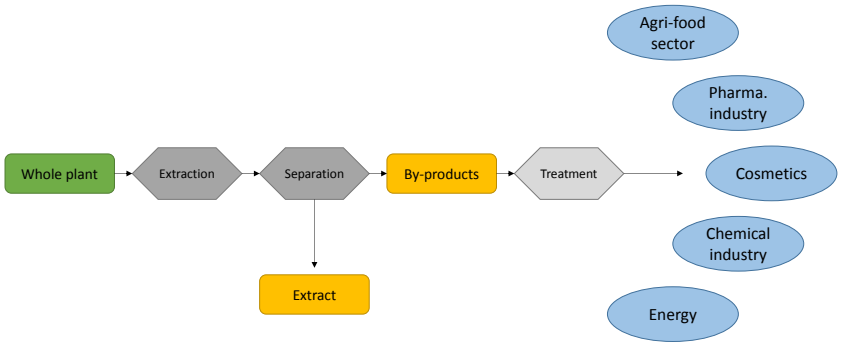


Figure I-32. The plant bio-refinery concept (adapted from [13]).

This concept is directly patterned on the petroleum refinery system **(Figure I-33)**. Indeed, in both cases, from the inputs (biomass or fossil resources) are generated several products (outputs) using a multi-step process. However, the products obtained with the conventional refinery are mainly dedicated to energy and fuel sector, unlike bio-refinery where the balance between bio-chemicals and bio-energy generated is more equilibrated. An ideal bio-refinery aims at producing high-value low volumes to enhance the profitability and low-value high volumes which will be converted in energy using further operation units. Currently, bio-refineries can be differenced in two categories according to the kind of raw material selected as input: the first and the second generation bio-refineries.

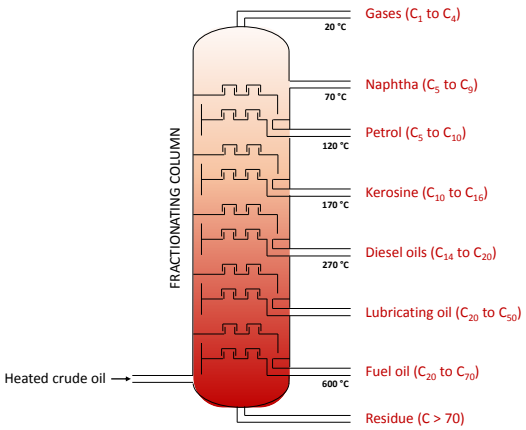


Figure I-33. Simplified diagram of petroleum refinery system.

As illustrated in **Figure I-34**, the first generation bio-refinery (or "whole-crop bio-refinery") uses directly agricultural biomass (as wheat, rice, maize or colza) and generates mainly bio-fuels such as bio-ethanol or bio-diesel. Bio-ethanol is produced from biomasses rich in starch or sugars (such as wheat, sugar beet or corn) via an enzymatic hydrolysis followed by a fermentation step. Using this process, some compounds can also be generated in a secondary way, as the polylactic or the succinic acids. Regarding bio-diesel, it is generated from biomasses rich in vegetable oil and triglycerides via a transesterification step, which can be also associated to the production of secondary bio-chemicals as methyl esters of fatty acids or surfactants. This efficient and alternative production of biofuels appears as a good way to meet the challenge of fossil fuels replacement. However, first generation bio-refineries are currently controversial since they encourage the competition between the production of agricultural raw material dedicated to the food sector and those dedicated to the

bio-refinery. Plus, the increase in agricultural raw material demand might result in intensive culture, intensive use of fertilizers and pesticides to improve production yields [100,128,129].

In the case of second generation bio-refinery (or "lignocellulose feedstock bio-refinery"), inputs correspond to non-food biomass. Bio-fuels and bio-chemicals are produced from lignocellulosic material including residues and waste from agriculture, forestry or food industry [100,128]. It corresponds to the hugest source of renewable carbon and the available biomasses are extremely diverse. Lignocellulosic biomass is composed of four fractions at various proportions: 40-60 % cellulose, 20-40 % hemicellulose, 10-25 % lignines and 10 % inorganic minerals and organic extractives (phenols, terpenes, alkaloids…) [130,131]. From these structural compounds are produced the second generation bio-fuels (or "cellulosic bio-fuels") and bio-chemicals, using thermochemical or biochemical ways **(Figure I-34)**. Thermochemical conversion includes essentially thermal processes such as combustion or pyrolysis. Regarding biochemical processes, the anaerobic digestion, the acid or enzymatic hydrolysis, followed by the fermentation of produced sugars are commonly performed. Generally, a previous mechanical or chemical pre-treatment is necessary to break down the lignocellulosic material and make available the cellulose, hemicellulose and lignins [132].

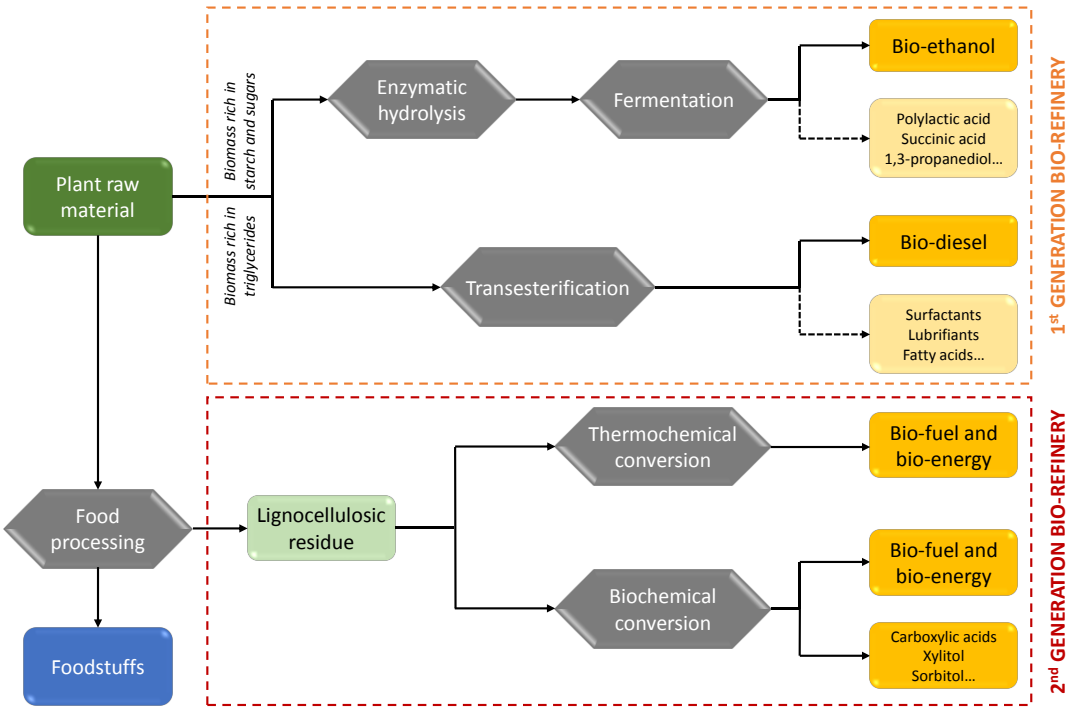
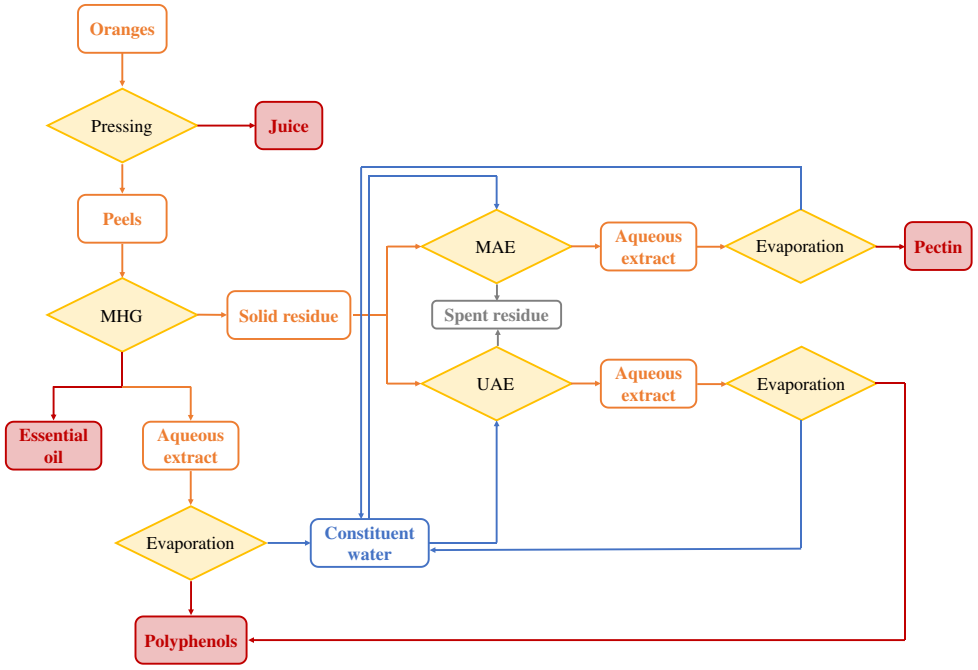


Figure I-34. Simplified diagram of first and second generation bio-refineries.

Current literature relates most successful cases of bio-refineries performed on by-products. As an example, Zhang *et al*. produced from cassava-based industrial waste bio-fuels (bio-ethanol, bio-butanol, bio-hydrogen, bio-methane), organic acids (fatty acids, citric, lactic and succinic acids), bio-surfactant, bio-fertilizers and polysaccharides [133]. In another case, palm-based waste was used to produce bio-compost, bio-plastics, bio-adsorbent, bio-diesel, bio-composite, bio-vanillin, bio-ethanol and bio-butanol [134].

Beyond the potential natural resources, the case of olive biomass was hugely investigated for bio-refinery. Olive oil processing generates huge amount of very varied waste (olive tree, leaves, olive pomace, olive stones or whether wastewater) which can be valorized. In their review, Romero-García *et al*. showed the large range of compounds which can be recovered from olive biomass. Conventional bio-refinery (CBR) processes such as fermentation or enzymatic hydrolysis can be used to obtain as examples ethanol, lignin, oligosaccharides or antioxidants (oleuropein, tyrosol and hydroxytyrosol). These compounds can be afterwards precursors to other conversion processes leading to high value bio-chemicals [135]. Such as olive biomass, orange peel waste is as well an ideal candidate for bio-refinery. Huge amounts are generated each year, due to the production of juice, and its composition offers a wide variety of valuable compounds. Indeed, after processing, the waste and by-products (about 50 % of initial fruit) are mainly composed of skin, pulp and seeds, which are source of EO, fatty acids, organic acids, sugars, enzymes, flavonoids or whether pectin. To valorize completely the orange peel waste, Boukroufa *et al.* developed a solvent-free bio-refinery with green processes and obtained successively EO, polyphenols and pectin [136]. The experimental diagram of this study is presented in **Figure I-35**. The use of MHG and US technologies rather than conventional extraction procedures enabled to reduce drastically extraction times and increase the polyphenols and pectin extraction yields. Fidalgo *et al*. also valorized pectin and D-limonene from orange and lemon peels waste using MW in a bio-refinery concept. They showed that their bio-refinery was an eco-friendly process which can be easily up-scaled at semi-industrial level [137]. MW and US were as well integrated in a bio-refinery process as alternative technologies by Jacotet-Navarro *et al.* to extract EO, antioxidants and dietary fibers from ginger by-products. In their study, their use reduced the environmental footprint of global process [138].



(MHG: Microwave Hydrodiffusion and Gravity; MAE: Microwave assisted extraction; UAE: Ultrasound assisted extraction)

Figure I-35. Towards a bio-refinery of orange peels (adapted from [136]).

I.6 PRINCIPLE 5: REDUCTION OF UNIT OPERATIONS NUMBER AND DEVELOPMENT OF SAFE, ROBUST AND CONTROLLED PROCESSES.

I.6.1 Context

With current economic and environmental concerns, plant extraction industry must develop efficient processes in terms of extraction yields, cleanliness to environment, safety of operators and use of space. This approach is part of process intensification demarche illustrated in **Figure I-2**. More precisely, intensification aims at increasing extraction yield, extract purity and quality, while decreasing extraction time, number of unit operations, energy consumption, environmental impacts, economical costs, quantity of solvent and total waste.

Generally, a whole extraction process is composed of following essential steps [13]:

(1) Plant material pre-treatment: raw material must be previously dried and ground to increase surface contact area and solvent penetration level;
(2) Solid-liquid extraction with appropriated solvent;
(3) Solid-liquid separation by filtration or centrifugation;
(4) Solvent removal and recycling under vacuum to eliminate every trace of residual solvent in final extract.

Such process is often very long, and results in high energy consumption, particularly due to fourth step. To overcome this problem, intensification may be achieved, for example by designing smaller size units and by reducing the number of unit operations. Ideally, it may result in a better use of process inputs (raw material, energy…), a superior control of global process and a reduced global environmental footprint.

I.6.2 Good practices guidelines

Beyond energy efficiency described (Principle 3), a process must present other essential characteristics to meet the definition of green extraction process [13,15]:

- Intensification of global process using innovative technologies
- Reduction of the number of unit operations by deep study of the process
- Development of more compact unit operations
- Ensure the global safety and robustness of the process
- Total control of the process and flux related

I.6.3 Success stories

This part provides examples of processes and equipment in accordance with principle 5 specifications. A focus is done more particularly on reduction of unit operations number and process automatization.

I.6.3.1 Elimination of unit operations

In a whole extraction process, solvent elimination from both extract and spent plant residue after extraction is one of the longest stage. It is even longer and energy consuming if boiling point of solvent is high. Moreover, it may cause the degradation of sensitive compounds in final extract, particularly when too hard experimental conditions are applied. Therefore, the question of solvent removal is a real concern in current extraction field: it may result in a loss of final extract quality, but it is nevertheless a necessary step, particularly when extraction is performed with organic solvents. However, in certain cases, some alternative solutions can be adopted to skip this step, particularly:

- Using solvent which can be removed directly after extraction process without concentration step;
- Using solvent that forms part of final extract formulation.

Plant extraction with supercritical CO_2 as solvent is a perfect example to illustrate the first solution. As shown in **Figure I-36**, CO_2 is gaseous at atmospheric pressure. Therefore, after extraction under supercritical conditions (T_c=31 °C; P_c=73.8 bar), pressure is brought back to atmospheric level, and it results in direct removal of gaseous CO_2 from the extract [139]. Instantaneously, final extract is safe, exempt from any residual solvent and ready for further purification or formulation steps. As an example, valuable products extraction from rosemary and ginger (matrices of main interest in this thesis) using supercritical CO_2 is hugely reported in current literature, particularly the recovery of EO, oleoresin or low polarity antioxidants [46,139–144].

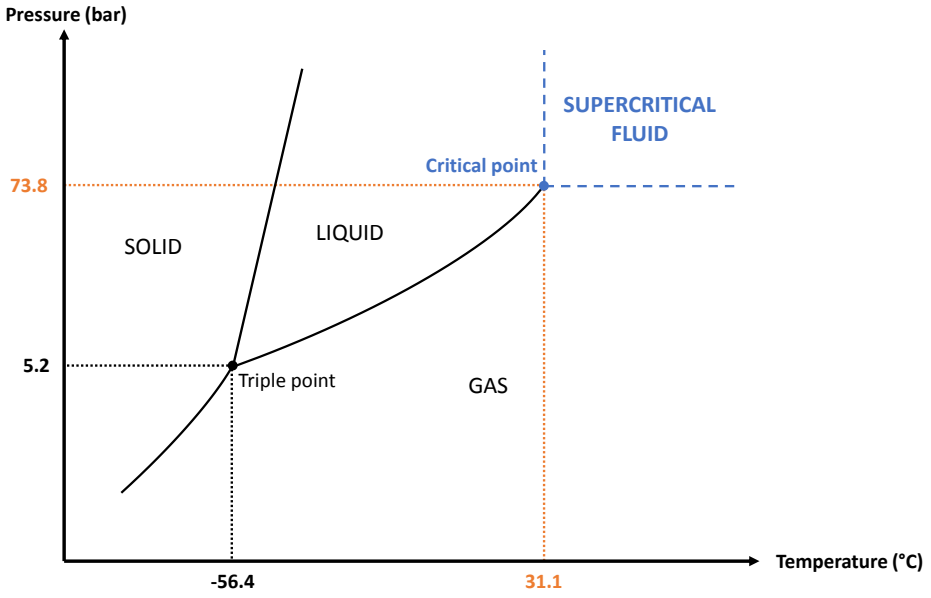


Figure I-36. Simplified pressure/temperature phase diagram for carbon dioxide.

In other cases, it is possible to perform plant extraction directly in final formulation solvent. Actually, compounds of interest are extracted from plant material and immediately solubilized in final formulation solvent. This approach enables to skip not only solvent removal step after extraction, but also that of formulation. First of all, the example of aromatized oils can be cited. In most cases, vegetable oils are aromatized with EO previously extracted with organic solvents or by HD. However, in 2010, Veillet *et al.* proposed an original procedure for direct aromatisation of olive oil with basil [145]. Basil leaves were directly immersed in olive oil and US were applied to the mixture in order to accelerate diffusion of basil volatile into the oil. Their use enabled to reduce extraction time from hours or days with conventional maceration (CM) to 20 min. Aromatization using this procedure accounts only one unit operation against several using conventional way. The same approach was adopted by Li *et al*. in 2012 to produce vegetable oil enriched with carotenoids [146]. As illustrated in **Figure I-37**, number of unit operations was drastically reduced compared to conventional procedure, particularly with the elimination of carotenoids extraction with organic solvent and solvent removal steps.

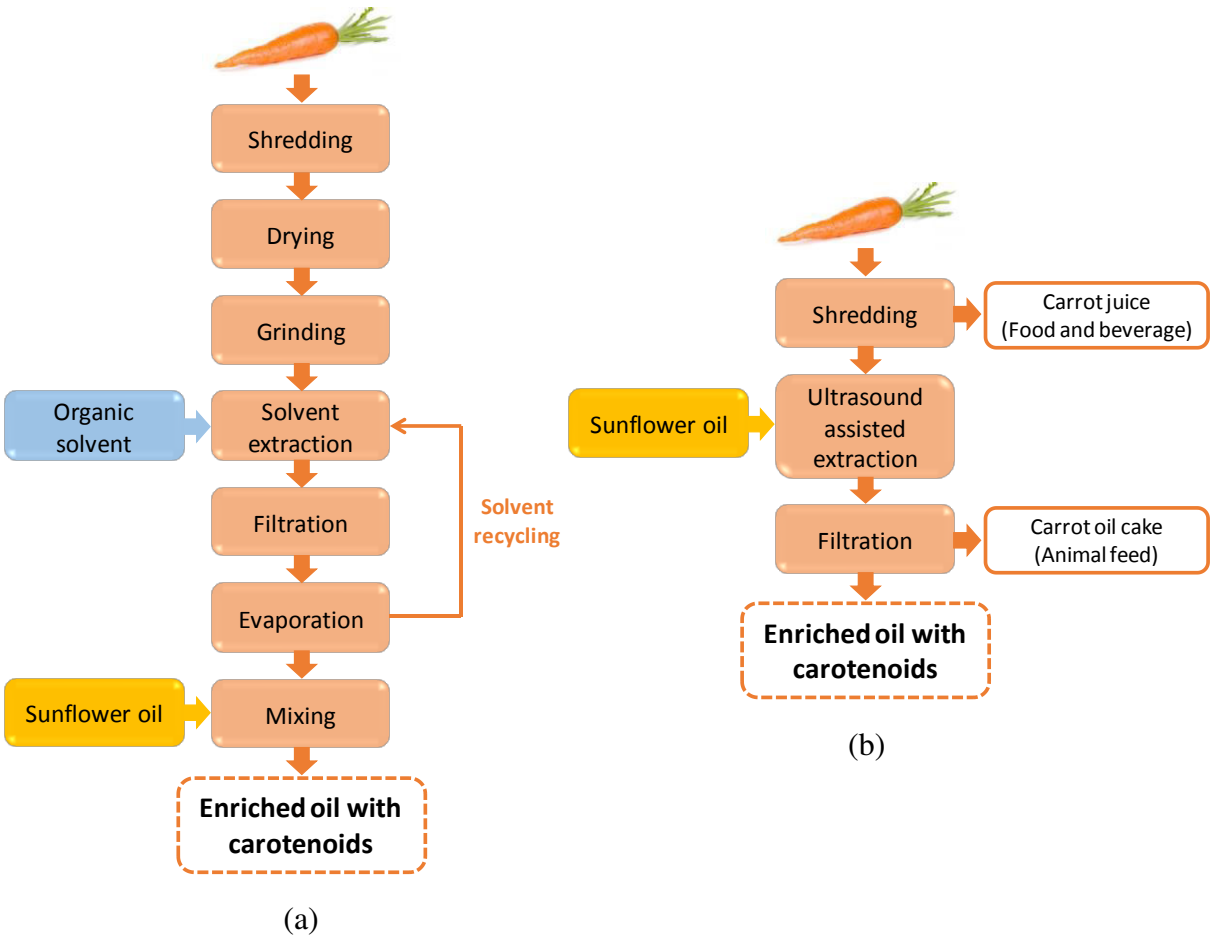


Figure I-37. Processing procedures to obtain enriched oil with carotenoids by (a) conventional solvent extraction and (b) ultrasound assisted extraction (adapted from [146]).

I.6.3.2 Automatized and compacted equipment

Conventionally, characterization of plant raw material is performed using a Soxhlet extractor. This method is very time-consuming (extraction lasts at least 8 h), and consequently requires a lot of energy [147]. Moreover, extractions must be performed one after another, and the elimination of solvent in next step requires long concentration time, particularly when large volumes are involved. In order to intensify the process, Ankom Technology proposes an automated Soxhlet extractor (Ankom^{XT15} extractor) to determine crude fat and oil content in samples **(Figure I-38)**. This equipment is fully automatized and consequently handlings errors are avoided. Initial raw materials are encapsulated in specific filters which are afterwards placed in the reactor (until 15 samples at a time). The process is accelerated by performing the extraction under pressure at elevated temperatures. This extractor enables to perform 15 extractions at a time and a total daily volume of more than 150 samples. After

extraction, crude fat and oil content is determined by weight difference of plant material before/after extraction. Regarding extraction solvent, recovery and recycling is done automatically at a rate of around 97 % or greater.

Figure I-38. Ankom^{XT15} extractor.

Table I-9 compares the performances of conventional Soxhlet extraction and extraction with Ankom^{XT15} extractor for the determination of crude fat and oil content. In conclusion, this equipment is efficient, compacted, sure, well-controlled and enables to save time, money and place, and therefore in accordance with principle 5 specifications. The main disadvantage is that final extracts are not recovered at the end of the process, which avoid potential composition determination.

As another example, VELP® society developed an automatic solvent extractor (SER 158) to extract a wide range of plant materials. Processing steps of such equipment are presented in **Figure I-39**. First of all, sample is placed in extraction thimble and immersed in boiling solvent for an effective extraction of active compounds (1-). The choice of solvent and extraction duration is free. After extraction, solvent level is decreased below extraction thimble. A part of solvent is collected in recovery tank (2-) and the other part continues to flow through the sample to complete previous extraction step (3-). Then, solvent is completely collected in recovery tank (4-). Finally, heaters are switched off and glassware is lifted to prevent burning of dried extract (5-).

	Conventional Soxhlet	Ankom^{XT15} extractor
Extraction time	≤ 8 h	30 min – 1 h
Temperature	under boiling point	90 °C
Pressure	atmospheric	under pressure
Number of sample per batch	1	Up to 15
Extraction yield determination	weighting final extract	by weight difference of raw material before/after extraction
Possibility to determine final extract composition	yes	no

Table I-9. Comparison between conventional Soxhlet and Ankom^{XT15} extractor for the determination of crude fat content.

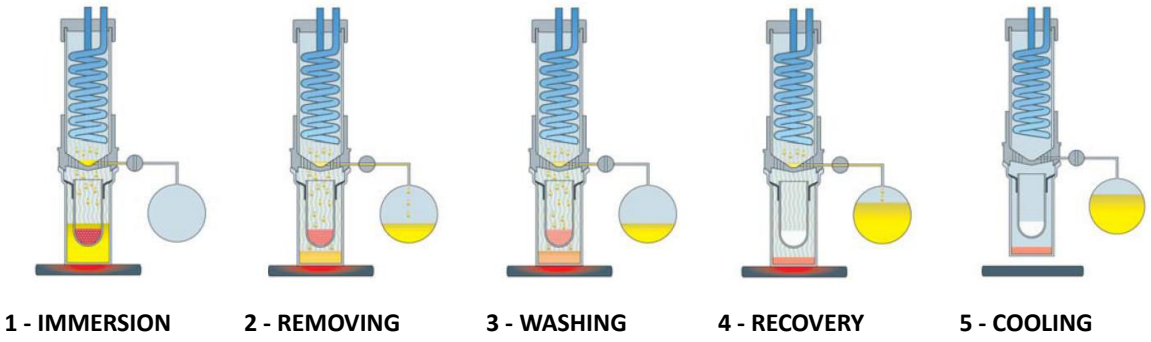


Figure I-39. Extraction progressing with SER 158 Solvent AutoExtractor.

This equipment proves a perfect match with principle 5 requirements. Indeed, it is extremely compact as six extractions can be performed at a time in a reduced space. Moreover, it enables to obtain reliable results with excellent reproducibility. Solvent recovery is about 90 % and water consumption is limited, which permit to save energy and money. Finally, last but not least, the process is operator-friendly since it is easy to use and safe (user exposure to solvent is minimized).

I.7 PRINCIPLE 6: AIM FOR A NON-DENATURED AND BIODEGRADABLE EXTRACT WITHOUT CONTAMINANTS.

I.7.1 Context

Conventionally, natural extracts are obtained from plant material by solid/liquid extraction with organic solvent. However, because of contaminated plant material, potential use of petroleum solvents, drastic extraction conditions or too long extraction time, resulting extracts may contain contaminants, denatured compounds and solvent residues. This aspect questions the interest of using natural extracts compared to synthetic additives. Therefore, it is necessary to determine the characteristics of an ideal extract or “eco-extract” in order to guide plant extraction industrials when developing a new standard.

I.7.2 Good practices guidelines

To be considered as an "eco-extract", an extract must present most of the following characteristics **(Figure I-40)**:

- Naturalness;
- High quality with active and non-denatured compounds;
- High functionality (antioxidant, antimicrobial, flavoring, coloring properties…);
- Complete safety;
- Accordance with specific legislations regarding the application sector (food processing, cosmetics, pharmaceutical industry…);
- Low environmental footprint (determination with the LCA approach).

Figure I-40. Characteristics defining an "eco-extract".

For a better understanding of these different characteristics, each of them are further developed below.

I.7.2.1 Naturalness

The concept of naturalness is quite difficult to define, particularly in the case of plant extracts where the definition of a genuine natural extract is not clear. According to REACH legislation [148], a natural substance is "a naturally occurring substance as such, unprocessed

or processed only by manual, mechanical or gravitational means, by dissolution in water, by flotation, by extraction with water, by steam distillation or by heating solely to remove water, or which is extracted from air by any means. This definition includes that the substance must not be chemically modified, in other words its molecular structure must remain unchanged independently of the process performed (even chemical)." Thus, according to this legislation, olive oil or fruits and vegetables juices for example can be considered as "natural". However, this definition does not bring any details about solvent extraction process, excepted that it can be performed with water to ensure the naturalness of final extract. Thus, the naturalness of extracts obtained with such process is controversial and different points of view are represented. In some cases, solvent extraction is considered as a physical process, and the generated extract is considered as "natural", provided that solvent is eliminated at the end of the process. It is the case for favoring substances dedicated to foodstuffs governed by Decree n°91-366 (April 11th 1991). In other cases, the naturalness of final extract is commanded by the type of solvent used for extraction which must be natural or natural origin to ensure naturalness. Still others refute the naturalness of extracts when they are obtained by solvent extraction other than water [149]. Therefore, naturalness of extract obtained by solvent extraction is open to debate. Finally, last but not least, the global naturalness of an extract is also governed by the nature of initial plant material, which must be provided by a well-reasoned sourcing as far as possible.

It can be noticed that naturalness is very often associated to "organic" aspects. As an example, Ecocert and Cosmos certifications are essential in cosmetic sector and deal with both aspects. They are associated to detailed guides governing the chemicals and solvents authorized, as well as the processes and facilities adapted to produce certified organic and natural extracts or product [150,151]. Various other appellations or labels referring to naturalness can be found on extracts or final products packaging in the different sector activities **(Figure I-41)**. Each of them are associated to a specific charter of good practices, relating to the type of raw material, chemicals, processes and additives authorized. All of these labels aim principally at sending a particular message to the consumer which feels more secure about what he buys.

Figure I-41. Examples of labels with naturalness values.

I.7.2.2 Quality

Extract quality is generally determined considering the content in active compounds and the absence of denatured molecules. During an extraction process, degradation of compounds of interest may occur depending on process conditions, such as high temperature, presence of oxygen or long extraction time. This phenomenon must be avoided as far as possible since degradation products can affect the organoleptic and the nutritive properties of final extract. For example, ultrasound assisted extraction (UAE) of raw material containing lipids may result in the formation of radicals and consequently off-flavors in the extract [152]. In the case of dyes, unsuitable extraction conditions (high temperature, light or presence of oxygen) may increase the kinetics of β-carotene oxidation [153,154]. Plus, at worst, degradation compounds may represent a threat for final consumer which is in direct contact with the product (ingestion or skin absorption).

I.7.2.3 Functionality

By definition, the functionality is “the quality of having a practical use”. Natural extracts are considered as functional when they aim at accomplishing a particular task in the final foodstuffs or cosmetics in which they are added (coloring, antioxidant, texturing…).

Hence, the formulation step is essential to ensure the best extract performance. For example, the polarity of the extract must be compatible with the polarity of final product.

Measurement of extract functionality is quite difficult to implement routinely at industrial level, that's why natural extracts are generally standardized according to their content in active compounds. However, it must be kept in mind that composition and functionality do not always point to the same direction. Moreover, when activity and functionality are assessed, it should be recognized that results are influenced by the nature of the tests carried out, depending on whether they take place in polar systems where polar compounds are favored over less polar molecules to react since they are more soluble, hence more available for reaction.

I.7.2.4 Safety

Safety is a capital criterion in "eco-extract" definition. First of all, the extract must be exempt from any microbial contamination. To this end, extracts can be pasteurized or submitted to sterilizing filtration at the end of the process. Besides microorganisms, the most common contaminants found in natural extracts include pesticides, polycyclic aromatic hydrocarbons (PAH) and heavy metals. Most of the cases, they are transferred from contaminated soils to plant material during agricultural production. After plant extraction, according to the solvent used, such compounds are found in very high quantity in the final extract and very often beyond any quantitative limits. Actually, extraction process tends to concentrate the contaminants initially present in raw material in the final extract since these molecules are also soluble in the solvent and extracted at the same time as active compounds. To limit or avoid this phenomenon, plant material sourcing must be well-reasoned as far as possible, for example using raw material proceeding from organic farming. Another solution is a previous step of plant material decontamination before extraction [155] or sometimes even during the extraction using absorbent materials [156].

I.7.2.5 Legislation

Development of extracts or "eco-extracts" cannot be performed without considering legislation aspects. Regarding the sector to which they are dedicated, extracts must be in accordance with specific legislations related. Indeed, a huge number of legal documents,

regulations or directives governs the foodstuffs, cosmetics or pharmaceuticals, whether regarding their composition, their safety or their labelling.

For instance, in food processing sector, the use of additives including some natural extracts are governed by various regulations such as n°1333/2008. More specific regulations exist for particular cases as Regulation (n° 1334/2008) dedicated to flavoring ingredients. The use of solvent for the production of foodstuffs and food ingredients is also regulated, particularly by Directive 2009/32/EC, which relates maximal solvent residue limits in the final foodstuffs.

Among the foodstuffs and food ingredients including some natural extracts, we can also find the category of "novel food". By definition, novel foods are foodstuffs or ingredients which were not consumed in European community before 1997. There are governed by Regulation n°258/97 from 27 January 1997. According to legislation, novel foods include:

- "Foods and food ingredients produced from genetically modified organisms (GMO), but which does not contain such compounds;
- Foods and food ingredients with a new or intentionally modified primary molecular structure;
- Foods and food ingredients consisting of or isolated from micro-organisms, fungi or algae;
- Foods and food ingredients consisting of or isolated from plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating or breeding practices and having a history of safe food use;
- Foods and food ingredients to which has been applied Article 3 a production process not currently used, where that process gives rise to significant changes in the composition or structure of the foods or food ingredients which affect their nutritional value, metabolism or level of undesirable substances."

As examples, phytosterols or extract of magnolia bark are included in this category of food ingredients.

Before being authorized for commercialization, the potential toxicity of such ingredients and the eventual nutritional imbalance induced by their introduction in the global diet have to be assessed by relevant institutions. Final decision rests with the European commission which, after consulting the EFSA (European Food Safety Authority), accepts or not the new ingredient as a novel food.

I.7.2.6 Life Cycle Assessment (LCA)

As last but not least requirement, an "eco-extract" must have a low environmental footprint. This parameter can be determined using a LCA approach.

LCA was born in a context of sustainability. It is a multi-criteria study aiming at quantifying the potential impacts of a product or a service during its whole life cycle, from the cradle to the grave [157]. Impacts (positive or negative) can be environmental, economic or social. The whole life cycle of a product is illustrated in **Figure I-42**. It includes the extraction of raw materials, the processing, the distribution and transport, the utilization and finally the end-of-life treatment.

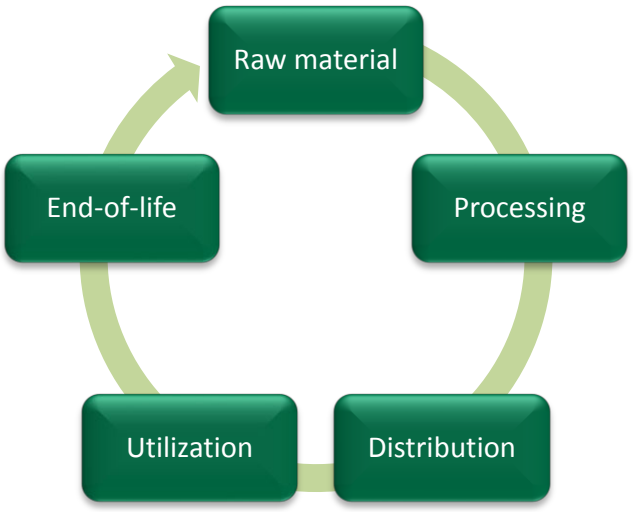


Figure I-42. Whole life cycle of a product.

The methodology to perform an LCA is described in norms ISO14040 to ISO14044. Actually, it is a multi-step approach presented in **Figure I-43**. The first step is the definition of the functional unit. It is a key component for the study. It must be determined carefully and the same functional unit must be kept if one aims at comparing different life cycles. If not, that doesn't make any sense: indeed, a process can have less negative impacts on environment but perhaps the final extract recovered is less performant or is a low quality extract compared to those from the more polluting process. Secondly, a Life Cycle Inventory (LCI) has to be done. It consists in an assessment of inputs and outputs for each step of the life cycle and they

must be reported to the functional unit. Inputs are energy and non-energy resources, and outputs are emissions into water, soil and air, waste and by-products.

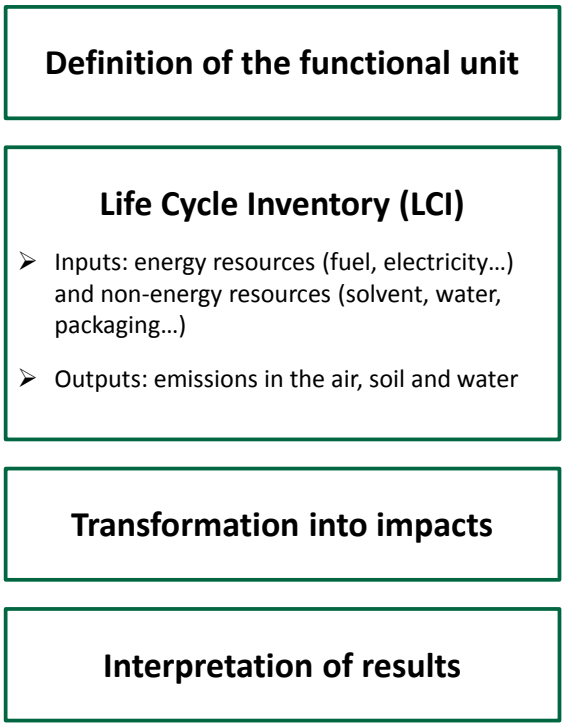


Figure I-43. Life Cycle Assessment methodology requirements.

A complete LCI from the cradle to the grave is often quite difficult and heavy to perform. Data are not always available. A gate to gate approach more focused on the process is sometimes adopted **(Figure I-44)** to facilitate the study. At industrial level, data can be collected directly in the production sites. This approach is useful particularly to compare processes in terms of environmental efficiency. However, in the case of natural extracts, it is recommended to consider also the agricultural step (plant growth and harvest before it use for extraction) and the recycling and treatment of extraction by-products [13].

The next step after LCI is the transformation of data into impacts. For this purpose, there are various LCA software available with implemented databases. Some of them are dedicated to specific sectors of activity. We can cite for example Food'Print v-1 for agri-business or BEE V3.1 for packaging. Open LCA and SimaPro are examples of more universal software which can be used in extraction field. Databases such as Ecoinvent 2.1, Agribalyse,

ELCD or LCA Food can be implemented by default, but it is generally possible to add the database of its choice in the program used.

Therefore, LCA software enables to convert data from LCI into impacts. Actually, depending on the program used, different types of impact can be chosen. The most common are greenhouse effect, euthrophisation, acidification, ozone depleting, eco-toxicity and fossil resources depletion. They correspond to "midpoint" impacts dedicated to scientific or advertised community. It is also possible to express the results into damages, particularly for communicating with non-advertised public. In this case, we can talk about human health, increase of cancer rate or loss of biodiversity for instance.

The mode of action of the software is very simple. The life cycle of the product is firstly separated into its different constitution blocks, in a more or less detailed way. For example, the block corresponding to the fabrication can be divided into the different operation units. Then, the contribution of each step regarding each impact is calculated using algorithms. Results can be expressed in a table or as a graphic. Besides determining the global environmental footprint of a product, it enables to see which step in the life cycle is more impacting for potential improvement.

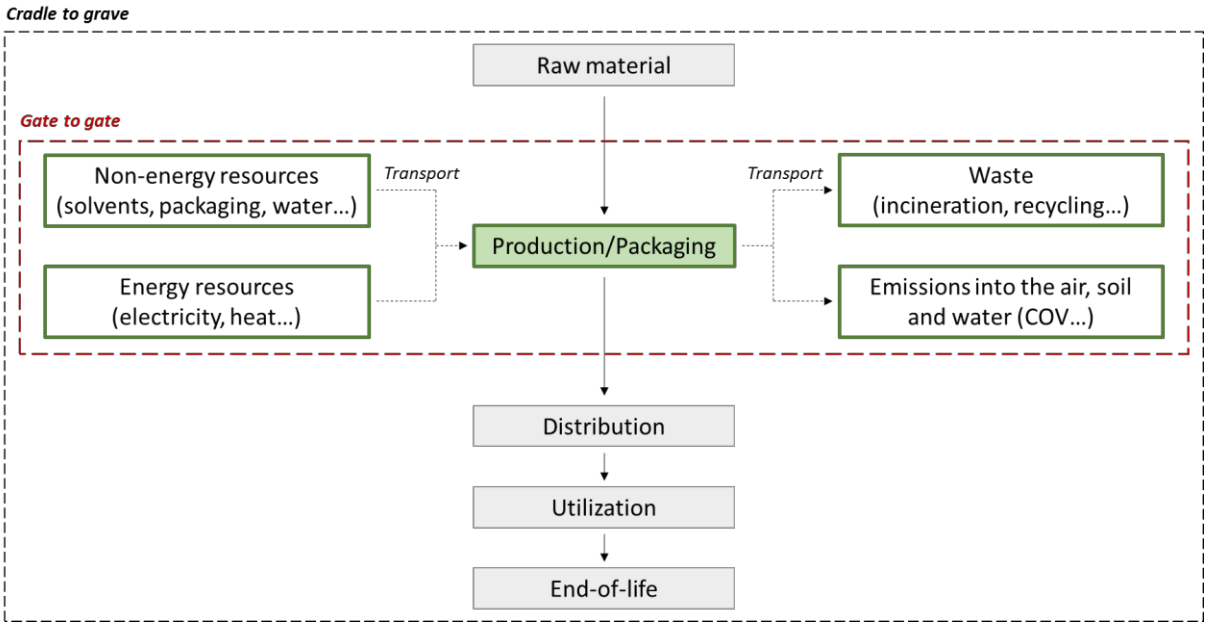


Figure I-44. "Gate to gate" approach in Life Cycle Assessment methodology.

I.7.3 Success stories

In this section, two examples of eco-extracts in accordance with principle 6 specifications are developed. Both present naturalness, high quality, high functionality, complete safety and a low environmental footprint

I.7.3.1 New generation "essential oils"

EO belong to one of the most sought compounds in global natural products extraction field. It is a very concentrated lipophilic and odoriferous liquid containing the volatile compounds of the plant. It is generally located in specific glands on the surface or inside plant material. Conventionally, it is extracted by steam distillation, HD or expression. However, in the last decade, alternative processes have been performed for this purpose and led to improved extraction yields and higher extract quality. In literature, MW are one of the main technologies which have been hugely used to intensify EO extraction. **Table I-10** relates some examples of success stories using MW as intensification process. In all cases, EO recovered may be considered as "eco-extracts" since the use of MW enables to:

- Increase or give similar extraction yields
- Shorter global extraction time (several hours for conventional process against several minutes under MW) and consequently reduce energy consumption and environmental footprint of resulting extract
- Increase functional properties of resulting EO (particularly antimicrobial)
- Avoid the presence of residual solvent in the final product
- Reduce waste water generated in the case of SFME
- Increase the quality of resulting EO with higher amount of oxygenated compounds in their final composition.

Indeed, an EO is mainly composed of monoterpenes (such as limonene or α-pinene), oxygenated monoterpenes (such as camphor or linalool), sesquiterpenes (such as γ-curcumene) and oxygenated sesquiterpenes. Oxygenated compounds are the most valuable since they are highly odoriferous and hence responsible of essential oil freshness. It has been demonstrated that such compounds are more present in EO obtained with MW assisted processes because thermal and hydrolytic effects are reduced compared to conventional HD. Plus, oxygenated compounds have a high dipolar moment and consequently interact

preferentially with MW, resulting in a better extraction compared to monoterpenes hydrocarbons which have a lower dipolar moment.

Even using MW in the process, resulting "eco-extracts" are commonly named "essential oils". However, according to REACH legislation, an essential oil is a volatile part of a natural product which can be obtained by HD and steam distillation, and more particularly by expression in the case of citruses, and extracts obtained with MW processes do not meet this definition. Hence, from a regulatory point of view, it is not convenient to name them "essential oils". Therefore, further demarches must be undertaken to define at best these new "eco-extract" (as Novel Food).

Plant material	Microwave assisted process	Solvent	Reference
Rosemary	Microwave assisted hydrodistillation (MAHD)	Water	[158]
Fennel (Foeniculum vulgare)	Microwave hydrodistillation (MWHD)	Water	[159]
Lavender	Microwave assisted steam distillation (MASD)	Steam	[160]
Calamintha nepeta (L.) Savi	Solvent-free microwave extraction (SFME)	/	[30]
Grapefruit peel	Solvent-free microwave extraction (SFME)	/	[161]
Citrus	Microwave accelerated distillation (MAD)	/	[162]
Rosemary	Microwave hydrodiffusion and gravity (MHG)	/	[33]

Table I-10. Examples of microwave assisted extraction to recover essential oil from various plant materials.

I.7.3.2 High resolution extract from plant core: Eutectys™ products

Plant materials are a huge source of very various active molecules synthetized at the very core of the plant cells. Some of these metabolites are neither hydrosoluble nor

liposoluble. However, they may be stored and transported in a third class of liquid phase named natural deep eutectic solvents (NaDES). By definition, a NaDES is a mixture of compounds, in specific proportions, which presents a lower melting point than any of the individual components. The mixture thus becomes liquid at room temperature.

Last year, inspired by nature, Naturex patented a new extraction process based on this phenomenon of "eutectigenesis" which mimics the intracellular environment. It consists of extracting the active compounds from the plant with the formation of a NaDES, a natural alternative solvent. Based on this process, they developed a new range of cosmetic products named "Eutectys™" (**Figure I-45**).

Figure I-45. Logo of Eutectys™ product range.

As illustrated in **Table I-11**, these new products present superior phytochemical profiles and improved performance compared to the corresponding conventional hydro-glycerin extracts. Considered as 100 % natural, Eutectys™ extracts are Ecocert and Cosmos approved and easily bio-degradable (OECD301b). Miscible in water and glycols, they are also very easy to formulate. Plus, they are completely safe as established by a toxicological expert according to European regulation (EC) n°1223/2009). In terms of regulation, Eutectys™ extracts are REACH compliant, and authorized in Europe, USA and China (IECIC 2014). Finally, last but not least, the environmental footprint related to their production is reduced compared to those of conventional hydro-glycerin extract **(Figure I-46)**. Therefore, Eutectys® extracts are fully in line with the principles of green chemistry and can be considered completely as "eco-extracts".

Products	Improved chemical profile	Improved properties
Saffron flower Eutectys™	Richer flavonoid composition	Anti-aging (+ 91 %) Radiance and whitening
Sea fennel Eutectys™	Higher content in phenolic compounds	Radiance and whitening
Rose of Jericho Eutectys™	Higher content in phenolic compounds	Radiance and whitening
Olive leaf Eutectys™	+ 400 % oleuropein	Anti-wrinkle, regenenatory, and firming (stimulation of collagen synthesis x 17) Photo-protective (x 3.7)
Horsetail Eutectys™	+ 18 % phenolic acids	Soothing (x 5) Antioxidant (+ 61 %)
Rosemary Eutectys™	Rosmarinic acid content x 120	Antioxidant (+ 100 %)

Table I-11. Improved chemical profiles and properties of Eutectys™ extracts compared to the corresponding hydro-glycerin extracts.

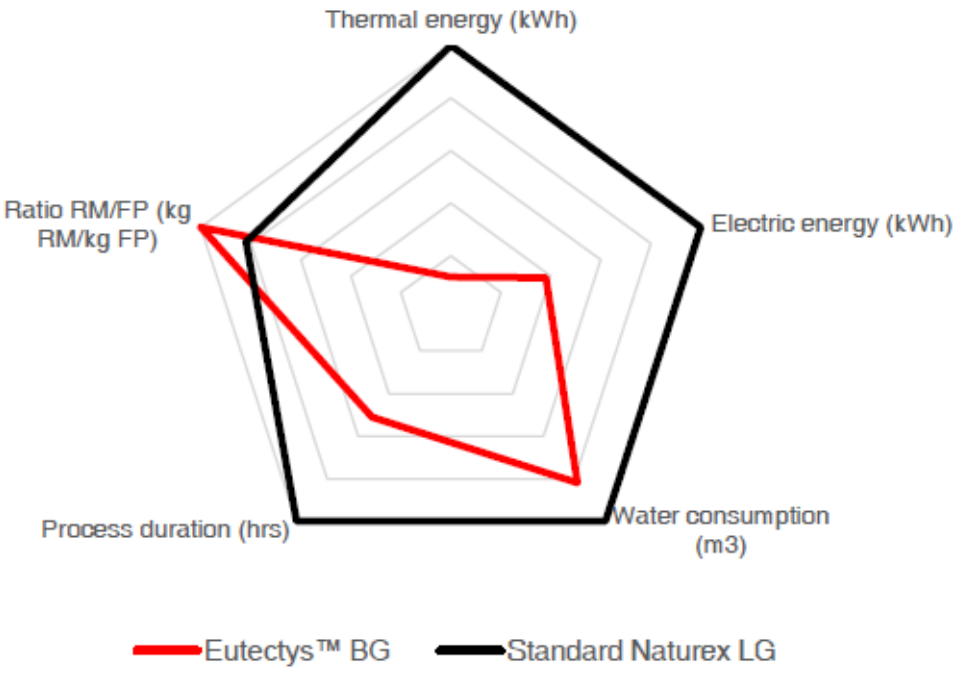


Figure I-46. Environmental footprints of olive leaf Eutectys™ and standard (conventional) extracts according to selected parameters.

I.8 CONCLUSION

In conclusion, this chapter clearly shows that the demarche of green extraction is not limited to one type of natural extract. It is accessible for all businesses (food processing, cosmetics, pharmaceuticals…) thanks to well-defined guidelines related to each principle. Proposed success stories testify that the adoption of such demarche is possible at laboratory and industrial levels, and provide solutions and tools for:

- Well-reasoned sourcing of plant material
- Selection of alternative solvents, less harmful towards users and environment, but equally effective for extraction
- Limitation of total energy consumption
- Development of compact, robust and safe processes
- Management and valorization of by-products generated during the process
- Recovery of eco-extract with green values (non-denatured, bio-degradable and without contaminants).

This chapter also shows that adopting such initiative presents economical, societal and environmental advantages for industrials. Moreover, even if some points remain at the stage of research and development, it enables to apprehend potential legislation evolution regarding these aspects. This thesis, centered on process intensification using innovative technologies, solvent substitution and solvent elimination using a bio-refinery concept, fits perfectly with the issues raised in this chapter.

CHAPTER II . MATERIALS AND METHODS

II.1 PLANT MATERIALS

In this thesis, studies have been performed principally on two kinds of raw materials: rosemary leaves and ginger press cake.

Rosemary was selected because it is a strategic and historic raw material for Naturex. This plant material can provide essential oil by hydrodistillation or extraction with supercritical CO_2, and active compounds (as RA and CA) by solvent extraction.

Regarding ginger press cake, it is one of the by-products obtained after juice processing of ginger rhizomes. Since it still contains valuable products (essential oil and gingerols), we chose to valorize this raw material into high valued compounds.

II.1.1 Rosemary

Rosemary (*Rosmarinus officinalis* L.) was provided by Naturex **(Figure II-1)**. The batches used were collected in Morocco in 2012, 2013 and 2015. For rosemary used in **CHAPTER III**, initial moisture content was 8.93 ± 0.01 % and initial content in rosmarinic acid (RA) and carnosic acid (CA) was 0.21 % and 1.70 % respectively. For rosemary used in **CHAPTER IV**, initial moisture content was 8.33 ± 0.01 % and initial content in RA and CA was 0.91 % and 2.4 % respectively.

Figure II-1. Rosemary (*Rosmarinus officinalis* L.) leaves.

II.1.2 Ginger

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.

(a) (b)

Figure II-2. Ginger rhizomes (a) and ginger press cake (b) used in the study.

II.2 CHEMICALS AND REAGENTS

II.2.1 Solvents

For extraction solvent, only demineralized water, 96 % ethanol and absolute ethanol (Deulep, France or ACS reagent, VWR, France) were used.

For analysis, water, methanol, acetonitrile, acetone, phosphoric acid 85 %, pentane 98 % and diethyl ether >99 % were analytical grade and purchased from Sigma Aldrich.

II.2.2 Reagents

Folin-Ciocalteu's phenol reagent and sodium carbonate (Na_2CO_3) were purchased from Emsure Merck Millipore (France)

Randomly methylated *β*-cyclodextrin (RMCD), Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), APPH (2,2'-azobis(2-methylpropionamidine) dihydrochloride), potassium phosphate monobasic (HPLC), potassium phosphate dibasic (PBS), 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) were from Sigma-Aldrich (France).

II.2.3 Standards

RA (97 %) and CA (98 %) standards were from Sigma-Aldrich (France).

II.3 SOLUBILITY STUDIES

II.3.1 Computational method: COSMO-RS calculation

The Conductor-like Screening Model for Real Solvents (COSMO-RS) is a method for molecular description and solvent screening based on a combination of quantum chemistry (COSMO) and statistical thermodynamics (RS) to determine and predict thermodynamic properties without experimental data [65].

COSMO-RS is a two-step procedure: microscopic and macroscopic steps.
(i) First, the COSMO model is applied to simulate a virtual conductor environment for the molecule of interest which is then embedded into a virtual conductor. In such an environment, the molecule induces a polarization charge density σ on the interface between the molecule and the conductor, (i.e. on the molecular surface). During the quantum calculation through self-consistency algorithm, the solute molecule converged to its energetically optimal state in the conductor with respect to electron density and geometry. In this study, the standard quantum chemical method for the COSMO-RS approach is the density functional theory (DFT) at the B88-PW86 level with a triple zeta valence polarized basis set (TZVP).
(ii) The second step relies on the statistical thermodynamics calculation. The polarization charge density was used for the quantification of the interaction energy of pair-wise interacting surface segments with regard to the most important molecular interaction modes, i.e. electrostatics and hydrogen bonding. The 3D distribution of the polarization charges σ on the surface of each molecule was converted into a surface composition function (σ-profiles). Such σ-profiles provided detailed information about the molecular polarity distribution. The chemical potential of the surface segment (σ-potential) was calculated from thermodynamics of the molecular interactions based on the obtained σ-profile. . In practice, the smiles formats of rosmarinic and carnosic acids were implemented in the software and both molecules were generated and added to the available database.

All calculations were performed using the COSMOtherm X program (Version C30 Release 16.01). Various ethanol:water mixtures were modeled wherein the percentage of ethanol varied from 0 to 100 % with 10 %-increments. For each mixture, calculations were specifically conducted at ambient temperature (20 °C). The solvent screening option works similar to the solubility option and is based on the same thermodynamics calculations. It allows predicting the solubility of one solute in a list of solvents. The logarithm of the best

solubility was set to 0 and all other solvents were ranked relative to the best one. A solvent with a log(x_solubility) (where x is mole fraction) value of -1.00 corresponds to a solubility decreased by a factor 10 compared to the best solvent.

II.3.2 Experimental solubilities of RA and CA into various ethanol:water mixtures

For convenience, experiments were performed at ambient temperature (20 °C) with high purity standards (97 % for RA and 98 % for CA). Various quantities of each standard were weighted into microtubes and a precise volume of each solvent mixture was added. Microtubes were vortexed (5000 rpm) for 3 min and centrifuged for 10 min (MiniStar silverline, VWR, France). Then a quantity of supernatant was sampled and diluted into methanol with 0.5 % of H_3PO_4 for CA and methanol:water 50:50 (v/v) for RA. Each experiment was performed in duplicate. Samples were subsequently injected into the HPLC system.

II.3.3 Degradation study of CA

The solubility of CA was investigated depending on the solvent and temperature. The standard was weighed and solubilized in 100 % ethanol, 50 % ethanol and 100 % water in order to reach the same concentration as in the extraction media. Resulting solutions were magnetically stirred (500 rpm) for 1 h under reflux (78 °C for 100 % ethanol, 82.2 °C for 50 % ethanol and 100 °C for 100 % water). However, since a gap exists between solvents' boiling points (until 20 °C between 100 % ethanol and 100 % water), experiments were also performed at 78 °C with 50 % ethanol and at 78 °C and 82.5 °C with 100 % water to assess the effect of temperature on compound stability. After experiment, samples were directly injected into the HPLC system.

II.4 EXTRACTION PROCEDURES

II.4.1 Conventional processes

II.4.1.1 Heat reflux extraction (HRE)

To assess the best solvent for extraction, rosemary leaves were submitted to reflux for 60 min with various solvents. The solvent was composed of ethanol:water mixture in different proportions: 0:100, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, 96:4 or

100:0 (v/v). In all cases, extraction was performed at ebullition temperature using a solid/liquid ratio of 1/20 (m/v). After extraction, the plant matrix was removed by filtration to vacuum (n°4 filter, Auchan, France) and rinsed with 100 mL solvent. Then, the extract was concentrated until dryness using a rotary evaporator (Laborota 4001, Heidolph, Germany). The dried extract was stored at -18 °C until used. Each experiment was performed in triplicate.

For the intensification study, the same experimental conditions were applied to rosemary leaves for 30 min or 5 h, using 90:10 (v/v) ethanol:water mixture as extraction solvent. This process was thereafter compared with alternative ones. To evaluate a potential effect of a fine dispersion in the solvent prior to extraction, they were as well applied to rosemary leaves previously ground into the solvent during 3 min with a homogenizer (Ika T25 digital Ultra-Turrax, Germany). Each experiment was performed in triplicate.

II.4.1.2 Maceration procedures

In this thesis, maceration was used as a conventional process for the extraction of RA, CA and UA from rosemary. Maceration was also performed as reference process to drain ginger material from its valuable phenolics.

II.4.1.2.1 Application to rosemary leaves

Rosemary leaves were submitted to conventional maceration (CM) in double jacket reactor during 30 min with ethanol:water 90:10 (v/v) as extraction solvent. The solid/liquid ratio was 1/20 (m/v) to limit the saturation of the solvent. Extraction temperature was maintained at 40 °C using a cryostat (Ministat 125, Huber, Germany). Matrix was homogenized into the solvent with a motorised stirrer (Ika Labortechnik RW16 basis, Germany). After extraction, the solvent was separated from the matrix by filtration to vacuum using a filter paper. The extract was concentrated until dryness by solvent evaporation under vacuum (Laborota 4001, Heidolph, Germany). Each experiment was performed in triplicate.

II.4.1.2.2 Application to ginger plant materials

GR, GP and GPMHG were submitted to CM 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.* [163] 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 min 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.

II.4.1.3 Hydrodistillation (HD)

Figure II-3. Hydrodistillation apparatus.

HD **(Figure II-3)** was performed as reference process for ginger essential oil (EO) extraction. 1 kg of GP was submitted to HD using a Clevenger-type apparatus [164]. Extraction was performed with 4 L of water for 360 min until no more EO was obtained. Then EO was recovered and stored at 4 °C before analysis.

II.4.2 Alternative processes

Six alternative extraction processes were performed to extract RA, CA and UA from rosemary: ultrasound (US) bath, US reactor, US probe, reflux under microwaves (MW), MW under nitrogen pressure and MW under vapor pressure. Those processes are illustrated in **Figure II-4**.

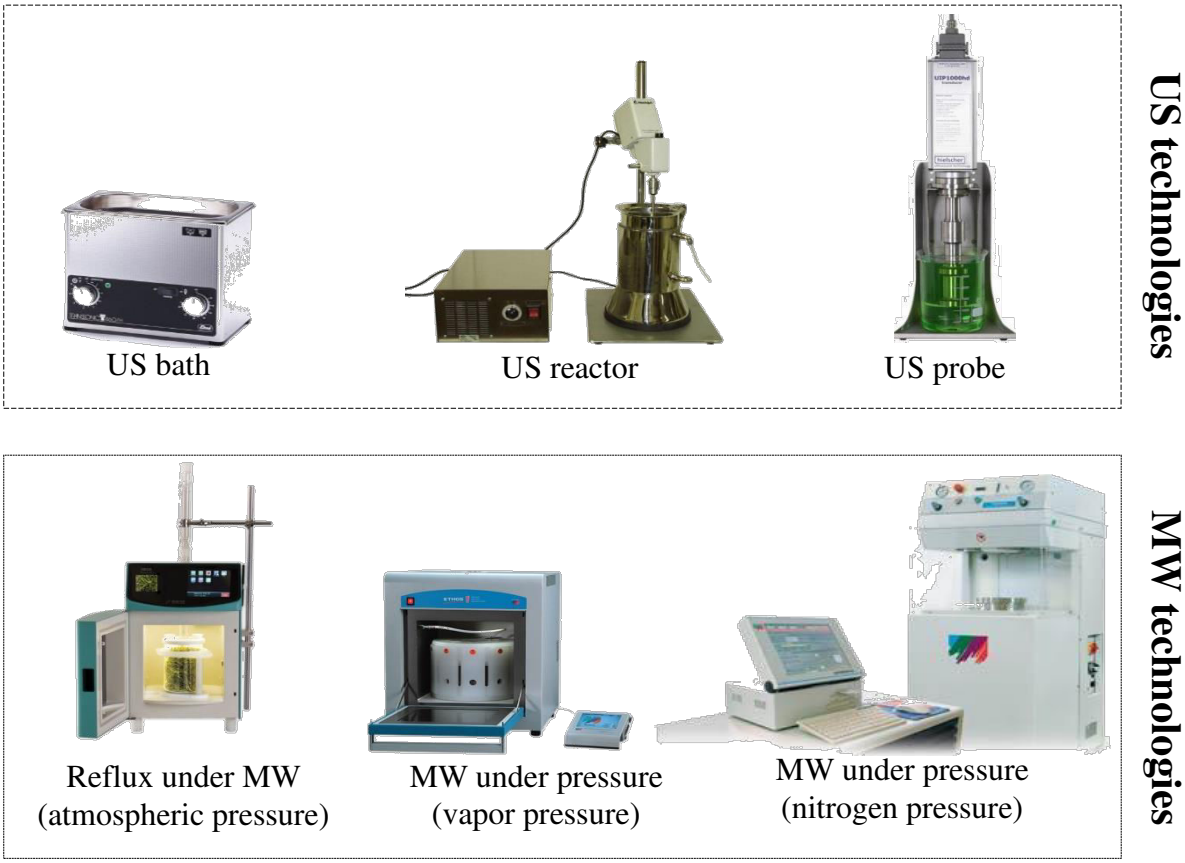


Figure II-4. Equipments assessed for the ultrasound and microwave assisted extraction study.

All the extractions were performed using a solid/liquid ratio of 1/20 (m/v). The extraction solvent was ethanol/water mixture in a proportion of 90/10 (v/v). After extraction, the solvent was separated from the matrix by filtration to vacuum using a filter paper. The extract was concentrated until dryness by solvent evaporation under vacuum (Laborota 4001, Heidolph, Germany).

II.4.2.1 Ultrasound assisted extraction (UAE) of active compounds from rosemary leaves

II.4.2.1.1 Ultrasound bath

Ground rosemary leaves were immersed into the solvent and the mixture was introduced in an ultrasonic bath (Prolabo, Labover, France) during 30 min. Temperature of water bath was maintained at 40 °C and checked during extraction with an external temperature probe. Each experiment was performed in triplicate.

II.4.2.1.2 Ultrasound reactor

Ground rosemary leaves were immersed into the solvent and submitted to US during 30 min using an US reactor (150 W, Pex1, REUS, France). Matrix was homogenized into the solvent with a motorised stirrer (Ika Labortechnik RW16 basis, Germany). Extraction temperature was kept constant at 40 ± 1 °C using a cooling system (Ministat 125, Huber, Germany) connected to the double jacket of the reactor. Each experiment was performed in triplicate.

II.4.2.1.3 Ultrasound probe

Rosemary leaves were placed in a double jacket reactor with the solvent and the whole was submitted to US (1 kW, UIP 1000 hdT, Hielscher Ultrasonics GmbH, Germany) during 30 min. US were applied to the system using a sonotrode immerged in the solvent approximately 2 cm. Extraction temperature was measured with an external sensor and controlled at 40 ± 1 °C with a cryostat (Ministat 125, Huber, Germany). Each experiment was performed in triplicate.

II.4.2.2 Microwave assisted extraction (MAE) of active compounds from rosemary leaves

II.4.2.2.1 Microwave assisted extraction under pressure (nitrogen pressure)

Rosemary leaves were packaged in gauze in order to be totally immersed in the solvent. The whole was placed in a reactor. Aiming at temperature homogeneity, the reactor was immersed in 700 mL of distilled water and introduced in the MW cavity. Extraction was performed using a high performance MW reactor (1,2 kW, UltraClave, Milestone, Italy). Before starting extraction, oxygen in the apparatus was flushed using a nitrogen flow.

Pressure was reached using a nitrogen flow and temperature was reached with a MW heating. Extraction temperature was set at 70 °C and initial pressure at 100 bar. Pressure and temperature were controlled by external sensors. At the set temperature, extraction was performed during 30 min. MW power was not fixed, it varied as a function of temperature, firstly to reach the set temperature and then to keep it constant during the extraction step. Each experiment was performed in triplicate.

II.4.2.2.2 Microwave assisted extraction

Rosemary leaves were immersed into the solvent and submitted to MW during 30 min, using a MW reactor (900 W, EOS-GR, Milestone, Italy) and a reflux apparatus. MW power was fixed at 210 W (1 W/g). Extraction was done at boiling temperature (78 °C) and atmospheric pressure. Each experiment was performed in triplicate.

II.4.2.2.3 Microwave assisted extraction under pressure (vapor pressure)

Rosemary leaves were packaged in gauze in order to be totally immersed in the solvent, and placed in a closed Teflon reactor. The whole was introduced in a MW reactor (1 kW, Ethos 1, Milestone, Italy) and heated using MW until the fixed temperature. Extraction was done at 125 °C and at 150 °C during 30 min. MW power was fixed at 300 W but it varied depending on temperature, firstly to reach the set temperature and then to keep it constant during the extraction step. Temperature and pressure were measured with external sensors. Each experiment was performed in triplicate.

II.4.3 Bio-refinery-inspired processes

In this thesis, two processes inspired from the bio-refinery concept were developed to valorize completely rosemary and ginger by-products. Both designed processes are presented in this section.

II.4.3.1 Towards a bio-refinery of rosemary leaves

The process developed is presented in **Figure II-5**. This process aims at extracting consecutively RA and CA from rosemary in a single process.

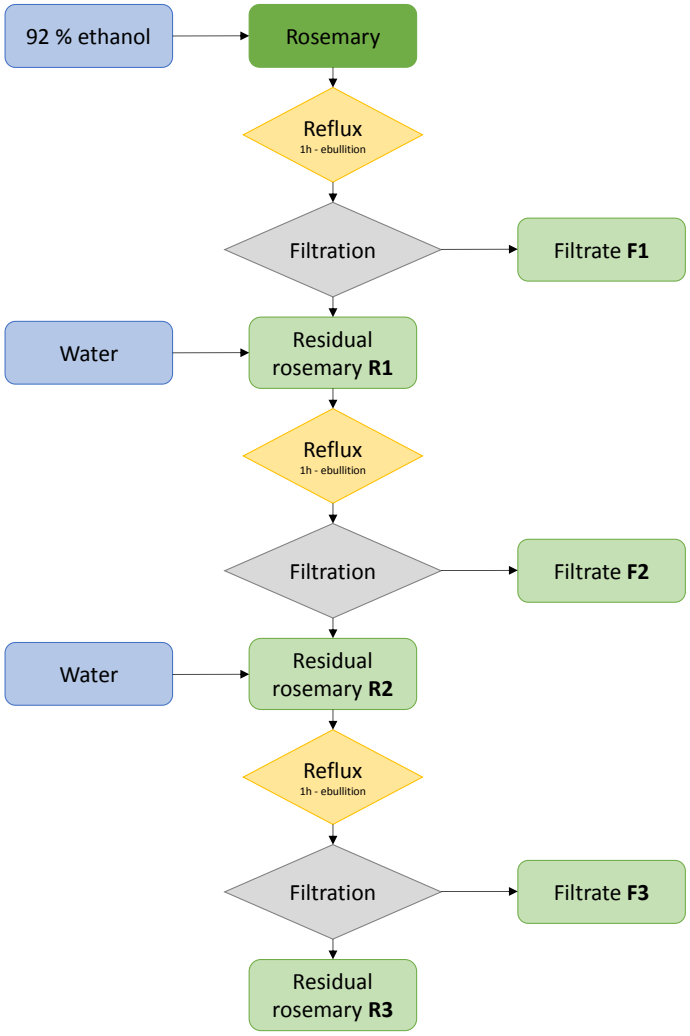


Figure II-5. Bio-refinery-inspired process developed for the valorization of RA and CA from rosemary.

167 g of rosemary leaves were submitted to reflux with 1 L of 92 % ethanol for 60 min. The solid to liquid ratio was 1 to 6 (m/v). Mechanical stirring (200 rpm) was added to the system. After this first extraction, the plant matrix R1 was removed by filtration to vacuum (retention rate 35-15 µm). The filtrate F1 (rich in CA), was stored at 4 °C until further purification step. 1 L of pure water was added to the remaining plant material R1, resulting in 20 % ethanol because of residual ethanol in R1 (ratio determined by mass balance between the amount of ethanol engaged as solvent and the amount of ethanol recovered in F1). The whole was submitted to reflux for 60 min with mechanical stirring (200 rpm). After this second extraction, the plant matrix R2 was removed by filtration to vacuum (retention rate 35-15 µm) and the filtrate F2 (rich in RA), was stored at 4 °C. 1 L of pure water was added to R2, resulting in 8 % ethanol because of residual ethanol in R2 (ratio determined by

mass balance between the amount of ethanol engaged as solvent and the amount of ethanol recovered in F2). The whole was submitted to a third reflux for 60 min with mechanical stirring. After this third extraction, the plant matrix R3 was removed by filtration to vacuum (retention rate 35-15 µm) and the filtrate F3 (rich in RA) was stored at 4 °C. After determination of dry matter, F1, F2 and F3 were injected in the HPLC system to determine their content in CA (F1) and RA (F2 and F3).

II.4.3.2 Towards a bio-refinery of ginger by-products

A bio-refinery concept was developed for total valorization of ginger by-products. The aim of this bio-refinery was the recovery of several high valued compounds at the end of each consecutive step, without addition of any external solvent or water. The "dry" bio-refinery (DBR) pattern is illustrated in **Figure II-6**.

As described in the flow sheet, after pressing, GP was firstly submitted to microwave hydrodiffusion and gravity (MHG), followed by UAE. To characterize ginger by-products and to assess the performance of DBR, conventional processes (HD and maceration) were performed as reference **(Figure II-6)**. MW and US equipments used in this study are presented in **Figure II-7** and **Figure II-8**.

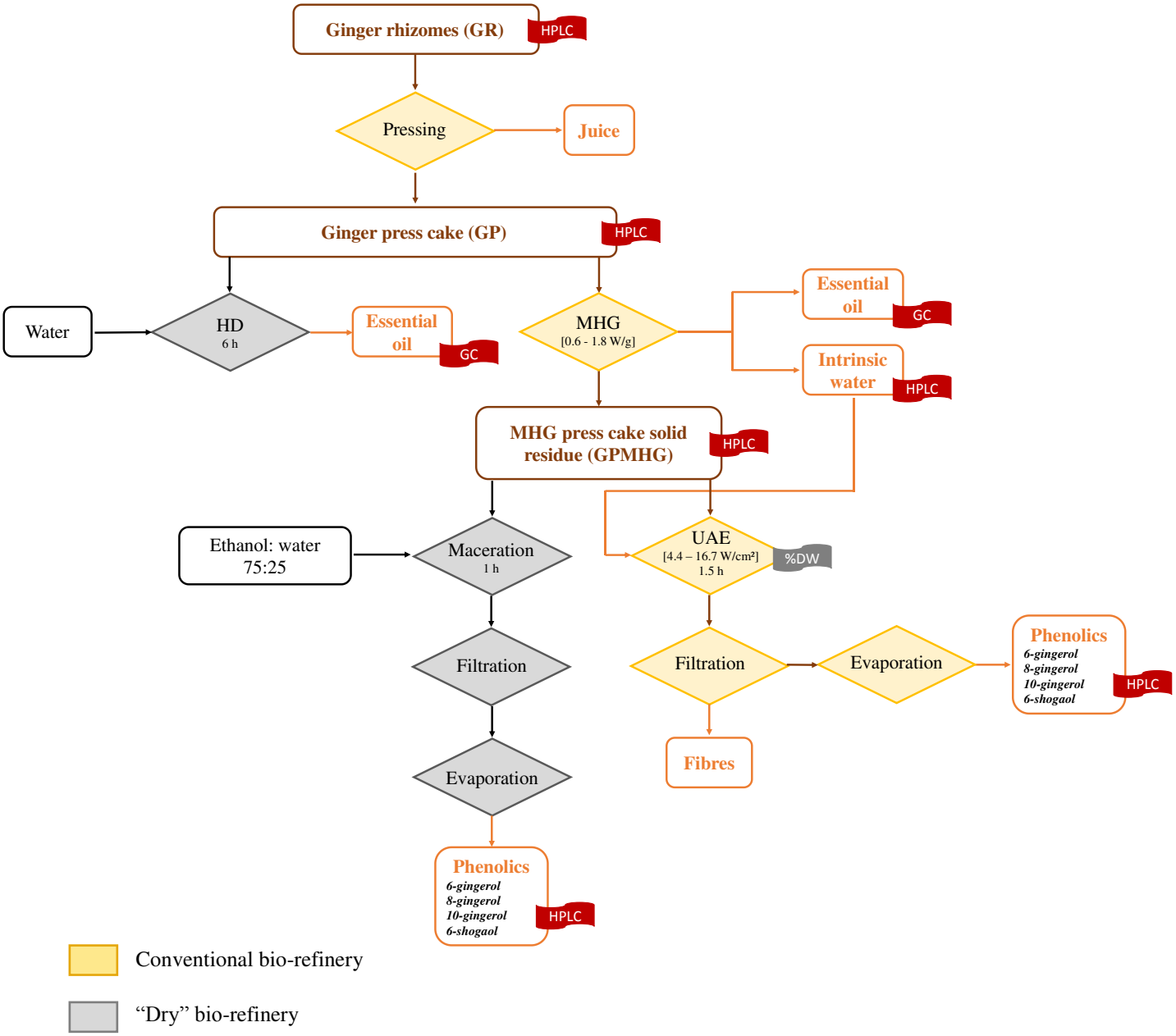


Figure II-6. Flow sheet of processes used in the study for total valorization of ginger by-products.

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

II.4.3.2.1 Microwave Hydrodiffusion and Gravity apparatus and procedure

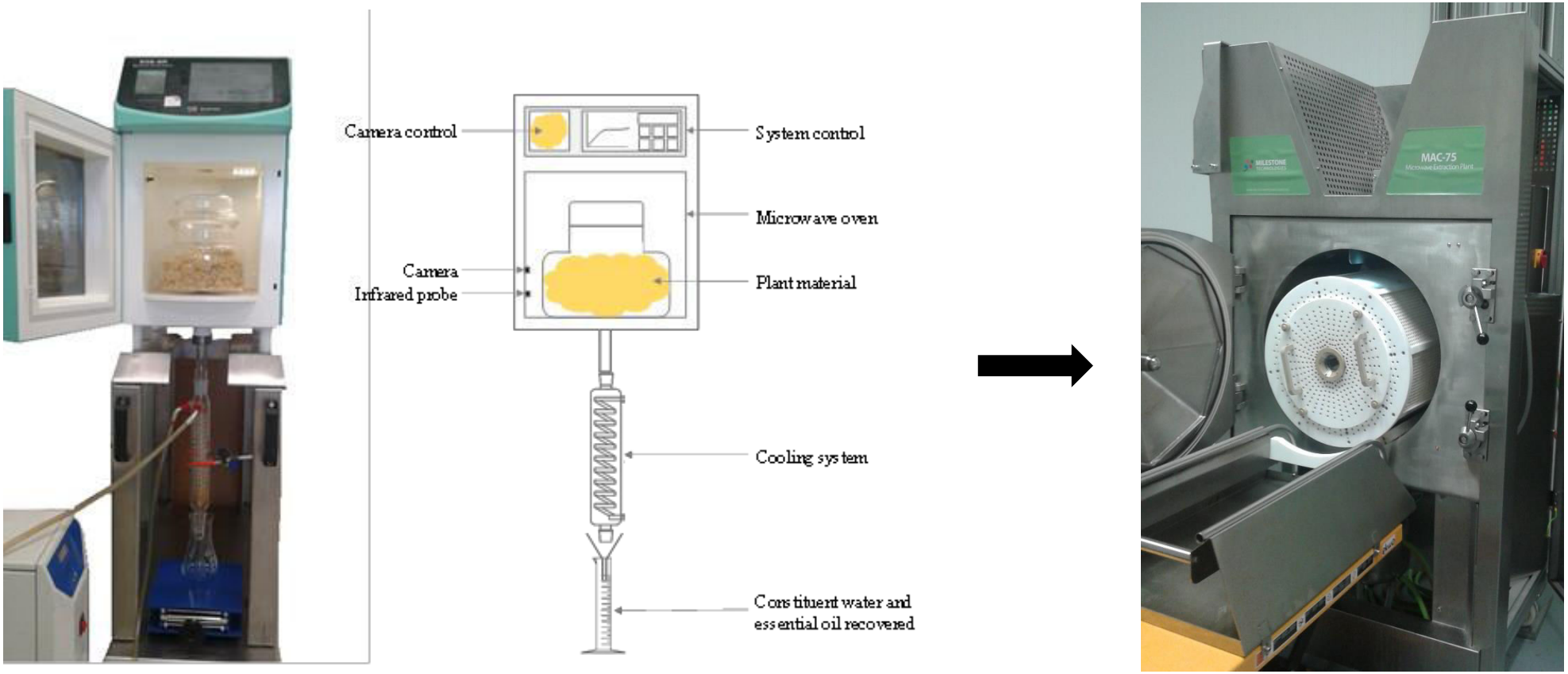
For each experiment using MHG, 500 g of GP were treated. Principle and apparatus are described in previous studies [172,173] and in **section I.3.3.1**. Extraction was performed in a MW laboratory oven (900 W, EOS-GR Microwave Gravity Station, Milestone, Italy) at atmospheric pressure. MW power delivered 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 GPMHG were recovered and stored at 4 °C before use. Each experiment was performed in duplicate.

II.4.3.2.2 Ultrasound Assisted Extraction (UAE)

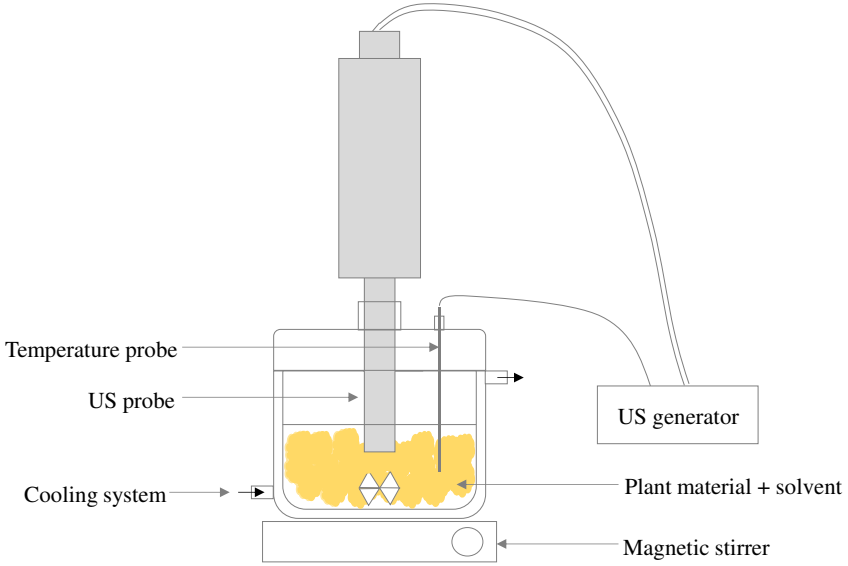
20 g of GPMHG were placed in a double jacket reactor with 500 g of constituent water. The whole was submitted to US (1 kW, UIP 1000 hdT, Hielscher Ultrasonics GmbH, Germany) for 90 min. UI in W/cm^2 and PD in W/cm^3 were both considered to evaluate the ultrasonic power since literature shows that they were both adapted for such type of extraction [91,174–176]. 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*. [177]. 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 min (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 CM was performed by mechanical stirring using identical extraction conditions as UAE.



(a) (b)

Figure II-7. Microwave Hydrodiffusion and Gravity: from laboratory (a) to pilot scale (b).



(a) (b)

Figure II-8. Ultrasound assisted extraction: from laboratory (a) to pilot scale (b).

II.5 ANALYSIS

II.5.1 High performance liquid chromatography (HPLC-DAD)

II.5.1.1 Rosmarinic, carnosic and ursolic acids

Quantification of RA, CA and ursolic acid (UA) in rosemary extracts was conducted by HPLC (Agilent 1100, France) equipped with a diode array detector (DAD). Specific analytical procedures are described below.

(i) For RA, the column was a C18 (5 µm, 4.6 mm x 250 mm, Zorbax SB, Agilent Technologies, France); the mobile phase (isocratic mode) was composed of 68 % water with 0.1 % TFA and 32 % acetonitrile (v/v) and the flow rate was set at 1 mL/min; the column oven temperature was set at 20 °C and the run time was 10 min. 5 µL were injected. RA was detected at 328 nm.

(ii) For CA, the column was a C18 (1.8 µm, 4.6 mm x 50 mm, Zorbax Eclipse XBD-C18, Agilent Technologies, France); the mobile phase (isocratic mode) was composed of 65 % acetonitrile and 35 % water with 0.5 % H_3PO_4 (v/v), and the flow rate was set at 1.5 mL/min; the column oven temperature was set at 25 °C. 5 µL were injected. CA was detected at 230 nm.

(iii) For UA, the column was a C18 (3 µm, 4 mm x 150 mm, All C18, Agilent Technologies, France); the mobile phase (isocratic mode) was composed of 90 % acetonitrile and 10 % water with 0.1 % H_3PO_4 (v/v) and the flow rate was set at 0.6 mL/min; the column oven temperature was set at 30 °C. Run time was 15 min. 5 µL were injected. Detection was performed at 210 nm.

II.5.1.2 Gingerols and 6-shogaol

Quantification of gingerols (6-gingerol, 8-gingerol, 10-gingerol) and 6-shogaol in ginger extracts was done by HPLC (Agilent 1100, France) equipped with DAD. The method described below was developed and validated internally.

The column used was a C18 column (5 µm, 4.6 mm x 250 mm, Advanced Chromatography Technologies ACE, Scotland). The mobile phase was composed of two solvents: (A) 100 % acetonitrile and (B) 100 % water with 0.05 % phosphoric acid (v/v). The

gradient of solvent was used as follows: 0 minute, 45 % (A), 55 % (B); 5 min, 45 % (A), 55 % (B); 10 min, 50 % (A), 50 % (B); 20 min, 55 % (A), 45 % (B); 40 min, 90 % (A), 10 % (B); 45 min, 45 % (A), 55 % (B); 55 min, 45 % (A), 55 % (B). The flow rate was set at 1 mL/min. The column oven temperature was 20 °C and the run time was 30 min. 20 µL were injected. Gingerols and 6-shogaol were detected at a wavelength of 282 nm and quantified using external calibration with standards.

II.5.2 Gas chromatography analysis (GC-FID)

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

II.5.3 Determination of total phenolic content (TPC)

II.5.3.1 Principle

TPC of a plant extract is generally determined using the non-competitive Folin-Ciocalteu method. It is based on the reduction of Folin-Ciocalteu reagent (composed of hexavalent phosphomolybdic/phosphotungstic acid complexes [178]) by phenolic compounds. The reaction forms a blue chromophore constituted by a phosphotungsticphosphomolybdenum complex where the maximum absorption of the chromophores (λ_{max} = 760 nm) depends on the alkaline solution (that's why Na_2CO_3 is added during experiment) and the concentration of phenolic compounds [179].

Folin-Ciocalteu method is generally admitted to determine TPC is an extract, but in practice, it is not absolutely right. Actually, this method is not specific to phenolic compounds and any reducing species (including phenolic compounds) can react with the reagent. It is therefore the global reducing activity which is determined. Contribution of phenolic compounds can be more or less important depending on the extract.

II.5.3.2 Experimental protocol

TPC in rosemary extracts was determined by Folin-Ciocalteu method [180,181] with some modifications. Briefly, 50 µL of sample previously filtered on 0.45 µm were mixed with 1250 µL of a 5-fold diluted Folin-Ciocalteu's reagent into water. The solutions were mixed thoroughly and incubated at room temperature (22 °C) for 1 min. Then 1 mL of 10 % sodium carbonate (Na_2CO_3) solution was added and the solutions were mixed thoroughly again. Solutions were incubated at room temperature (22 °C) for 30 min sheltered from light. Absorbances were measured at 760 nm using an UV-vis spectrophotometer (UV-1800, Shimadzu, Japan). Standardization curves were performed with RA and CA solutions at different concentrations. The TPC was expressed as mg of RA equivalent/ gram of extract powder on dry weight basis. The data were presented as the mean of duplicate analysis.

II.5.4 Oxygen radical absorbance capacity assay (ORAC)

II.5.4.1 Chemical mechanism

This method is a competitive method based on the fluorescence properties of fluorescein sodium salts (FLH) **(Figure II-9)**.

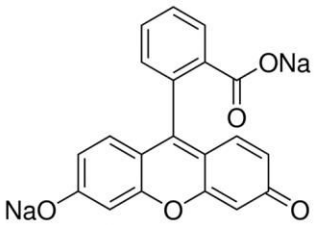


Figure II-9. Fluorescein sodium salt (FLH).

Actually, fluorescein sodium salts emit at 515 nm after excitation at almost 490 nm. When this substrate is oxidized by peroxyradicals generated by 2,2'-azobis(2-amidinopropane)dihydrochloride (AAPH) in PBS at pH 7.4 **(Figure II-10)**, it losses its spectral properties, resulting in a decrease of global fluorescence **(Eq. II-1)**. If external antioxidants (A) are added to the system (for example a plant extract), they reduce the peroxyradicals **(Eq. II-2)**, preventing their reaction with the fluorescein ($k_1 > k_2$). Consequently, fluorescence loss declined **(Figure II-11)** [182].

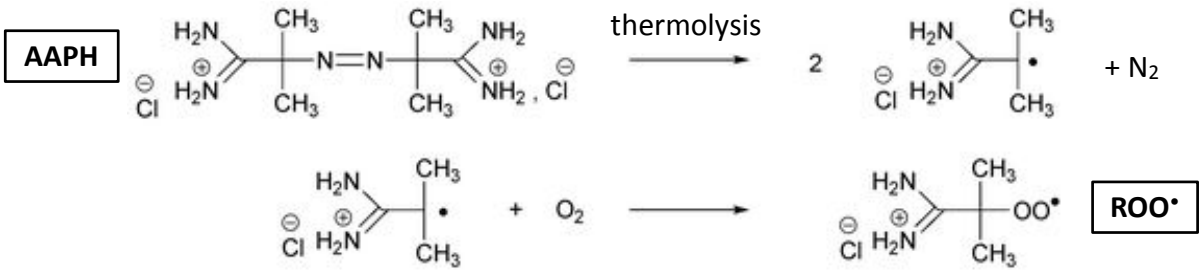


Figure II-10. Peroxyradicals formation from AAPH (from [183]).

Eq. II-1. $FL—H + ROO^{\bullet} \rightarrow FL^{\bullet} + ROOH$ **(k_1)**

Eq. II-2. $A—H + ROO^{\bullet} \rightarrow A^{\bullet} + ROOH$ **(k_2)**

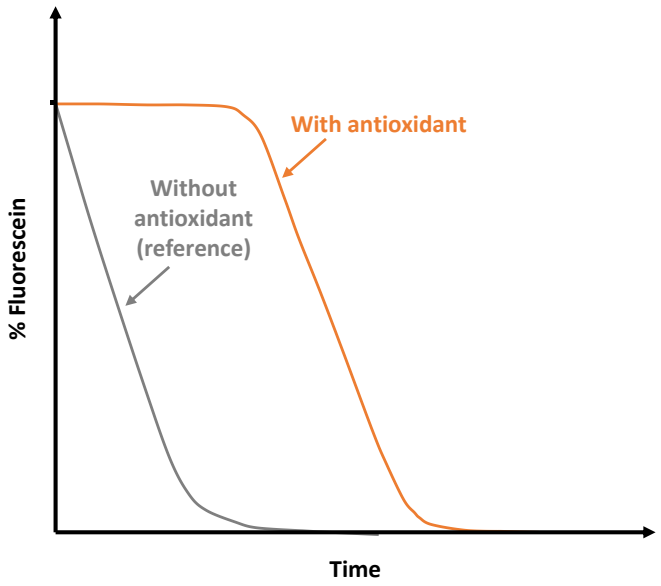


Figure II-11. Protection of fluorescein elicited by an antioxidant.

The benchmark antioxidant used in this method is Trolox **(Figure II-12)**. To obtain the ORAC value of an extract (corresponding to the antioxidant activity), information on fluorescence decay is extracted through the calculation of the area under the curve (AUC) and expressed as Trolox equivalent using **Eq. II-3**.

Eq. II-3. $$\text{Relative ORAC value} = \frac{(\textbf{AUC sample} - \textbf{AUC blank}) \times \textbf{molarity of trolox}}{(\textbf{AUC trolox} - \textbf{AUC blank}) \times \textbf{concentration of sample}}$$

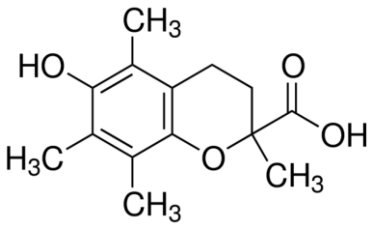


Figure II-12. Trolox molecule.

Currently, two ORAC protocols are available to test hydrophilic or lipophilic molecules [184].

II.5.4.2 Experimental protocol

Hydrophilic ORAC was conducted following the procedure of Ou *et al.* (2001) wherein dry samples are solubilized in 50:50 (v/v) acetone:water mixture [185]. Then, dilutions were prepared in PBS (75 mM, pH 7.0) for each sample. Twenty-five µL of these solutions and PBS alone (blank) were transferred automatically into a 96-well microplate (BRAND, Germany). Outer wells were not used for measurement and were filled with water (200 µL). The plate was refrigerated (6 °C) before the sequence. After introducing the microplate in the reader (Infinite, Tecan, Switzerland), 150 µL of fluorescein sodium salt solution (0.1 µM) were added into each well. The plate was shaken for 8 sec (2 mm amplitude) and incubated for 30 min at 37 °C. After zero value measurement, 25 µL of AAPH solution (152 mM) were added into each well, and the reaction kinetics was measured for 90 min every 90 sec (60 measurements), corresponding to the decrease of fluorescein fluorescence (λ_{ex}: 485 nm/λ_{em}: 535 nm). The antioxidant value of a sample was calculated through the difference between the AUC of this sample and that of the blank (without antioxidant). The result of this operation gave the net AUC which was then plotted on a graph as a function of the concentration. Only the linear part of the curve was taken into account to calculate the slope which was then divided by the slope of the Trolox (standard) calculated in the same conditions and analyzed on the same microplate. As such, ORAC values were expressed as µmol Trolox equivalent/g extract.

II.5.5 Determination of free radical scavenging activity (DPPH)

II.5.5.1 Chemical mechanism

Free radical scavenging activity is determined using an artificial and stable free radical, the 2,2-diphényl-1-picrylhydrazyl (DPPH$^{\bullet}$) (λ_{max} = 510-520 nm) **(Figure II-13)**.

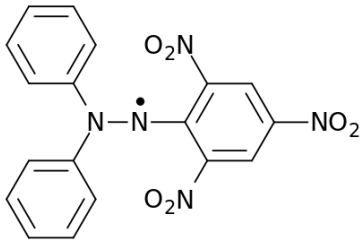


Figure II-13. DPPH$^{\bullet}$ radical.

This method is non-competitive and consists in the quenching of this free radical by an anti-radical (A). This phenomenon occurred either by transfer of electron from the anti-radical agent to DPPH$^{\bullet}$ to generate DPPH^{-} which will capture afterwards a proton in the system **(Eq. II-4)**; or directly by transfer of an hydrogen atom to generate DPPH-H molecule **(Eq. II-5)** [186]. It results in the discoloration of the reaction media (from purple to yellow) according to the amount of reacted DPPH$^{\bullet}$.

Eq. II-4.

$$A—H \rightarrow A^{-} + H^{+}$$

$$A^{-} + DPPH^{\bullet} \rightarrow A^{\bullet} + DPPH^{-}$$

$$DPPH^{-} + H^{+} \rightarrow DPPH—H$$

Eq. II-5.

$$AH + DPPH^{\bullet} \rightarrow A^{\bullet} + DPPH—H$$

II.5.5.2 Experimental protocol

Free radical scavenging activity of each extract was determined using DPPH free radical. The method, consisting in the reduction of DPPH free radicals by antioxidants contained in the extract, was based on Brand-Williams *et al.* procedure [187]. Briefly, 25 mg of DPPH were solubilized into 100 mL methanol, then diluted 1:10 with methanol to reach 0.025 g/L. Different solutions of rosemary extract were prepared at several concentrations with methanol: 0.1875, 0.375, 0.75, and 1.5 g/L. One hundred µL of sample were thoroughly mixed with 3.9 mL DPPH solution, and incubated at 22 °C for 30 min sheltered from light. Concentrations of extract in final solutions were 4.69, 9.38, 18.75 and 37.5 mg/L, and that of DPPH was 0.024 g/L (61 µM).

Then, absorbance of the reaction mixtures was measured at 518 nm using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan). The absorbance were converted into percentage of antioxidant activity (%AA) according to the following equation:

Eq. II-6.
$$\%AA = 100 - \left(\frac{\text{Abs sample-Abs blank}}{\text{Abs reference}} \times 100 \right)$$

Where Abs_{sample} is the absorbance of the sample at a given concentration, Abs_{blank} is the absorbance of the pure solvent (methanol) and $Abs_{reference}$ is the absorbance of the DPPH solution without any extract.

The IC_{50} was determined using the equation of the linear curves obtained for all extract concentrations that corresponds to the extract concentration which shows a % AA of 50 % (extract concentration necessary to scavenge 50 % of DPPH). RA was considered as reference, therefore the DPPH scavenging capacity of the extract was expressed in g RA equivalent/g extract.

II.5.6 High performance thin layer chromatography (HPTLC) coupled to DPPH free radical scavenging activity measurement

Ethyl acetate:glacial acetic acid:methanol:water in 60:15:15:10 (v:v:v:v) proportions was used as mobile phase. HPTLC was conducted on 20.0 x 10.0 cm plates composed of silica gel 60 F 254. Various rosemary extracts were diluted between 1.4 and 2.7 g/L and were

deposited using a CAMAG Automatic TLC Sampler (CAMAG, Switzerland) equipped with a 25-µL syringe. The bands were 6 mm-long. Plates were developed in a CAMAG twin trough chamber (CAMAG, Switzerland). Humidity control was performed with $MgCl_2$ (33 %) during 10 min and the chamber was previously saturated with mobile phase vapor for 20 min at room temperature. After development and drying in the chamber, the plates were immersed during 1 sec in a 0.05 % DPPH solution (0.1 g DPPH in 200 mL methanol), dried at room temperature and observed in a CAMAG UV cabinet (CAMAG, Switzerland) **(Figure II-14)**.

Figure II-14. HPTLC modules.

II.5.7 LC-MS identification

Two rosemary extracts (obtained using 100 % water and 30 % ethanol) were analyzed by LC-MS to characterize their phenolic profile. Each sample was dissolved in water and 30 % ethanol (respectively) and 5 µL of this solution were injected in an Agilent 1200 HPLC instrument equipped with a DAD. The column used was an Atlantis T3 (3 µm, 3.0 mm x 150 mm, Waters, France); the mobile phase was composed of two solvents mixtures: (A) 90 % ammonium formate buffer pH 3.0 and 10 % acetonitrile, and (B) 90 % acetonitrile and 10 % 10 mM ammonium formate buffer pH 3.0. The gradient was used as follows: from 0 to 2 min, 100 % (A); from 2 to 30 min, linear gradient from 100 % (A) to 100 % (B). The flow rate was 0.6 mL/min and the column oven temperature was set at 30 °C. Negative ion mode ESI-MS was acquired using a MS-TOF instrument (Agilent 6230 model, Santa Clara, Californie), in full scan mode. Analysis conditions were fixed as follows: ionization voltage

3.5 kV; capillary temperature , 100 °C; desolvation temperature, 325 °C; nitrogen flow, 8 (arbitrary units); cone voltage, 190 V.

II.6 CALCULATIONS

In this manuscript, results are expressed in different ways, corresponding to:

Eq. II-7. $$\text{Extraction yield (\%)} = \left(\frac{\text{weight of extract (DW)}}{\text{weight of initial raw material (DW)}} \times 100 \right)$$

Eq. II-8. $$\text{Purity (\%)} = \left(\frac{\text{weight of compound (DW)}}{\text{weight of extract (DW)}} \times 100 \right)$$

Eq. II-9. $$\text{Content in active (mg/g)} = \left(\text{purity} \times \frac{\text{weight of extract (mg)}}{\text{weight of initial raw material (g)}} \right)$$

Eq. II-10. $$\text{Active recovery (\%)} = \left(\frac{\text{weight of compound extracted (DW)}}{\text{weight of compound available (DW)}} \times 100 \right)$$

In the process intensification study **(CHAPTER III)**, estimation of specific carbon emissions resulting from electric consumption was determined considering that 1 kWh = 800 g CO_2 [22]. Energy consumption of each process was measured using an electrical meter (Cost Control, La Crosse Technology, France).

CHAPTER III . PROCESS INTENSIFICATION

ULTRASOUND VERSUS MICROWAVE AS GREEN PROCESSES FOR EXTRACTION OF ROSMARINIC, CARNOSIC AND URSOLIC ACIDS FROM ROSEMARY

M. Jacotet-Navarro, N. Rombaut, A.-S. Fabiano-Tixier, M. Danguien, A. Bily, F. Chemat, Ultrasound versus microwave as green processes for extraction of rosmarinic, carnosic and ursolic acids from rosemary, Ultrason. Sonochem. 27 (2015) 102–109. doi:10.1016/j.ultsonch.2015.05.006.

CONTEXT

Antioxidant extraction from rosemary have been hugely studied in recent years. Extraction selectivity is generally solvent-dependent and based on “like dissolves like” principle. However, few studies investigated extraction selectivity according to the process. In this context, it would be interesting to assess both intensification capacity and extraction selectivity of specific processes (ultrasound (US) and microwave (MW)) towards rosemary antioxidants.

ABSTRACT

US and MW as green processes are investigated in this study, focusing on the extraction selectivity towards antioxidant extraction from rosemary leaves. Due to its richness in valuable compounds such as rosmarinic (RA), carnosic (CA) and ursolic (UA) acids, rosemary is a reference matrix for extraction study. In this work, six alternative processes are compared: US (bath, reactor and probe), MW (reflux under MW, MW under nitrogen pressure and MW under vapor pressure). The main result of this study is that selective extraction can be achieved according to extraction techniques and therefore to the extraction process.

III.1 INTRODUCTION

The growing demand for natural products leads to constant developments of natural extracts. In the field of food preservation, compounds such as tocopherols and flavonoids are broadly used as antioxidants [188]. Natural antioxidants are extracted from plants, more specifically from herbs or spices, where numerous compounds have been identified as potential antioxidants such as vitamins, lipids, and predominantly polyphenols [188]. Due to its polyphenol composition, rosemary can be considered as a reference matrix for the production of natural antioxidant extracts [188,189].

Rosemary (*Rosmarinus officinalis* L.) is native to the Mediterranean region. Rosemary belongs to the Lamiaceae family and possesses needle-like leaves which contain a powerful fragrance and polyphenolic compounds: phenolic acids such as rosmarinic acid (RA) and caffeic acid; phenolic diterpenes such as carnosic acid (CA), carnosol (CO) and triterpenoids such as ursolic acid (UA) **(Figure III-1)**. This polyphenol profile induces antioxidant [190–192], antibacterial [192,193] and antimutagenic properties to rosemary extracts. RA and CA are more specifically used in the food industry as natural antioxidants [194,195]. Apart from the antioxidant properties of rosemary compounds, UA is another valuable natural compound which is studied for its pharmacological effects (e.g. antitumor property [196]).

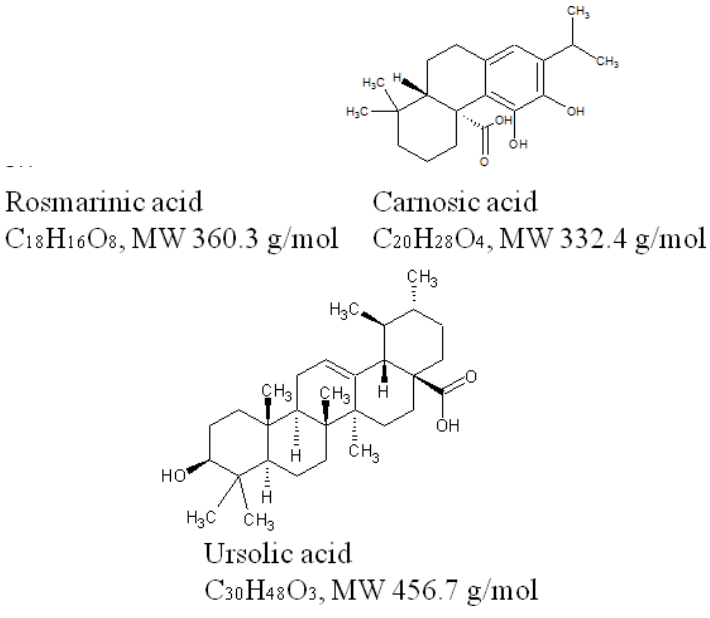


Figure III-1. Chemical structures of major valuable compounds in rosemary.

Throughout literature, at a laboratory scale, extraction of RA and CA from rosemary leaves has been investigated using different technologies: conventional solvent extraction (CSE) [197], microwave (MW) [198,199], ultrasound assisted extraction (UAE) [143,198,200–202], supercritical and subcritical fluid extraction [139,142,143], pressurized liquid extraction (PLE) [139,203], deodorization by Instant Controlled Pressure drop [94] or extraction with ionic liquids (IL) [204]. **Table III-1** details the experimental conditions of the mentioned processes. Some extraction processes, particularly conventional ones, are sometimes accompanied by several drawbacks, such as the use of harmful solvents, degradation of compounds of interest due to high temperature, long extraction time, difficulty to implement or high economic and energetic costs. That way, during the last few years, concepts of "Green chemistry" and "green extraction" emerged [14,205]. Extraction processes have been studied to be more energy saving, safe for users and environmental friendly than yesterday, without reducing extraction efficiency. Intensification of extraction processes taking in account those different aspects should become a new challenge for the design of extraction processes.

Due to their chemical structure, CA and RA are conventionally extracted by methanol and acetone [202,206,207]. Other solvents have been used such as ethanol and water or a mixture of both [206]. Considering sustainable and safe extraction, there is a major interest in the use of a mixture of ethanol and water as an extraction solvent, each of these solvents being classified as GRAS solvents.

Aiming at intensification of extraction taking into account concept of "Green Chemistry", a comparative study is carried out between ultrasound (US) and MW to extract RA, CA and UA from rosemary leaves. To evaluate those innovative processes, results are compared to conventional solid/liquid extraction (reflux extraction and maceration). Within the objectives of green extraction [14,205], all extractions were performed in a mixture of ethanol/water (90/10, v/v). Results are compared quantitatively on the basis of extraction yield and on the contents of RA, CA and UA recovered in the extracts. Ultimately, the processes assessed are compared according to the energy consumption required to achieve extraction.

Extraction techniques	Extracted compounds	Solvents	Experimental conditions	Analysis	Ref.
USAE	RA, CA	water, ethanol	ratio 1:6 (w/w) P_{US}= 300 J/g T= 40 °C t= 7 min	TPC, DPPH, HPLC-UV	[198]
CSE	RA, CA, CO	methanol/water 80/20 (v/v)	ratio 1:1 (w/v) t= 2 min	HPLC-UV	[197]
DIC pre-treatment + CSE	RA, CO	ethanol/water 80/20 (v/v)	ratio 1:1 (w/v) t= 2 min	HPLC-UV	[94]
PLE	RA,CA, CO	water, ethanol	T= 50-200 °C t= 20 min	TPC, DPPH, UPLC-MS	[139]
MW pre-treatment + CSE	RA,CA	ethanol/water 80/20 (v/v)	ratio 1:10 (w/v) P_{MW} = 8 W/g t_{MW}= 15 min t_{CSE}= 4 min	HPLC-UV	[199]
SWE	CA, CO	subcritical water	T= 25-200 °C t= 30 min	DPPH, LC-MS	[46]
SFE	CA, CO	supercritical CO_2	P = 355 bar T= 100 °C t= 20 min	HPLC-UV, MS	[143]
IL	RA,CA	$[C_8mim]Br$ 1M	ratio 1:20 (w/v) P_{US}= 221 W $t_{soaking}$= 2 h t_{US}= 30 min	HPLC-UV	[204]

(RA: Rosmarinic Acid; CA: Carnosic Acid; CO: carnosol; USAE: Ultrasounds Assisted Extraction; CSE: Conventional Solvent Extraction; DIC: Deodorization by instant controlled pressure drop; PLE: Pressurized Liquid Extraction; MW: Microwaves; SWE: Subcritical Water Extraction; SFE: Supercritical Fluid Extraction; IL: Ionic Liquids)

Table III-1. Examples of extraction conditions to recover RA, CA and CO from rosemary.

III.2 MATERIALS AND METHODS

Rosemary (*Rosmarinus officinalis* L.) was provided by Naturex. The batch used was collected in Morocco in 2013 and previously hydrodistillated by the supplier. Only leaves were used and were ground for 10 s before extraction using a coffee grinder (Severin, France). Initial moisture content was 8.93 ± 0.01 % and initial content in rosemary in RA and CA is 0.21 % w/w and 1.70 % w/w respectively.

Ten different extraction processes were applied in this study: four conventional processes (reflux 30 min, reflux 5 h, grinding 3 min followed by reflux 30 min, and maceration) and six innovative (US bath, US reactor, US probe, reflux under MW, MW under nitrogen pressure and MW under vapor pressure). Those processes are detailed in **CHAPTER II** and illustrated in **Figure II-4**.

All the extractions were performed using a solid/liquid ratio of 1/20 (m/v). The extraction solvent was ethanol/water mixture in a proportion of 90/10 (v/v). After extraction, the solvent was separated from the matrix by filtration to vacuum using a filter paper. The extract was concentrated until dryness by solvent evaporation under vacuum (Laborota 4001, Heidolph, Germany).

III.3 RESULTS AND DISCUSSION

III.3.1 Comparison of extraction processes in terms of mass extraction yields

Extraction yields obtained by heat reflux extraction (HRE), maceration, UAE and microwave assisted extraction (MAE) are presented in **Figure III-2**. Regarding HRE extractions, it can be identified that most of the extraction is achieved within 30 min. Increasing the duration of HRE up to 5 h does not lead to a drastic increase of the extraction yield (20 % against 19 % for 5 h and 30 min extraction duration respectively). Moreover, adding a preliminary step of homogenization prior to HRE does not improve significantly the yield (18.8 ± 0.2 % and 19.0 ± 0.5 % respectively). Maceration at 40 °C for 30 min results in a much lower extraction yield (10.0 ± 0.3 %). These differences in yield between HRE and maceration are attributed to the temperature difference during extraction.

For UAE, extraction temperatures were maintained at 40 °C. It can be identified that similar extraction yield are obtained for extraction performed with the US reactor and for the US probe (18.1 ± 2.3 % and 18.8 ± 2.2 % respectively). Lower yields are obtained with the US bath (13.1 ± 0.1 %). Those results may be explained by a low ultrasonic power delivered by the bath compared to the ultrasonic reactor and the probe. Compared to HRE, it can be noted that equivalent yields are achieved at 40 °C using UAE.

MAE was performed at 70 °C and higher temperatures (boiling temperature, 125 °C and 150 °C). For these extractions **(Figure III-2)**, increasing temperatures lead to an increase of the extraction yield, the highest yield (25.2 %) being reached at 150 °C. It is a classical observation in extraction that extraction yields increase with increasing temperatures.

If the extraction yield gives an indication of a process performance, the composition of the extracts has to be studied to assess the selectivity of extraction.

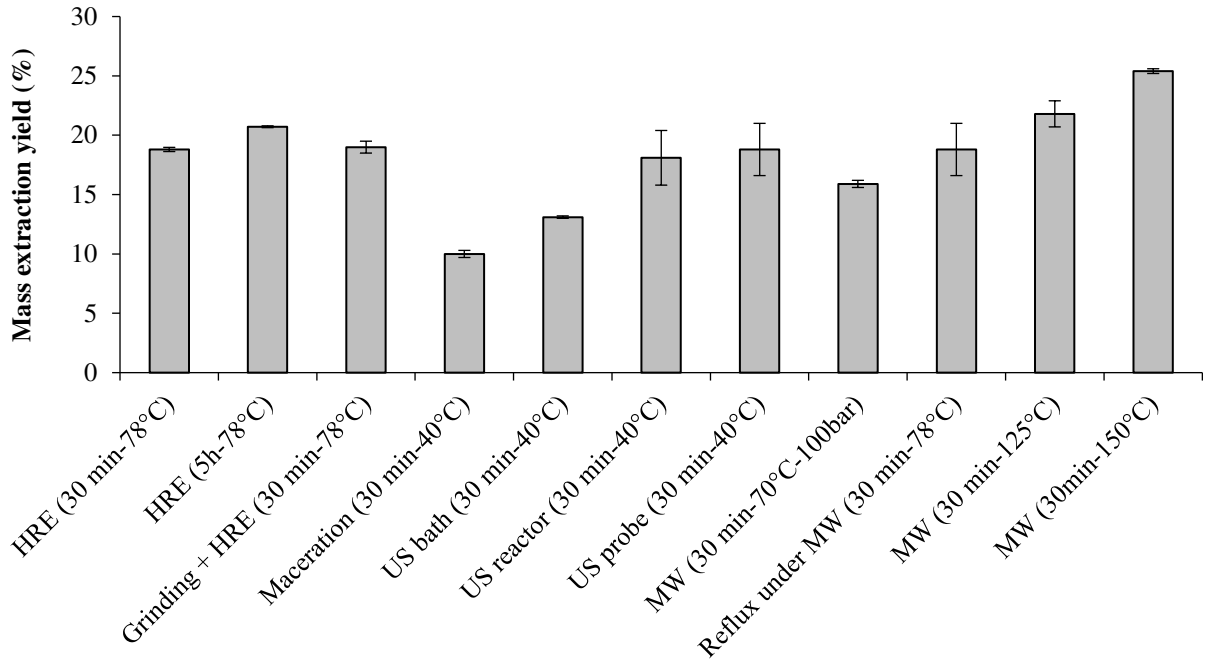


Figure III-2. Comparison of mass extraction yields according to HRE, maceration, ultrasound and microwave assisted extraction.

III.3.2 Processing impact towards the extraction efficiency of rosmarinic, carnosic and ursolic acids

The amount of RA, CA and UA extracted are compared in **Figure III-3**, according to HRE, UAE and MAE. It can be noted that among the three compounds of interest, CA and UA were extracted from 5.5 to 15.4 mg/g rosemary and 20.5 to 35.3 mg/g rosemary respectively and RA from 0.4 to 2.2 mg/g rosemary, no matter the extraction technology. Differences among the extraction are noticed according to the extraction process used.

Modification of the profile of extracts obtained by HRE could be noticed. The extraction of RA seems to be enhanced with extraction duration from 30 min to 5 h (from 1.4 ± 0.1 to 2.1 ± 0.1 mg/g rosemary, **Figure III-3**). Extraction being performed at the boiling point of the solvent, RA does not appear to be a thermo-sensitive compound. Some authors indicate that an increase of temperature favors extraction until a critical value [139,208]. The opposite tendency is obtained for CA, which extraction efficiency decreases with the increase of the extraction duration at high temperature. The tendency observed tend to show a degradation of CA with temperature. However, different conclusions are obtained throughout literature: either enhanced extraction with temperature (from 100 to 200 °C using pressurized

water extraction [46,139]) while others report a degradation with mild temperatures in stability studies (40 °C or less; [209,210]). Overall, it seems that temperature is not the sole factor impacting on the extraction of CA. Homogenization prior to extraction does not lead to an enhanced extraction for RA, CA and UA **(Figure III-3)**, however, RA is more rapidly extracted.

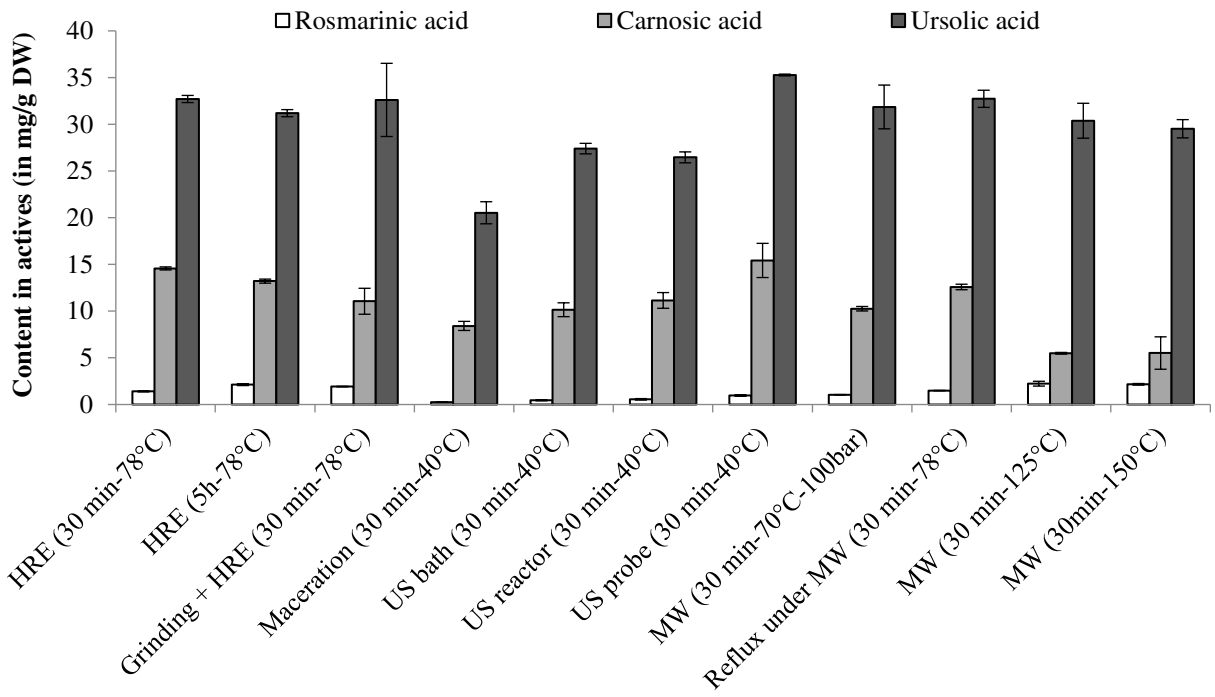


Figure III-3. Comparison of RA, CA and UA extraction from rosemary according to the process assessed.

For UAE, temperature was lower (40 °C) than HRE. It can be identified that the level of RA extracted (from 0.2 to 1 mg/g rosemary, **Figure III-3**) is much lower than for HRE. Among the US technologies, sonication by the US probe during 30 min appears as the most efficient process for the extraction of CA and UA. This effect may be explained by a more effective treatment due to specific ultrasonic power delivered using the US probe. Within a shorter duration of extraction and lower extraction temperature, the yields obtained are higher: for CA, contents are 15.4 ± 1.8 mg/g and 13.2 ± 0.2 mg/g for US probe and 5 h HRE respectively.

With experiments performed using MAE, two parameters are examined: effect of pressure and temperature. Comparing extraction at 100 bars and 70 °C (MW) and 30 min

HRE **(Figure III-3)**, it was noticed that high pressure does not enhance compounds extraction. RA yields obtained with MW process at 125 °C and 150 °C (2.2 ± 0.1 mg/g) are equivalent to the reference one (2.1 ± 0.1 mg/g for 5 h HRE). RA was increasingly extracted at high temperatures (from 1.5 mg/g at 78 °C to 2.1 mg/g at 150 °C, **Figure III-3**), as for HRE extractions. MAE appears to be are more adapted for extraction of RA. A decrease of CA is noted for all extraction assisted by MW. A degradation of CA was noticed with the increase of temperature: concentration decreased from 12.6 ± 0.3 mg/g for reflux under MW (30 min-78 °C) to 5.5 ± 1.7 mg/g for MW (30 min-150 °C). Since CA was degraded at high temperatures, it may have been transformed in degradation products. Additionally, the extraction yield reached the highest level using an extraction temperature of 150 °C (25.4 %, **Figure III-2**).

III.3.3 Investigation on the conversion of CA into carnosol

Factors such as temperature or light can induce a degradation of rosemary antioxidants into several compounds. A conversion of CA into CO **(Figure III-4)** is reported by several authors [210,211]. Additionally, CO also has antioxidant properties [212,213].

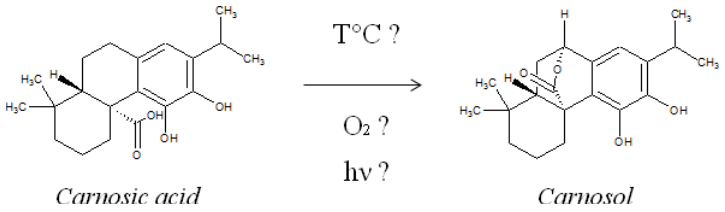


Figure III-4. Hypothesis of the conversion of carnosic acid into carnosol.

In order to assess if CA was converted into CO during extraction, we examined the purities of CA and CO in the extracts **(Table III-2)**. When extraction is performed at 40 °C or at boiling temperature (78 °C), the proportions of CA are higher than CO. The extracts obtained by MW at 125 °C contain more CO than CA: 3.36 ± 0.07 % and 2.53 ± 0.17 % respectively **(Table III-2)**. At 150 °C, purities in CA and CO are very similar: 2.17 ± 0.66 % and 2.04 ± 0.02 % for CA and CO respectively. It can be concluded that the decrease of CA in extracts does not result in a systematic increase of CO. Other minor degradation derivatives of CA are epirosmanol [214], 7-methy, l-epirosmanol [211] and probably rosmanol 9-ethyl ether

[215]. Other degradation products of CA such as rosmanol and rosmaridiphenol are also generated from CA during process. Moreover, it has to be underlined that CO naturally occurs in rosemary leaves [191], which could explain the concentration of CO found at lower extraction temperatures (**Table III-2**). Globally, our results indicate that higher pressure and intensification through MW favors a higher ratio of CO compared to CA.

		Purity of compounds in extracts	
Extraction process	Experimental conditions (temperature – extraction duration)	CA (%)	Carnosol (%)
HRE	78 °C - 30 min	7.75 ± 0.01	2.22 ± 0.02
HRE	78 °C - 5 h	6.38 ± 0.08	2.10 ± 0.01
Grinding + HRE	78 °C - 30 min	5.32 ± 0.58	2.09 ± 0.07
Maceration	40 °C - 30 min	8.41 ± 0.71	2.50 ± 0.12
US bath	40 °C - 30 min	7.73 ± 0.63	2.26 ± 0.16
US reactor	40 °C - 30 min	6.27 ± 0.92	2.02 ± 0.27
US probe	40 °C - 30 min	8.21 ± 0.00	2.37 ± 0.03
Ultraclave 100bar	70 °C - 30 min	6.45 ± 0.02	2.24 ± 0.03
Reflux under MW	78 °C - 30 min	6.19 ± 0.49	1.93 ± 0.10
MW under pressure (vapour pressure)	125 °C - 30 min	2.53 ± 0.17	3.36 ± 0.07
	150 °C - 30 min	2.17 ± 0.66	2.04 ± 0.02

HRE: Heat reflux extraction; US: ultrasound; MW: microwave; DW: dry weight

Table III-2. Purities of carnosic acid and carnosol in the extracts obtained by the different processes.

III.3.4 Energy consumption

An energy consumption monitoring of the different experiments was performed. **Table III-3** indicates the measures obtained per process. In all cases the evaluation was performed on 10 g of rosemary leaves and 200 mL of solvent. It appears clearly that 5 h reflux is the most energy-consuming technique (850 kWh/kg extract) and consequently the process with the highest carbon emissions associated (680 kg CO_2/kg extract). It is mainly due to the long extraction duration (5 h). US processes applied during 30 min present the lowest values compared to MW processes and HRE: US probe resulted in an energetic consumption of 23 kWh/kg extract and 19 kg CO_2/kg extract. Therefore, at laboratory scale, this process appears as the best compromise between yield and energy consumption. MW treatments also show reduced values compared to HRE, with few differences between MW processes.

This energetic assessment was carried out at laboratory scale using an electrical meter, allowing a comparison on the basis of the sole process. For upscaling and industrial considerations, a Life Cycle Assessment (LCA) could be established to obtain the energetic and environmental profiles of different products or processes [203,216]. That way, LCA could be a tool for industrial decision-making since it could determine which process is the most eco-friendly and economical.

Extraction process	Experimental conditions (temperature - extraction duration)	Energy consumption (kWh/kg extract)	Carbon emissions (kg CO_2/kg extract)
HRE	78 °C - 30 min	94	75
HRE	78 °C - 5 h	850	680
Grinding + HRE	78 °C - 30 min	94	75
Maceration	40 °C - 30 min	79	63
US bath	40 °C - 30 min	15	12
US reactor	40 °C - 30 min	39	31
US probe	40 °C - 30 min	23	19
Ultraclave 100bar	70 °C - 30 min	154	123
Reflux under MW	78 °C - 30 min	85	68
MW under vapour pressure	125 °C - 30 min	171	137
	150 °C - 30 min	157	125

HRE: Heat reflux extraction; US: ultrasound; MW: microwave

Table III-3. Energy consumption and carbon emissions of the different extraction processes.

III.4 CONCLUSION

This study was carried out to compare different processes for extraction of RA, CA and UA from rosemary leaves. The main conclusion is that selective extraction of RA and CA can be achieved by modification of the extraction technique and procedure. The use of intensified extraction processes at different extraction temperatures enabled to achieve similar yields compared to conventional extraction processes (HRE and maceration). It has been demonstrated that CA and UA extraction is enhanced using US processes at lower temperature whereas MW are more adapted to extract RA at higher temperature **(Figure III-5)**. Moreover, this study revealed that US and MW technologies are good alternatives to conventional processes regarding energy consumption and carbon emissions at laboratory scale. Further research are required to investigate the choice of the most appropriate technology for scale up and industrialization [217].

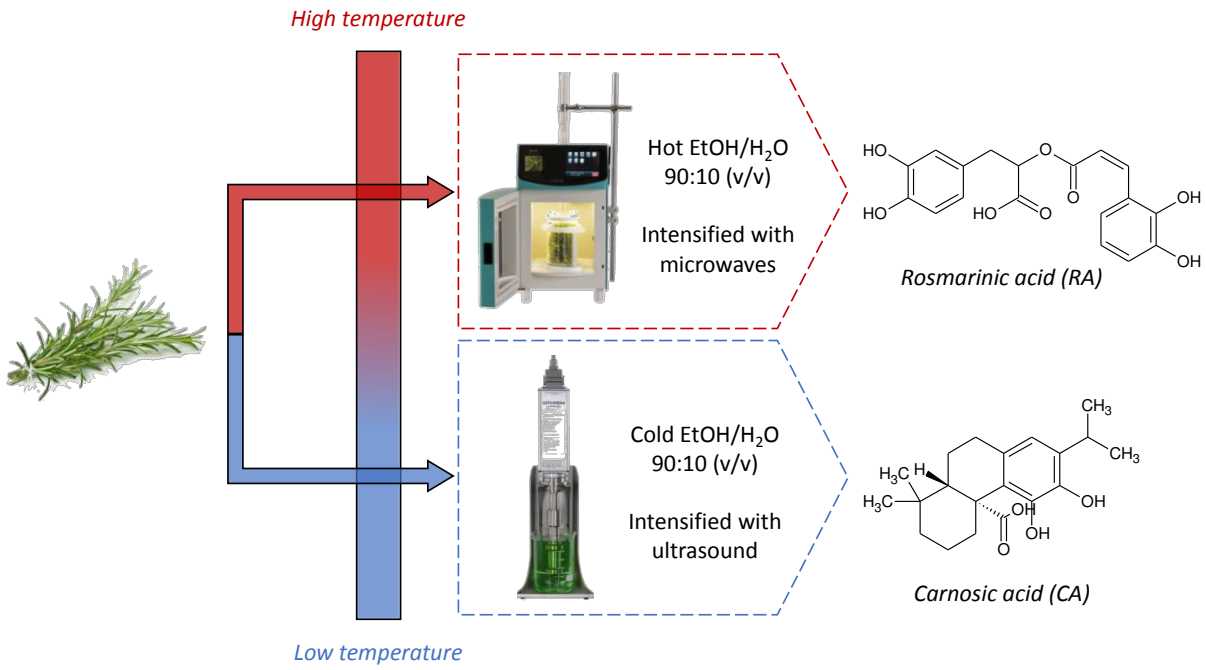


Figure III-5. Selectivity towards RA acid or CA according to temperature and intensification technology.

CHAPTER IV . SUBSTITUTION OF SOLVENT

ASSESSMENT OF THE BEST SOLVENT FOR THE EXTRACTION OF ROSMARINIC AND CARNOSIC ACIDS FROM ROSEMARY

M. Jacotet-Navarro, M. Laguerre, N. Feuillère, A.-S. Fabiano-Tixier, M. Tenon, A. Bily, F.Chemat, What is the best ethanol-water ratio for the extraction of antioxidants from rosemary? Impact of the solvent on yield, composition and activity of the extracts. *(Submitted)*

CONTEXT

Rosemary extracts are widely used in current food processing industry. Lipophilic extracts are obtained with acetone or ethanol whereas hydrophilic extracts are obtained with ethanol or water. At industrial level, both extracts are generally standardized regarding their content in active compounds, but rarely regarding their activity. In this context, it would be interesting to assess the effect of solvent (and more particularly the effect of ethanol ratio in the solvent since it is safer than acetone) on extracts composition but also on extract activity.

ABSTRACT

Extracts rich in antioxidants, such as rosemary extracts, are currently obtained by extraction of the plant material using hydro-alcoholic mixtures with high ethanol content. As this ratio is generally chosen by default and scarcely optimized, we intended to investigate the impact of the hydro-alcoholic composition on extract characteristics such as extraction yield, composition profile in selected compounds, and antioxidant/reducing activity such as Folin-Ciocalteu, DPPH, and ORAC. A theoretical determination of rosmarinic (RA) and carnosic (CA) acid solubilities in ethanol:water mixtures was also performed using COSMO-RS and was confronted to experiments. While the best solubilizing solvent (100% ethanol) was also the best extracting solvent for CA, it was not the case with RA since pure ethanol appeared as a poor solvent compared to 30% ethanol which was optimal. Finally, the best antioxidant activities were obtained with 30% ethanol.

IV.1 INTRODUCTION

The choice of the solvent is crucial in extraction processes, since it directly impacts the selectivity, and consequently, affects the chemical composition and functional properties of the final extract. In general, this selection depends on the solubility of the targeted compound in the said solvent. Since solubilization implies electrostatic repulsions and attractions between the solvent and the solute, it is roughly admitted that a polar solvent would favor the solubilization and extraction of polar compounds, whereas a less polar solvent would fit for less polar molecules.

Beyond the alternative solvents available for natural products extraction, hydro-alcoholic mixtures are good candidates since they are rather few selective and scanned a wide range of polarities regarding the compounds to be extracted. Surprisingly, most extractions using hydro-alcoholic mixtures are performed by default with high amount of ethanol (between 70 and 90 % ethanol) [218–221]. This is particularly exemplified with rosemary extracts which are currently used as antioxidants in the food sector. At the industrial level, oil soluble rosemary extracts are obtained via an extraction using liquid solvents or supercritical carbon dioxide. Generally, acetone [222], pure ethanol or hydro-alcoholic mixtures with high content in ethanol (80 % to 100 %) are selected to extract both carnosic (CA) and rosmarinic (RA) acids [223,201,198,199,94,221,90]. These molecules are known as the main antioxidants in rosemary [191] and are of special interest due to a strong market demand. Apart from Oliveira *et al.,* who optimized the ethanol content to extract RA, CA and carnosol (CO) from rosemary [75], very few studies were carried out to rationalize the ethanol:water mixture. Furthermore, the resulting extracts are generally characterized by their content in active compounds using HPLC, for reasons of simplicity and speed of analysis. Their global activity (antioxidant, antimicrobial…) in food formulations or in cells can be evaluated as well, but both profile and activity characterizations are scarcely performed for the same extract. This raises the question of which methodology should be adopted to determine the best solvent for extraction. Should industrials focus on solubility, profile, or activity? Is there a common solvent to maximize these three responses?

The present study addresses two important aspects. The first one is about a better understanding of solubilization and extraction phenomenon. It is implemented for the case of rosemary and more particularly its antioxidants (RA and CA). To this end, solvent effects were not only evaluated in terms of global mass extraction yield, total phenolic content (TPC)

(through their reducing capacity), and specific RA and CA composition, but also in terms of free radical scavenging (DPPH method) and antioxidant (ORAC method) activities of final extracts. Theoretical comprehension of dissolving mechanisms was performed using the COSMO-RS (Conductor like screening model for real solvents) approach and was compared with experimental solubilities of targeted compounds measured in various ethanol:water mixtures. The procedure is summarized in **Figure IV-1**.

In a second part, based on the previous results, a process inspired from the bio-refinery concept was developed to extract separately and consecutively both RA and CA from rosemary with hydro-alcoholic mixtures.

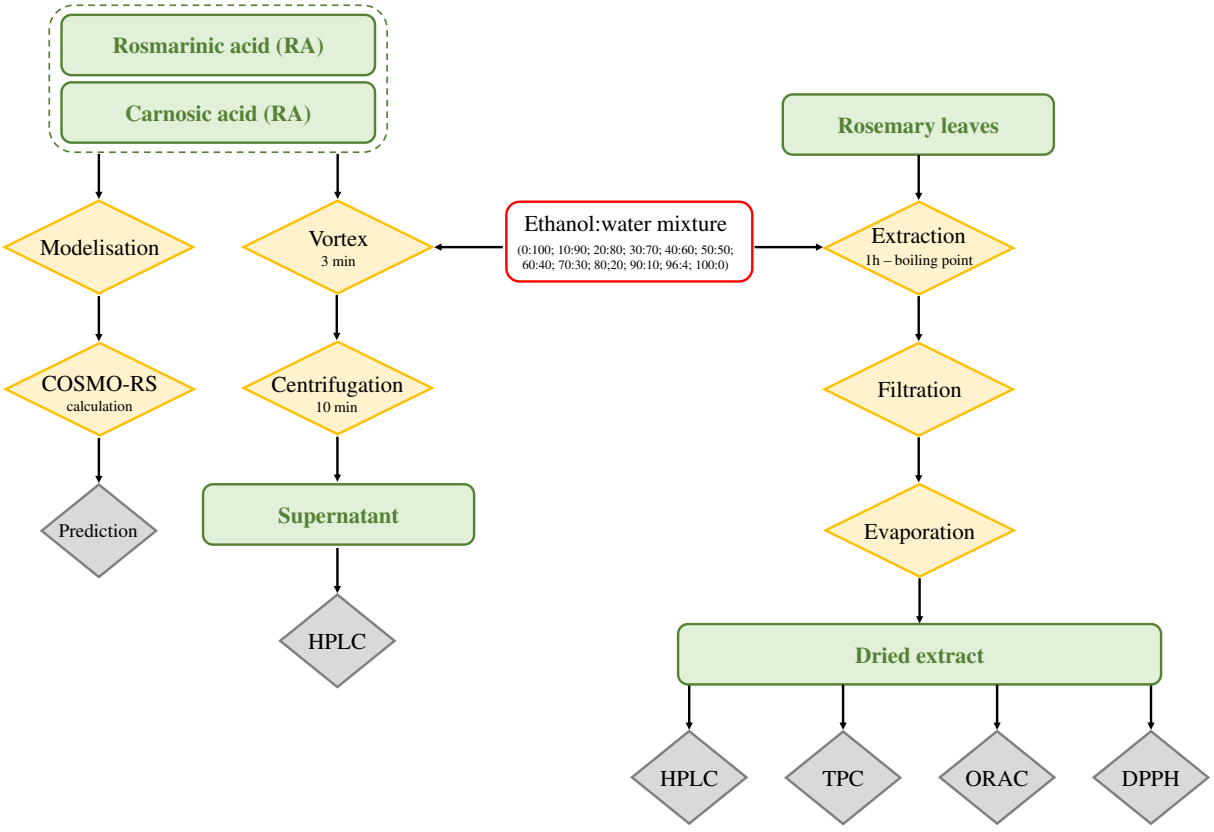


Figure IV-1. Assessment of processes and analysis performed in the study.

IV.2 MATERIALS AND METHODS

Rosemary (*Rosmarinus officinalis* L.) was provided by Naturex. The batches used were collected in Morocco in 2012 and 2015. Only leaves were used in this study. Initial moisture was 8.33 ± 0.01 % and initial content of rosemary in RA and CA was 0.91 % and 2.4 % respectively.

Various ethanol:water mixtures were evaluated as extraction solvent to obtain RA and CA from rosemary. In all cases, extraction was performed under reflux for 60 min. For a better understanding of extraction and solubilization phenomenon, the COSMO-RS software was used to predict theoretical solubilities of RA and CA in the various solvents. They were compared afterwards with experiment. Procedures are described in detail in **CHAPTER II**.

IV.3 RESULTS AND DISCUSSION

IV.3.1 Solubility of targeted compounds in ethanol:water mixtures

When it comes to the extraction process of specific compounds from a plant material, the solvent choice is crucial. It is current belief that a solvent which solubilizes a compound would be a good phase for its extraction. Hence, a coarse screen to sift good extraction solvents from thousands of possibilities is usually to consider the following concepts:

(i) "like dissolves like" is a general principle (i.e. two molecules or solvents having similar polarity are soluble or miscible)

(ii) solubility is higher when the temperature of the system increases;

(iii) the higher the molecular weight of a solute, the lower its solubility in a given solvent;

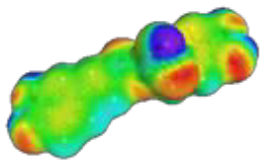
(iv) extraction is predominantly determined by solubility phenomena

Today, prediction tools are available to evaluate the solubilizing capacities of several solvents. For example, COSMO-RS software is increasingly used in the field of extraction [68,224,225] since it enables to classify solvents against each other on the basis of how well they can solubilize a compound of interest. The predictions can be confirmed afterwards by experiments.

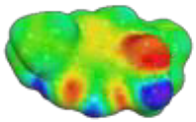
IV.3.1.1 Theoretical solubilities: COSMO-RS simulations

Solvent selection is usually based on polarity-based solubility parameters between targeted compounds and solvents. In contrast to standard quantum chemistry which considers molecules in vacuum, COSMO-RS calculations are done in a virtual conductor embedding the molecules. Calculations provide the conductor-polarization charge density σ on the surface of the molecules, which turns out to be a good descriptor of the local surface polarity. The σ-surface—that is the molecular surface color-coded by σ—and the σ-profile—that is the histograms of the molecular surface with respect to the surface polarity σ—describe similarities and differences between RA, CA, ethanol, and water. From these data, relative solubilities of RA and CA in several ethanol:water mixtures were determined. Predicted values were compared with experimental data obtained in the previous part. Note that the same trend was observed with COSMO-RS predictions when boiling points of the mixtures

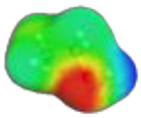
were considered for calculation. Regarding the pH of the extraction medium (~ 6-7), RA and CA are mostly under their deprotonated form (i.e. conjugated bases), since their pKa in pure water is 2.9 and 4.0, respectively (ACD-Lab data). However, for sake of convenience and because we do not have access to their pKa in all mixtures, we only considered the protonated form. In the previous work of Filly et al. [70], this aspect was not developed since the solutes were aromas not encompassing any carboxylic acid. Schröder et al. predicted the aqueous solubilities of carboxylic acids with COSMO-RS neglecting the effect of COOH dissociation [226]. In our case, RA and CA were considered as solid solutes and the relative solubilities of each compound in ethanol:water mixtures were determined (ratios were transformed from vol:vol to mol:mol). The best solubilizing solvent was found to be 100 % ethanol in all cases **(Figure IV-2)**.



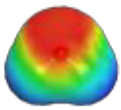
Rosmarinic acid (RA)



Carnosic acid (CA)



Water



Ethanol

% EtOH	Temperature	Rosmarinic acid	Carnosic acid
(v/v)	*°C*	*log10(x_RS)*	*log10(x_RS)*
0	100	-4.91	-5.18
10	93.5	-4.08	-4.29
20	88.5	-3.27	-3.42
30	85.5	-2.56	-2.67
40	83.8	-2.06	-2.13
50	82.5	-1.57	-1.60
60	81.5	-1.18	-1.19
70	80.6	-0.84	-0.84
80	79.5	-0.53	-0.52
90	78.5	-0.23	-0.22
96	78.3	-0.090	-0.087
100	78.3	0.0	0.0

Figure IV-2. COSMO-RS prediction regarding the solubilization of RA and CA into ethanol:water mixtures.

IV.3.1.2 Experimental solubilities

Experiments were carried out to determine RA and CA's solubilities in various ethanol:water mixtures **(Figure IV-3)**. They were performed at ambient temperature for convenience. In both cases, the solubility tends to increase with the ratio of ethanol in the solvent. Interestingly, experimental solubilities appears to be well correlated with the theoretical values calculated by COSMO-RS, even though CA and RA were implemented as non-deprotonated acids. For CA, solubility dramatically raised from 0.1 g/L (0-20 %) to 423.4 g/L (100 % ethanol). For RA, solubility increases from 17.7 g/L (0 % ethanol) to 472.2 g/L (90 % ethanol), with a further decrease to 392.7 g/L in pure ethanol. Based on these results, 90-100 % ethanol was selected as the best solvent composition to solubilize simultaneously RA and CA **(Figure IV-3)**. Hypothesizing that *the extraction process is predominantly determined by solubility phenomena*, we investigated whether or not pure ethanol is the best extraction solvent for these two antioxidants.

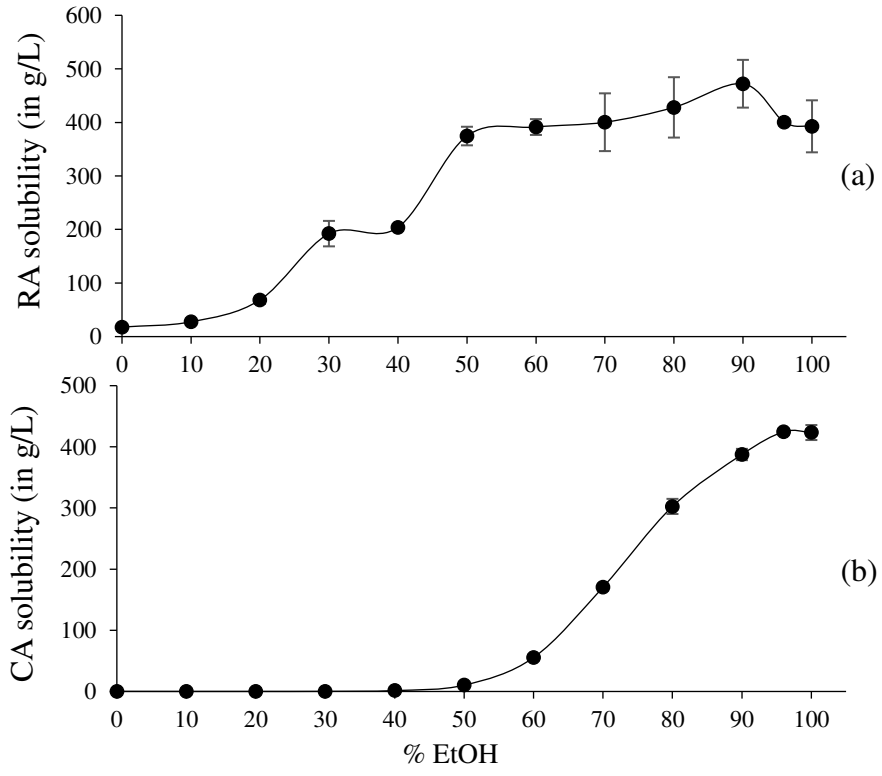


Figure IV-3. Experimental solubilities of RA (a) and CA (b) in various ethanol:water mixtures.

IV.3.2 Extraction of targeted compounds in ethanol:water mixtures

From an industrial standpoint, the determining responses to assess the performance of extraction are the quantity of extractives (corresponding to the mass extraction yield) and the recovery rate of active compounds. To determine which ratio must be selected for an optimal extraction, these two responses were assessed according to the ethanol ratio in the extraction solvent.

IV.3.2.1 Extraction yield and recovery rate

Figure 4a shows the influence of ethanol:water ratio on the mass extraction yield. From 0 to 60 % ethanol, extraction yield slightly rose from 19 to 28 % which is the highest value. Rising ethanol proportion from 60 to 80 % did not have any significant impact since results are quite similar. However, beyond 80 %, increasing ethanol level was accompanied by a decrease of extraction yield as values dropped from 26 to 15 % **(Figure IV-4 (a))**.

Specific results regarding RA and CA extraction are presented in **Figure IV-4 (b)** and **Figure IV-4 (c)**. The proportion of the molecule in the extract (purity in g molecule/100 g extract) and the extracted amount from rosemary leaves (molecule content in g molecule/100 g leaves) are reported as a function of the ethanol:water ratio of the extraction solvent. Selectivity of extraction partially depends on the intermolecular interactions taking place between solvent molecules and compounds to extract, which in turn, depends upon the polarity of both molecular species. Accordingly, different profiles in RA and CA can be obtained depending on the ratio **(Figure IV-4 (b) and Figure IV-4 (c))**.

When results are expressed as g molecule/100 g extract, it appeared that purity of RA slightly rose from 0 to 30 % ethanol. Beyond this threshold (30 %), a decrease of RA content is observed with a dramatic collapse after 80 % ethanol. Therefore, increasing the proportion of ethanol beyond 30 % contributes to a severe decrease of selectivity for RA **(Figure IV-4 (b))**. In contrast, selectivity of extraction for CA purity increased when ethanol proportion increased **(Figure IV-4 (c))**, which can be tentatively ascribed to the good solubility of CA in ethanol. It is also worth noting, as shown in **Table IV-1**, that the more the ethanol content in the hydro-alcoholic mixture, the more stable is CA. Thus, the increase in CA purity with increasing ethanol seems to be due not only to a better solubility but also to a better stability in ethanol than water.

The purities of RA and CA **(Figure IV-4 (b) and Figure IV-4 (c))** were combined with the mass extraction yields **(Figure IV-4 (a))** to determine the quantities recovered from rosemary leaves **(Figure IV-4 (b) and Figure IV-4 (c))**. 30 % ethanol appeared as the best extraction solvent to recover RA. The highest yield for CA was obtained with 70 % ethanol (~2.2 g CA/100 g leaves) even though quite similar yields were found with 50 and 60 %. Importantly, actives recovery from dried leaves for RA and CA were 100 % (at 30 % ethanol) and 91 % (at 70 % ethanol), respectively. Therefore, considering global extraction yields, a ratio ranging from 50 % ethanol to 80 % ethanol gives highest mass extraction yields (26.3-27.7 %) and total content in targeted compounds (3.0-3.1 g/100 g leaves).

	Degradation rate (%)		
	78 °C	82.5 °C	100 °C
100 % water	45.6 ± 12.2	70.1 ± 6.4	97.8 ± 3.1
50 % ethanol	2.3 ± 0.3	0.5 ± 0.1	/
100 % ethanol	0.2 ± 0.3	/	/

Table IV-1. Degradation rate of carnosic acid according to the solvent and the temperature.

IV.3.2.2 Is plant extraction all about solute solubility in the solvent?

It stems from the above, that the best solubilizing phase (100 % ethanol) is not the best extraction solvent, especially when we look at the RA purity. This results suggests that solubilization is not the predominant process that drives the extraction and that other important phenomena must be taken into consideration. A closer look at the definition of "*plant extraction*" brings a sequential molecular process roughly divided in three steps:

- diffusion of the solvent through the plant material core
- desorption of the targeted compound from the plant matrix due to chemical affinity with the solvent
- and mass transfer of the solute from the plant vicinity to the solvent bulk.

We should first mention that the solubility of the solute in the solvent does not impact the diffusion of this latter through the plant matrix. Consequently, it is not surprising that solubility–either given by an experimental protocol or by COSMO-RS calculations–is a poor descriptor of the overall extraction performances. Besides the solvent capacity to solubilize a

solute, the mass transport phenomena determined by the complex interactions occurring between the plant material and the solvent, and between the plant material and the molecule to extract, must be considered when extraction is performed. In other words, if the "*like dissolves like*" principle is experimentally verified by comparing COSMO-RS and experimental solubilities, it cannot be extrapolated to any "*like extracts like"* principle.

Another parameters which must be taken into account to explain the difference between solubilization and extraction is the temperature. Indeed, both theoretical (COSMO-RS) and experimental solubility values were determined at ambient temperature, whereas extractions were performed at boiling point.

Regarding selectivity of extraction (corresponding to the purity and expressed in g molecule/100 g extract), the result for CA is in accordance with predicted and experimental data (100 % ethanol in all three cases) **(Figure IV-4 (c))**. CA is suspected to be mainly located in the trichromes of rosemary leaves [227,228], suggesting that the molecule is easily accessible for extraction with 100 % ethanol in which it is most soluble. In contrast, for RA, the theoretical and experimental solubilities (100 % ethanol) were not correlated with purity values (30 % ethanol) **(Figure IV-4 (c))** which can be tentatively explained by the histolocalisation of RA into the rosemary leaves. Like most of polyphenols, RA mainly accumulates in the vacuole of plant cells [229,230]. Crossing several cell compartments such as walls and membranes to get to the vacuole may be particularly difficult for pure ethanol compared to water:ethanol mixtures. Unlike these latters, ethanol may indeed denaturate proteins that will eventually form a physical barrier hindering the solvent access to vacuolar polyphenols [77].

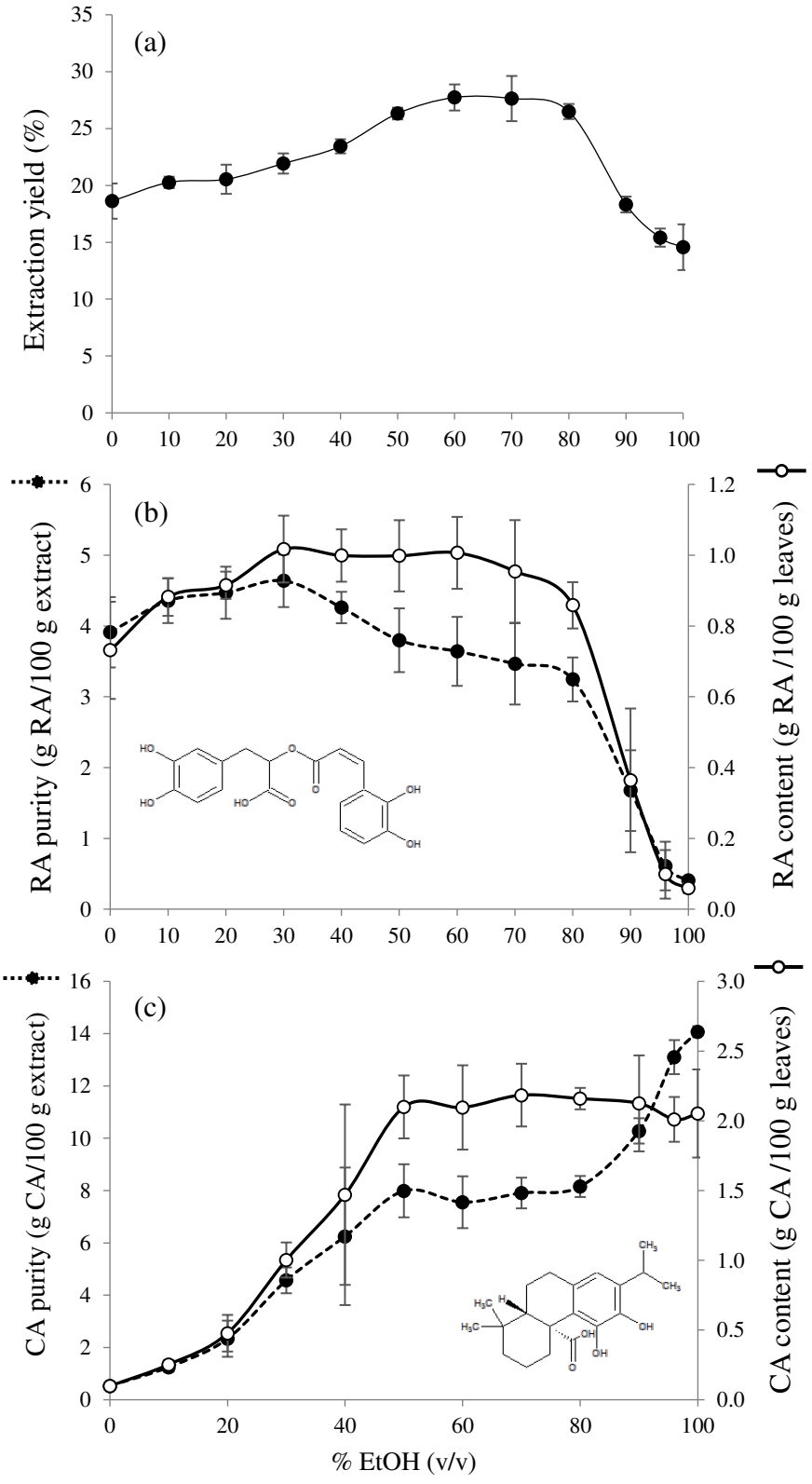


Figure IV-4. Total yields of targeted compounds from rosemary leaves as a function of the ethanol:water ratio of the extraction solvent: Extraction yield (expressed in %) (a); RA content expressed in g RA/100 g extract and g RA/100 g leaves (b); CA content expressed in g CA/100 g extract and g CA/100 g leaves (c).

IV.3.3 Antioxidant activity of rosemary extracts obtained using various ethanol:water mixtures

This work aims at determining not only the impact of the ethanol content on CA and RA extraction, but also on activities of the final extracts to get the highest reducing, radical scavenging and antioxidant properties. Specific activities of RA and CA were also evaluated. "CA-like compounds" are introduced here because CA is a thermosensitive molecule readily transformable under high temperature into CO and other CA derivatives [210,231,232] which still have antioxidant properties provided by the catechol group (*ortho*-diphenol) which is not affected.

IV.3.3.1 Determination of the total phenolic content (TPC) through the reducing activity

TPC was determined in the extracts using Folin-Ciocalteu method. Calibration curves **(Figure IV-5 (a), insert)** show that RA is 2.8 fold more reducing than CA. This can be partially explained by the fact that RA **(Figure IV-4 (b))** encompasses two catechol groups exhibiting high reducing activity, whereas CA only bears one **(Figure IV-4 (c))**. From a reactivity standpoint, RA should exhibit higher reducing activity than CA. It could also be supposed that CA is poorly soluble in the test system since the reduction of Folin-Ciocalteu reagent takes place in water. Despite CA standard was prepared in ethanolic stock solution, once in the water, it is not necessarily well-solubilized. It is possible that a significant part of CA (the insoluble part) self-aggregates to form association colloids in water, hindering its homogeneous distribution throughout the system, hence preventing an optimal reduction of the reagent, contrary to RA for which the aqueous phase is optimal. The solubility issues of antioxidants has already been mentioned by Laguerre *et al.* who postulated in a series of articles that the physical chemical environment of a molecule significantly modulates its reducing potential [233,234].

Figure IV-5 (a) shows that the highest TPC was obtained for 30 % ethanol. This ratio is different from the ones which were selected considering solubility (100 % ethanol) and extraction yields (50-80 % ethanol). However, as shown in Figure 4b, the highest yield for RA purity in the extract was obtained for the same ethanol:water ratio (30 % ethanol). Since RA is a better reducer than CA, it is logical to consider that TPC evolution parallels the RA amount into the extract. This can be seen when comparing the trends of RA purity **(Figure IV-4 (b))** and TPC **(Figure IV-5 (a))** as a function of the ethanol:water ratio in the extraction

solvent. It should also be kept in mind that RA and CA are present in rosemary extracts along with other reducers, mostly polyphenols. Consequently, a significant part of the reducing activity of the extracts is contributed by compounds other than CA and RA which demonstrates the interest to use plant extracts with complex composition instead of simple mix of synthetic antioxidants.

IV.3.3.2 Hydrophilic ORAC assay

Figure IV-5 (b) presents the ORAC values as a function of the percentage of ethanol. Antioxidant activity of pure RA and CA were evaluated using hydro-ORAC protocol. RA appears to be 6.6 times better than CA as antioxidant **(Figure IV-5 (b), insert)** which can be due to the hydrophilic nature of the system wherein reactions take place. Indeed, RA is much more soluble (hence available) than CA to react and slow down the AAPH-mediated oxidation of fluorescein. It can be noticed that the ORAC system is not a partitioned one, therefore the two main influent parameters which must be considered are the *reactivity* and the *solubility* of the active molecule. The reactivity can be directly linked to the number of catechol, as well as its electron delocalization area, its phenolic ring freedom of rotation, and its steric hindrance. Mesomeric effects of electron-withdrawing groups such as carboxylic acid in RA (but not in CA) should also be taken into account.

Regarding ORAC assay, a plateau appeared between 0-40 % ethanol which corresponds to the optimal range of solvent to recover extracts with high ORAC values. The plateau also corresponds to extracts with high RA content **(Figure IV-4 (b))**, which could highlight a correlation between RA content in extracts and ORAC value. The same has been found by Walch *et al*. who suggested a correlation between ORAC and RA and its derivatives [235]. Beyond 40 % ethanol, the antioxidant activity decreased by almost 55 % with an increase of ethanol proportion.

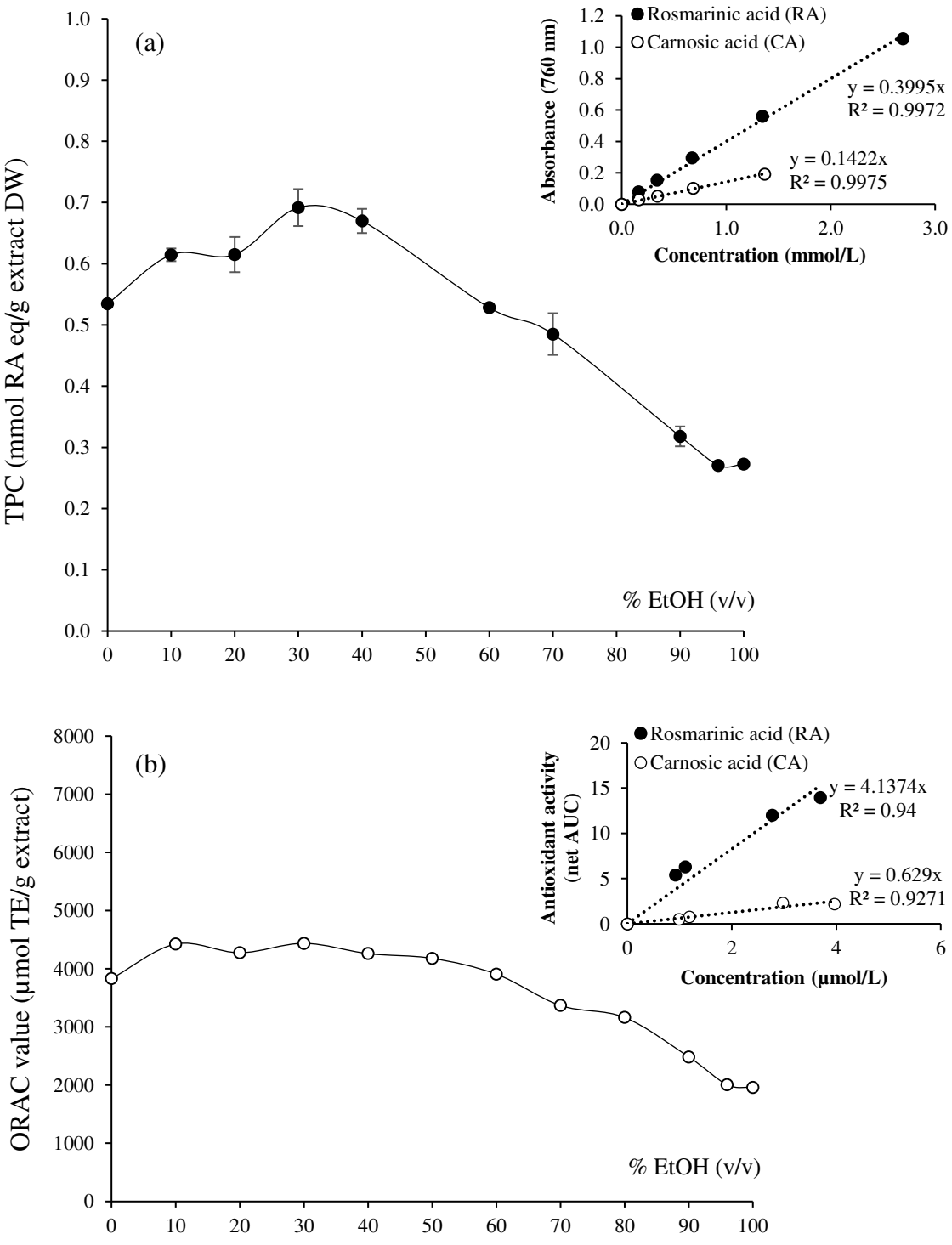


Figure IV-5. Total Phenolic Compounds in the extracts (expressed in mmol RA eq/g extract dry weight) as a function of the percentage of ethanol in the extraction solvent (a); Antioxidant activity (ORAC value expressed in µmol Trolox equivalent (TE)/g extract dry weight) of the extracts as a function of the percentage of ethanol in the extraction solvent (b).

IV.3.3.3 DPPH scavenging activity

Focusing our attention on RA and CA, the former appears as 2.4 times better than CA as DPPH scavenger **(Figure IV-6 (a), insert)**: IC50 of RA is 113 µM (0.041 g/L), while that of CA is 270 µM (0.090 g/L). Surprisingly, this is in contradiction with several reports in literature. For example, Erkan *et al*. found that CA was 2.2 times better than RA as DPPH free radical scavenger [190]. Others, such as Luis *et al.* showed that RA and CA have similar activity [236,237]. If the reason for such discrepancies is not yet clear for us, it is worth noting that our results can be well explained from a molecular standpoint. Indeed, as previously mentioned, RA encompasses two catechol groups, while CA has only one. The presence of a catechol is a key factor for antioxidant activity, in agreement with the low bond dissociation energy (homolytic breakdown) of the corresponding OH groups [238,239] which is due to the phenoxy radical (AO•) stabilization through the formation of an intramolecular hydrogen bond between the two phenolic hydroxyls in *ortho* position. In contrast, *meta* or *para*-dihydroxy substitutions would not favour the establishment of such a bond. Apart from the number of catechols, RA has another structural trait that makes it a better reducer than CA in polar and noncompartmentalized system such as DPPH in methanol, that is its larger electron delocalization area (throughout the caffeoyl moiety of RA). Mention should also be made that the phenolic hydroxyls of CA are sterically hindered by the vicinal isopropyl and the carboxylic acid as well. Finally, unlike for RA, the phenolic ring of CA cannot rotate freely with regard to the remaining part of the molecule, which should theoretically hinder the contact between DPPH and CA. Noteworthy, both molecules are readily soluble in methanol, so, the DPPH method does not seem to discriminate them on the basis of their solubility and mass transfer.

As shown in **Figure IV-6 (a)**, the global trend of DPPH scavenging activity of extracts decreases stepwise with the increase of ethanol proportion (decrease by almost 55 % was observed). A plateau appeared between 0 and 30 % ethanol, which is the optimal range to endow the corresponding extract with the highest DPPH scavenging activity. Similarly to TPC and ORAC, DPPH profile shares the same evolution that RA content in the extract. The several extracts were deposited on a TLC plate and after development step, the plate was immerged into a DPPH solution to show visually whether other compounds than RA and CA would contribute to the radical scavenging activity. A photo of the plate is presented in **Figure IV-6 (b)**. The decrease of ethanol ratio in extraction solvent leads to an increase of

other compounds contribution to the global activity (data not shown), particularly with the appearance of plots corresponding to other compounds than RA and CA.

Ethanol ranging from 10-30 % must be selected to maximize TPC, DPPH and ORAC values. This range of solvent differs from the optimal ratios found for RA and CA's solubility (100 % ethanol) and for the global extraction yields (50-80 % ethanol). It is obvious, here, that the results are influenced by the nature of the tests carried out: all of them take place in polar systems (aqueous or methanol) where polar compounds like RA are favored over less polar molecules like CA to react since they are more soluble, hence more available for oxidants.

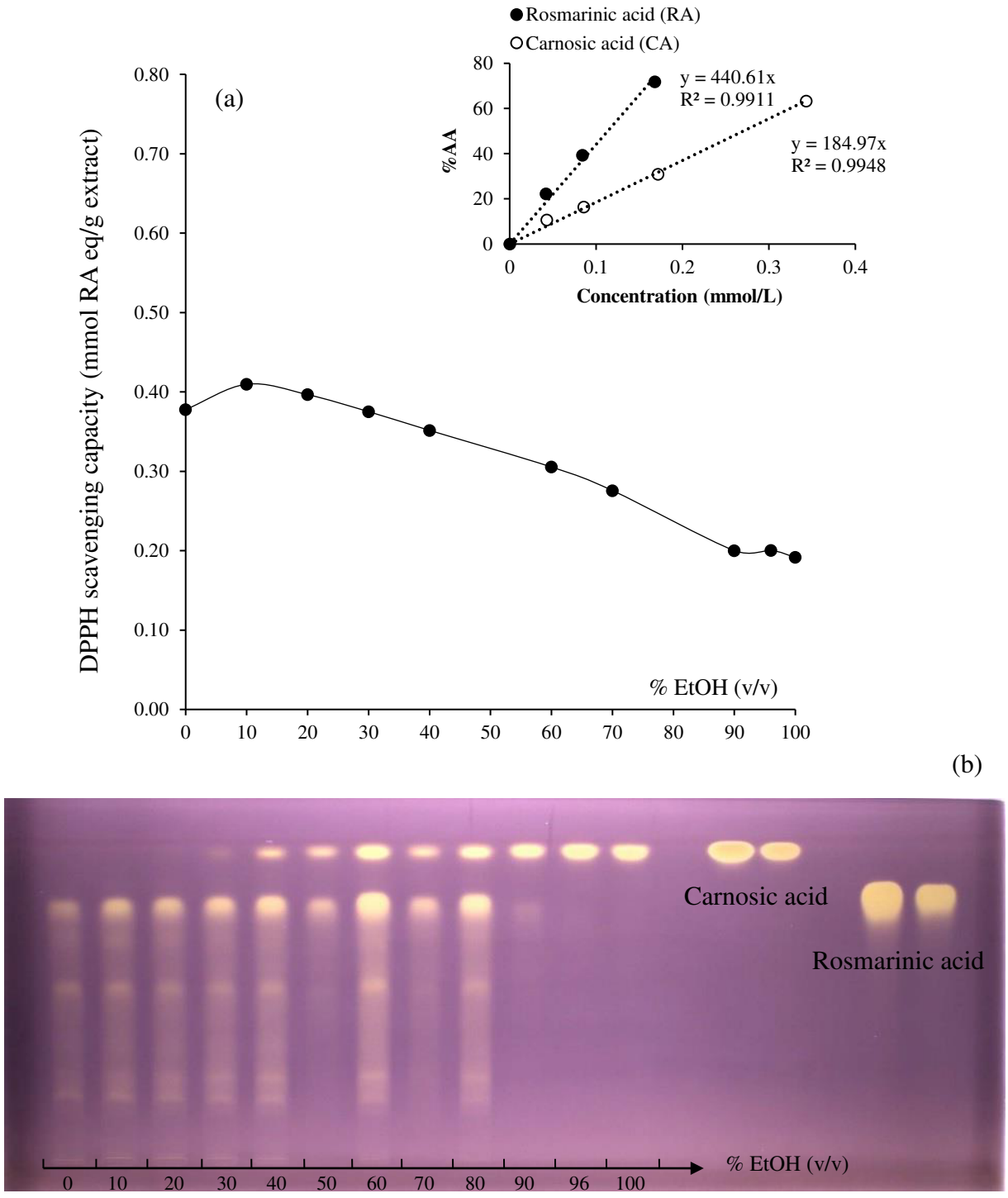


Figure IV-6. DPPH scavenging activity (expressed in mmol RA eq/g extract) of the extracts as a function of the percentage of ethanol in the extraction solvent (a); HPTLC profiles of the extracts after immersion into DPPH as a function of the percentage of ethanol in the extraction solvent (b).

IV.3.4 Contribution of RA and CA to extract activity

This section aims at a better understanding of extract activity. It was noticed in the previous part that the activity of extracts and more particularly the free radical scavenging activity could be ascribed to other molecules than RA and CA-like compounds **(Figure IV-6 (b))**. In this context, it would be interesting to know the respective contributions of RA and CA on global activity of the extract. Therefore they were evaluated regarding TPC, DPPH and ORAC assays.

Although it was demonstrated that RA and TPC profiles share the same evolution, RA has only a modest impact; its contribution to the TPC response accounts for less than 5-20 % **(Figure IV-7 (a))**. Until 40 % ethanol, the weight of RA on TPC response was much higher than the weight of CA-like compounds. The respective impacts were similar from 50 to 80 % ethanol. Beyond this threshold (80 %), the weight of CA-like compounds was much higher, since the study previously showed that the corresponding extracts were very low in RA and high in CA, and also because the overall TPC collapsed. The total weight from RA and CA-like compounds on TPC was kept constant from 0 to 40 % ethanol (~ 20-30 %), then increased beyond 40 % ethanol until pure ethanol (~ 45-65 %). From 0 to 40 % ethanol, two hypotheses can be put forward: *(i)* either, most of the reducing capacity of the extract toward Folin-Ciocalteu reagent is to be ascribed to molecules other than RA and CA-like compounds, *(ii)* or a strong synergistic action took place between CA and RA that boost their reducing activity from ~ 20-40 % of the TPC response (when isolated) to higher value % (when combined). Despite the observation of **Figure IV-6 (b)** suggests that the first hypothesis is more plausible, the second hypothesis was tested experimentally to detect any synergic effect between RA and CA standards in their reducing capacity toward Folin-Ciocalteu reagent. Each standard was diluted independently at different concentrations: 0.06, 0.125, 0.25 and 0.5 g/L. Solutions were then prepared with a mix of the two standards at the same concentrations: the first solution with 0.06 g/L of RA and CA each, the second with 0.125 g/L of RA and CA each, and so on. As shown in **Figure IV-7 (b)**, no synergistic effect was observed between the two molecules: when mixed in solution, the total absorbance corresponds to the addition of the absorbances of each molecule taken separately. To the best of our knowledge, there are only two reports of a synergistic effect involving RA. Remarkably, in both studies, the investigated system was a lipid dispersion. Peyrat-Maillard *et al.* indeed showed synergistic combination between RA and quercetin, and between RA and caffeic acid, in aqueous dispersion of linoleic acid.[240] More recently, it was found in

oil-in-water emulsion that RA exerted a strong synergistic effect when combined to α-tocopherol.[241] Interestingly, alkyl esters of RA produced additive and/or antagonistic effects with α-tocopherol in the same system, suggesting that the compartmentation of RA in the aqueous phase and that of α-tocopherol in the oily phase was required for a synergy to occur. In the present study, the TPC was evaluated in a non-compartmentalized system, which could explain why no synergy was observed between a water-soluble antioxidant such as RA and an oil-soluble antioxidant such as CA. Regardless of the mechanism of action, it is thus likely that in our case, reducers other than RA and CA-like compound impacts most of the overall reducing capacity of the extracts toward Folin-Ciocalteu reagent. Therefore, natural extracts of rosemary leaves exhibit much higher reducing activity than the one theoretically inferred from the individual effect of pure standards of CA and RA demonstrating the interest to use plant extracts with complex composition instead of simple mix of synthetic antioxidants.

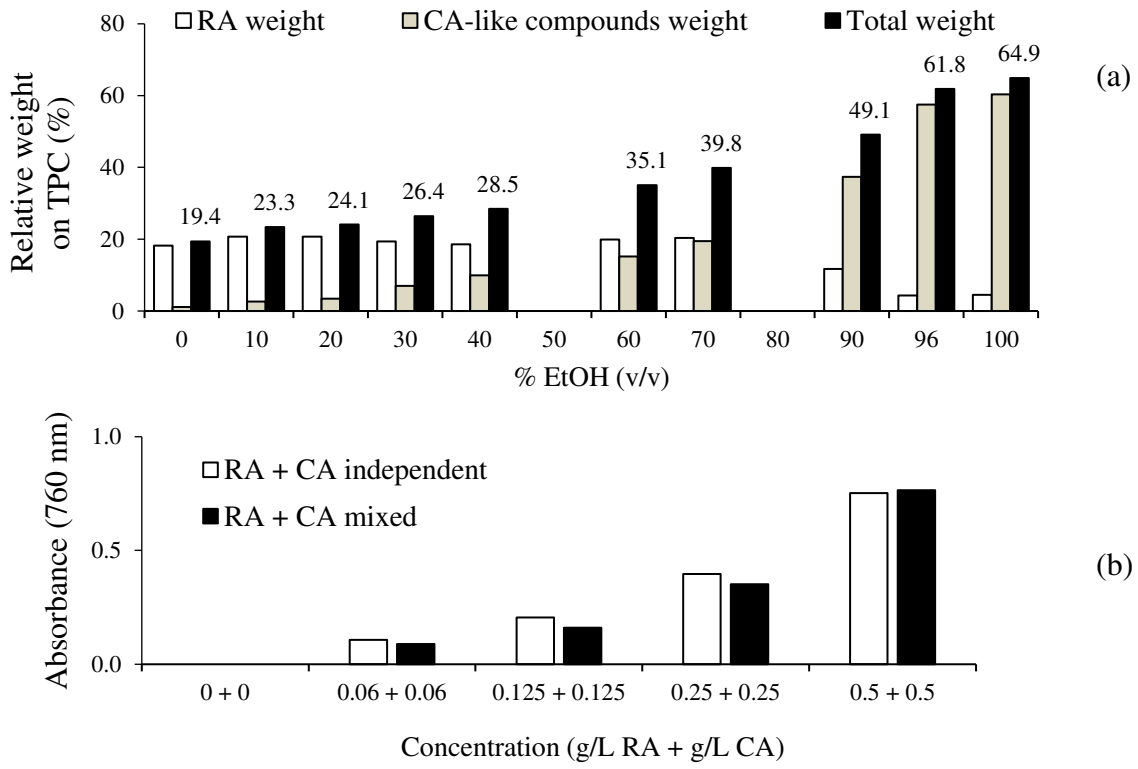


Figure IV-7. Relative weight of RA and CA on Total Phenolic Compounds (expressed in %) (a); Assessment of the combination effect of RA and CA in reducing capacity (b).

As for Folin-Ciocalteu method, the impact of RA and CA on the ORAC value of all extracts was calculated **(Figure IV-8)** and showed that they account for less than 50 % of the global response. Other compound(s) with free radical scavenging activity are thus supposed to exert a significant impact on the antioxidant activity of the extracts.

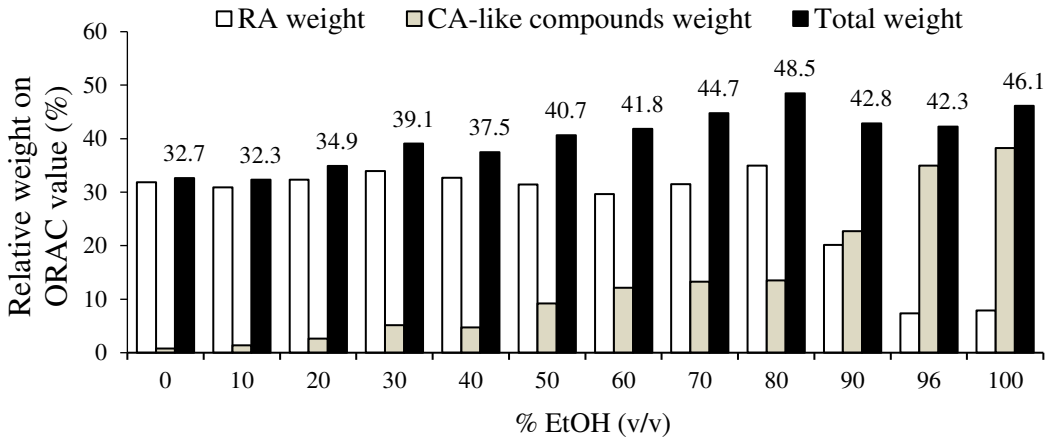


Figure IV-8. Relative weight of RA and CA on ORAC value (expressed in %).

Finally, similarly to TPC and ORAC, DPPH profile shares the same evolution that RA content in the extract, while the impact of RA on the global DPPH scavenging activity accounts for only 25-30 % (from 0 to 70 % ethanol, **Figure IV-9 (a)**) and even less than 5 % beyond 90 % ethanol. Considering CA-like compounds, global contribution on scavenging capacity increased with the proportion of ethanol, topping more than 90 % on total response with 100 % ethanol. This trend follows the amount of CA-like compounds in the extracts. The total weight from RA and CA-like compounds on DPPH scavenging capacity increased from ~ 30 to 98 % with the increase of ethanol proportion from 0 to 100 %. The contribution of other compounds than RA and CA-like compounds suggested by **Figure IV-6** for polar extracts below 40 % ethanol was confirmed since no synergistic effect on the global DPPH scavenging activity was observed between RA and CA **(Figure IV-9 (b))**.

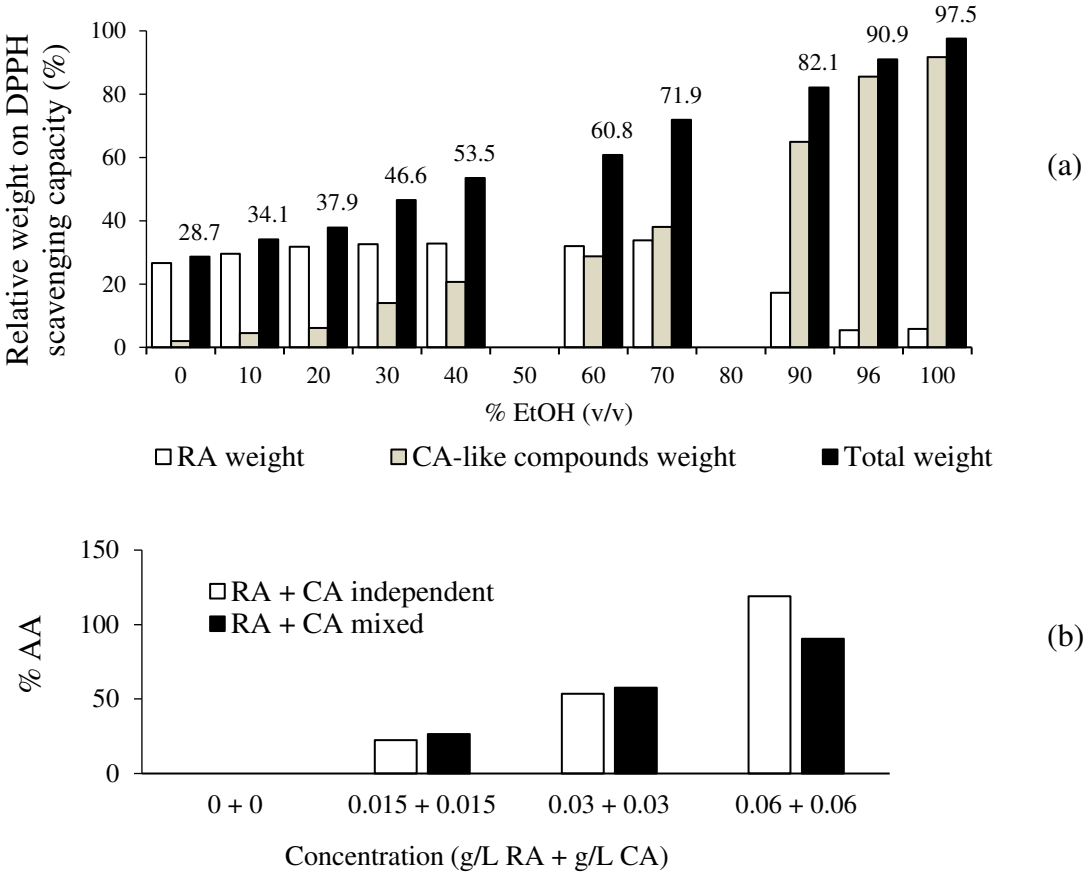
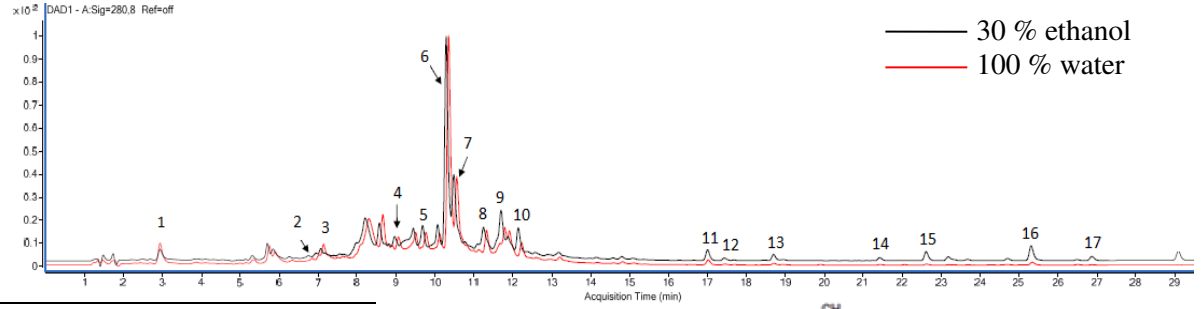


Figure IV-9. Relative weight of RA and CA on DPPH scavenging activity (expressed in %) (a); Assessment of the combination effect of RA and CA in DPPH scavenging response (b).

To investigate which kinds of compounds could contribute to the activity, extracts obtained with 100 % water and 30 % ethanol were analyzed by LC-MS. The identified compounds are presented in **Figure IV-10**. It reveals the presence of the following polar compounds (other than RA): syringic acid, medioresinol, gallocatechin, scutellarin, isorhamnetin-3o-glucoside, luteolin-3'-glucuronide and luteolin 3'-(O-acetyl)-*β*-D-glucuronide (isomers I, II, and III). Expectedly, their content is higher in extracts obtained with 100 % water compared to those obtained with 30 % ethanol (1.5 to 7 times higher). Less polar molecules others than CA such as flavonoid aglycones and phenolic diterpenes were also identified. Due to their better affinity for ethanol than water, their amounts are higher in 30 % ethanol (1.3 to 2.8 times higher). All of these molecules are potentially antioxidant and can contribute to the global activity of the extracts obtained with low amount of ethanol. Further work is thus needed to determine their specific antioxidant activity.



	Identified compounds	[M-H]-
Mainly extracted with 100 % water		
1	Syringic acid	197.0492
2	Medioresinol	387.1718
3	Gallocatechin	305.0754
4	Scutellarin	463.0905
5	Isorhamnetin 3-O-glucoside	477.1011
6	Rosmarinic acid	359.0828
7	Luteolin-3'-O-glucuronide	461.0805
8	Luteolin -3'-(O-acetyl)-b-D-glucuronide isomer I	503.0879
9	Luteolin -3'-(O-acetyl)-b-D-glucuronide isomer II	503.0886
10	Luteolin -3'-(O-acetyl)-b-D-glucuronide isomer III	503.0871
Mainly extracted with 30 % ethanol		
11	Rosmanol	345.1728
12	Epiisorosmanol	345.1739
13	Acacetin	283.0646
14	Epirosmanol	345.1742
15	Carnosol	329.1791
16	Carnosic acid	331.1908
17	12-O-methyl-carnosic acid	345.2114

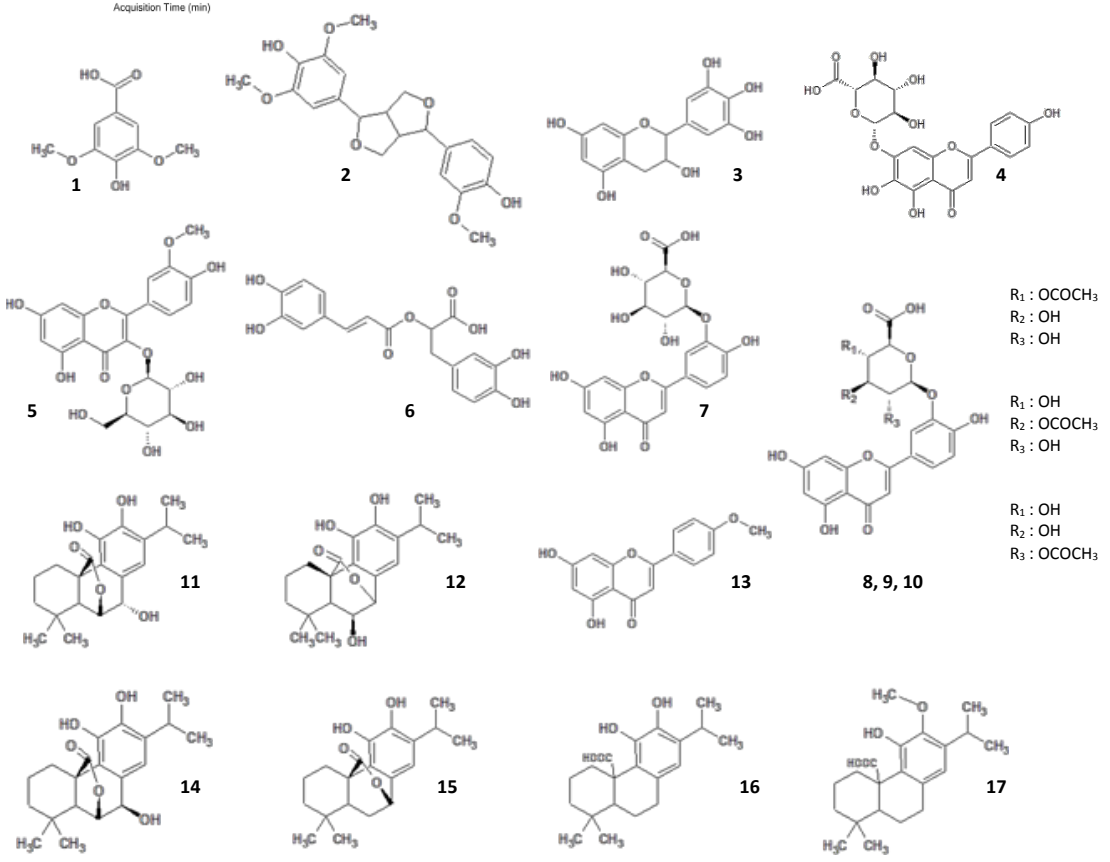


Figure IV-10. LC-DAD profiles of 100 % water and 30 % ethanol extracts and compounds identification by LC-MS.

IV.3.5 Towards a bio-refinery of rosemary

Currently, at industrial level, lipophilic (rich in CA) and hydrophilic (rich in RA) rosemary extracts are obtained using two independent processes. As illustrated in **Figure IV-11**, CA recovery is optimal using acetone as extraction solvent whereas RA is efficiently extracted with 80 % ethanol or 100 % water.

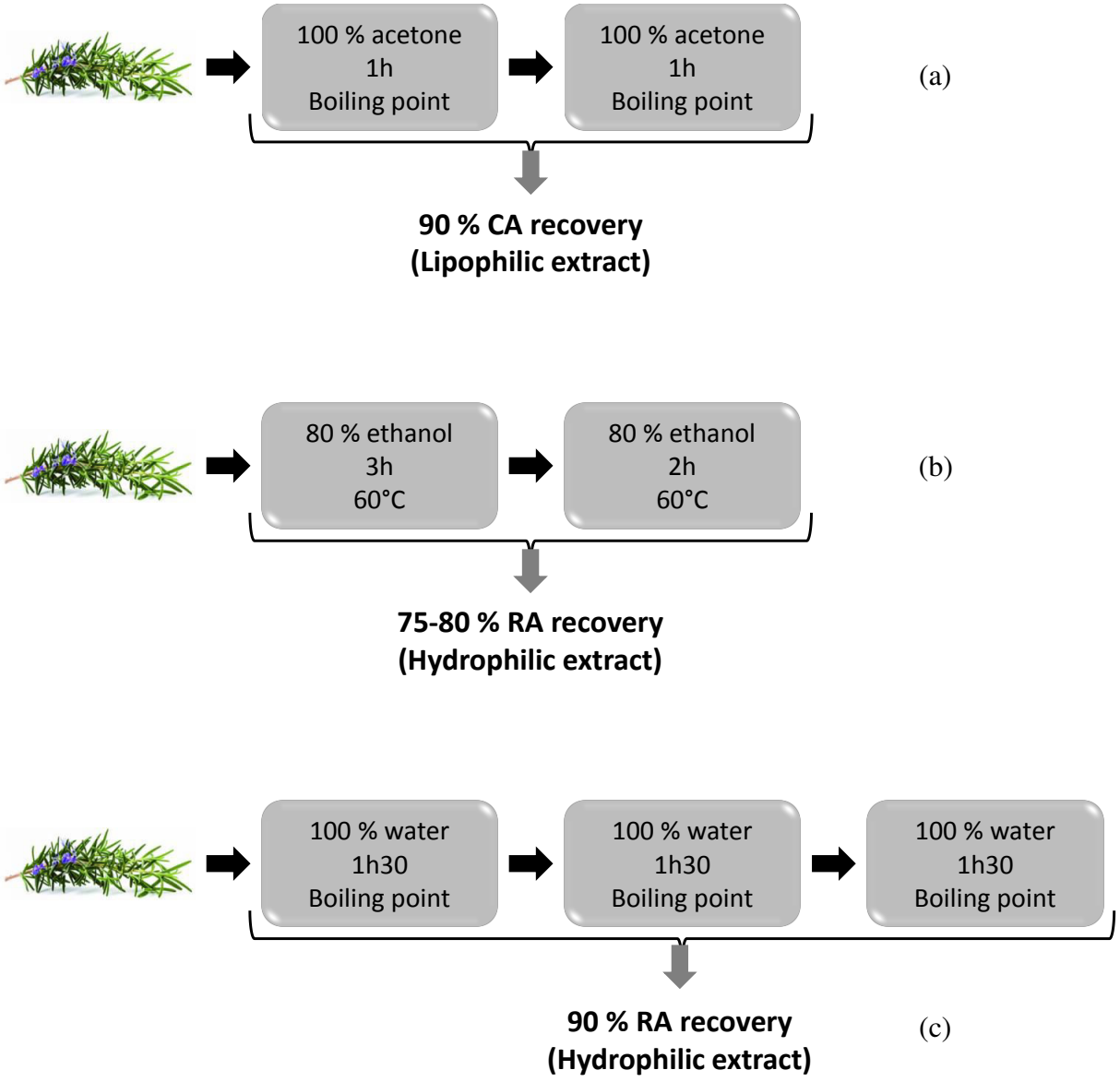


Figure IV-11. Conventional industrial processes to recover CA in acetone (a), RA in 80 % ethanol (b) and RA in water (c).

In previous part, we concluded that CA was better extracted with high amount of ethanol in extraction solvent whereas RA was more efficiently extracted with lower amount (30 % ethanol). Based on this result, we designed a bio-refinery process and proposed to extract consecutively RA and CA from rosemary in one single process. As described in section II.4.3, we decided to perform a first extraction with 92 % ethanol at boiling point during 60 min to recover CA, followed by an extraction with much lower amount of ethanol at boiling point for 60 min to recover RA **(Figure IV-12)**. The main objective is to recover separately maximal amounts of RA and CA from one batch of rosemary and in a single process.

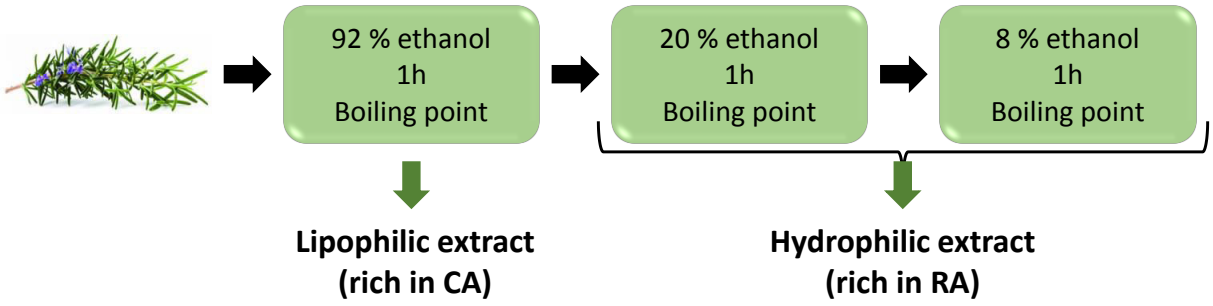


Figure IV-12. Simplified bio-refinery-inspired process for the valorization of RA and CA from rosemary.

IV.3.5.1 Development of bio-refinery process

IV.3.5.1.1 Preliminary assay

For the extraction of CA, 92 % ethanol was selected as solvent since higher content of ethanol (96 % or 99 %) is not up-scalable at industrial level. Actually, current solvent recycling conditions enable to reach maximally 92 % ethanol in the final recycled solvent. Laboratory tests were therefore carried out directly with this ratio of solvent. CA extraction was performed firstly since CA is a thermolabile compound which might be degraded if a first extraction with water is performed. After the first extraction to recover CA, water was added to the rosemary residue, and a second extraction was carried out to recover RA. The results of this first experiment are presented in **Table IV-2**. The percentage of recovery corresponds to the amount of compound extracted regarding the initial amount available in the plant.

The first extraction enables to recover 82.0 ± 6.6 % of available CA in initial rosemary. It is an encouraging result because it corresponds to the amount currently extracted at industrial level with acetone. Regarding RA, the amounts extracted during the first and the second extraction are approximately 20 % and 50 %, respectively, reaching a total recovery of 70 %. From this result, improvement must be performed at two levels:

- Reduction of RA recovery during the first extraction
- Increase of RA recovery during the second extraction

To deal with this problematic, extraction time and temperature were varied and their influence on extraction was assessed.

Process	Experimental parameters	CA recovery (%)	RA recovery (%)
1st extraction	92 % ethanol, 60 min, boiling point	82.0 ± 6.6	21.4 ± 1.0
2nd extraction	20 % ethanol, 60 min, boiling point	Not analyzed	50.3 ± 2.6

Table IV-2. Preliminary results regarding the consecutive recovery of CA and RA from rosemary.

IV.3.5.1.2 Impact of extraction time and temperature

Previous work about RA and CA extraction demonstrated that CA was extracted quickly than RA in the solvent, and that CA can be efficiently extracted at medium-low temperature contrary to RA which needs higher temperature [221]. It suggests that decreasing temperature or duration of the first extraction in our case might decrease RA recovery, without affecting CA extraction efficiency.

Therefore, a first experiment consisted in decreasing the first extraction temperature to 65 °C. Others parameters were kept constant compared to the preliminary test. In a second experiment, to assess the influence of extraction time on RA recovery, the strategy adopted was reducing the first extraction duration to 30 min and increasing the second one to 90 min. Results of both experiments are presented in **Table IV-3** and **Table IV-4**.

As wanted, decreasing temperature and duration of the first extraction caused the decrease of RA recovery from 21.4 % to 5.1 % and 10.6 %, respectively. However, CA recovery was also affected since the yield was decreased from 82 % (in preliminary experiment) to 67.6 % (lower temperature) and 71 % (lower extraction time). CA being the most important compound to valorize, the decrease of extraction temperature and time was abandoned.

Process	Experimental parameters	CA recovery (%)	RA recovery (%)
1st extraction	92 % ethanol, 60 min, 65 °C	67.6	5.1
2nd extraction	20 % ethanol, 60 min, boiling point	Not analyzed	54.0

Table IV-3. Impact of temperature on CA and RA recovery.

Process	Experimental parameters	CA recovery (%)	RA recovery (%)
1st extraction	92 % ethanol, 30 min, boiling point	71	10.6
2nd extraction	20 % ethanol, 90 min, boiling point	Not analyzed	48.2

Table IV-4. Impact of extraction time on CA and RA recovery.

Regarding the second extraction, increasing the duration from 60 min to 90 min did not enhance RA recovery: for both extraction durations, approximately 50 % of available RA was recovered at the end of process. Therefore, we chose to perform two covers for the second extraction, in order to see if RA recovery might be maximized in this way. Results are summarized in **Table IV-5**.

The performance of a second cover enabled to recover 20.5 ± 0.1 % more RA. Therefore, in the global process, 82.0 % of available CA and 71 % of available RA were

recovered separately in two independent extracts. Total amount of RA recovered in the whole process is about 92 %, which means that plant material was almost completely drained at the end of the bio-refinery. The only negative thing concerns the RA extracted with CA during the first extraction. However, it is not excluded that a further purification step performed on the extract permits its recovery and valorization.

Process	Experimental parameters	CA recovery (%)	RA recovery (%)
1st extraction	92 % ethanol, 60 min, boiling point	82.0 ± 6.6	21.4 ± 1.0
2nd extraction 1st cover	20 % ethanol, 60 min, boiling point	Not analyzed	50.3 ± 2.6
2nd extraction 2nd cover	8 % ethanol, 60 min, boiling point	Not analyzed	20.5 ± 0.1

Table IV-5. Optimal recovery of CA and RA from rosemary in three consecutive steps.

IV.3.5.2 Comparison with existing process

The bio-refinery developed in this study is compared with existing industrial processes in **Table IV-6** for the recovery of CA and RA. The bio-refinery appears as an efficient innovative process which is in accordance with the six principles of green extraction presented in **CHAPTER I**:

- Principle 1 (raw material): the quantity of raw material needed to recover both CA and RA is divided by two, since both compounds are extracted in one single process from the same batch of rosemary leaves.
- Principle 2 (solvent): for the extraction of CA, acetone was replaced by 92 % ethanol, which is greener and safer for users and environment.
- Principle 3 (energy): extraction duration was drastically reduced with the bio-refinery (3 h against 6 h 30-7 h with existing process) and consequently it would result in a decrease of energy consumption. Moreover, energy consumption related to spent raw material stripping (removal of residual solvent) was also reduced to almost zero: only one stripping

step is needed with the bio-refinery (and very few amounts of ethanol have to be removed as the solvent is 8 % ethanol) against two for existing process (one to remove acetone and one to remove 80 % ethanol). Solvent removal is often very energy consuming, particularly when the heat capacity C_p and the enthalpy of vaporization ΔH_{vap} are high.

- Principle 4 (by-products): CA extraction process did not generate any green waste at the end of the process as rosemary leaves were reused to obtain RA with a second extraction.
- Principle 5 (process): extraction duration was drastically reduced with the bio-refinery as well as the number of unit operations, particularly with the elimination of rosemary leaves stripping after CA extraction.
- Principle 6 (eco-extract): active recovery with the bio-refinery was quite acceptable with 82 % recovery for CA and 71 % recovery for RA from one single batch of rosemary leaves. CA and RA extracts can be considered as organic since hydro-alcoholic mixtures were used as solvent.

Globally, the challenge of rosemary bio-refinery was met successfully and we achieved to extract selectively CA and RA by varying the amount of ethanol in the solvent. Extraction of both CA and RA was performed in one single process from one single batch of rosemary, what makes the process economically and environmentally efficient.

	EXISTING PROCESSES			**BIO-REFINERY**	
	CA extraction	RA extraction (1)	RA extraction (2)	CA extraction	RA extraction
Quantity of raw material	100 kg	100 kg	100 kg	100 kg	0 kg
Solvent	Acetone	80 % ethanol	100 % water	92 % ethanol	20 % ethanol 8% ethanol
Volume of solvent	2000 L	2000 L	3000 L	1000 L	1000 L
Extraction duration	2 h	5 h	4 h 30	1 h	2 h
Active recovery	90 %	75-80 %	90 %	82 %	71 %
Green waste	> 100 kg	> 100 kg	> 100 kg	0 kg	> 100 kg
Stripping of residual rosemary	Yes	Yes	No	No	Yes (very few amount)

Table IV-6. Comparison between the bio-refinery and existing processes for the recovery of CA and RA from rosemary.

IV.4 CONCLUSION

This study shows how the solvent choice can be crucial for extraction since the solvent composition for which the highest recovery of targeted compounds is obtained does not necessarily gives the highest mass extraction yield nor the most active extract. Therefore, different solvents have to be selected depending on whether the extract performance is prioritized or a high mass extraction yield or a specific content in a targeted compound. Whereas highest mass extraction yield was obtained with 50-80 % ethanol, considering selectivity of extraction, RA was preferentially extracted with ethanol 30 %, and CA with pure ethanol. Furthermore, COSMO-RS predictions and experimental solubilities gave 100 % ethanol as the best solvent to solubilize both RA and CA. If solubility and selectivity were correlated for CA, it was not the case for RA, demonstrating that *extraction is not all about solubilization* and suggesting that the antioxidant histolocalisation strongly influences their extraction rates. Finally, the best performance of extract in terms of reducing, free radical scavenging and antioxidant activities was reached with ethanol 30 %, which is also the best conditions to recover high amounts of RA. At first sight, it would mean that RA content is a prime determinant of the global antioxidant/reducing performance of the extracts, while a more subtle observation reveals that other antioxidants are concomitantly present with RA and positively affect the global response. Taken together, these data point at crude rosemary extracts obtained with 0-30 % ethanol as promising antioxidant candidates for polar applications such as beverages.

In a second part, based on previous results, a bio-refinery concept was developed to extract both RA and CA in a single process. The challenge was achieved since 82.0 % of available CA and 71 % of available RA were recovered separately in two independent extracts from the same raw material. As perspective, it could be envisaged to valorize spent rosemary leaves after the three first extractions for their content in fibers or proteins (provided they are not degraded).

CHAPTER V . VALORIZATION OF BY-PRODUCTS

TOWARDS A "DRY" BIO-REFINERY WITHOUT SOLVENTS OR ADDED WATER USING MICROWAVES AND ULTRASOUND FOR TOTAL VALORIZATION OF FRUITS AND VEGETABLES BY-PRODUCTS

M. Jacotet-Navarro, N. Rombaut, S. Deslis, A.-S. Fabiano-Tixier, F.-X. Pierre, A. Bily, F. Chemat, Towards a "dry" bio-refinery without solvents or added water using microwaves and ultrasound for total valorization of fruit and vegetable by-products, Green Chem. 18 (2016) 3106–3115. doi:10.1039/C5GC02542G.

CONTEXT

Huge amounts of fruits and vegetables press cakes are daily generated by juice processing industry. They are generally considered as waste and their elimination is often complicated, polluting and expensive, particularly when large volumes are implicated. In this context, there are obvious economic and environmental interests to valorize this biomass into valuable products.

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, EO, extract rich in phenolics, and solid residue rich in fibers and phenolic acids were obtained from ginger rhizomes (GR) using DBR without solvent and added water. Finally, the performances of DBR and conventional bio-refinery (CBR) were compared in term of process time, energy consumption, quantity of waste and quantity of solvent.

V.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 [242]. 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 [242,243]. 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 [244] and phenolic compounds [245], orange peels from the orange juice industry for pectin and flavoring products [246,247]. More recently, the concept of bio-refinery of a plant is increasingly investigated for maximal valorization of natural products from a raw material [103,242,248]. 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 [14].

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 (MW) [249,250], ultrasound (US) [251,252], supercritical fluids [139,253], electro-technologies [254,255] or instantaneous controlled decompression DIC [21,256]. Those green extraction processes have proved their efficiency for extraction of natural products [3,205] but more rarely in the case of a bio-refinery [136]. 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 (EO) (1 – 4 %), phenolics

(gingerols and 6-shogaol, 1 – 2 %), and total carbohydrates (60 – 75 %) [257,258]. Ginger, and more specifically rhizomes are variously used as food product or traditional medicine [259]. 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 (MHG followed by UAE) and quality of the corresponding extracts was determined. Ultimately, the performances of bio-refinery using green extraction and conventional extraction were compared.

V.2 MATERIALS AND METHODS

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 DBR pattern is illustrated in **Figure II-6**. As described in the flow sheet, after pressing, GP was firstly submitted to MHG, followed by UAE. To characterize ginger by-products and to assess the performance of DBR, conventional processes (HD and maceration) were performed as reference **(Figure II-6)**. MW and US equipment used in this study are presented in **Figure II-7** and **Figure II-8**. Experimental conditions used for each process are described in **CHAPTER II**.

V.3 RESULTS AND DISCUSSION

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

In the concept of DBR developed in this study (**Figure II-6**), 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. MW extraction of EO using "constituent water" may occur by a mechanism based on the influence of molecules polarity. EO contain organic compounds that strongly absorb MW energy such as oxygenated monoterpenes. MW 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 EO to flow towards the gland layer. Compounds with high and low dipolar moments could be extracted in various proportions by MW extraction. Organic compounds that have a high dipolar moment will interact more vigorously with MW and can be extracted more easily in contrast with aromatic compounds, which have low dipolar moments.

V.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 [172]. Yet, this aspect can be discussed because of the large variety of plant material which has probably not the same behavior regarding MW 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 V-1**). 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 V-2**, during MW heating, (i) temperature into the biomass firstly increases linearly -more or less quickly depending on MW 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 MW.

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 V-1**, 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 min against 20 min 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.

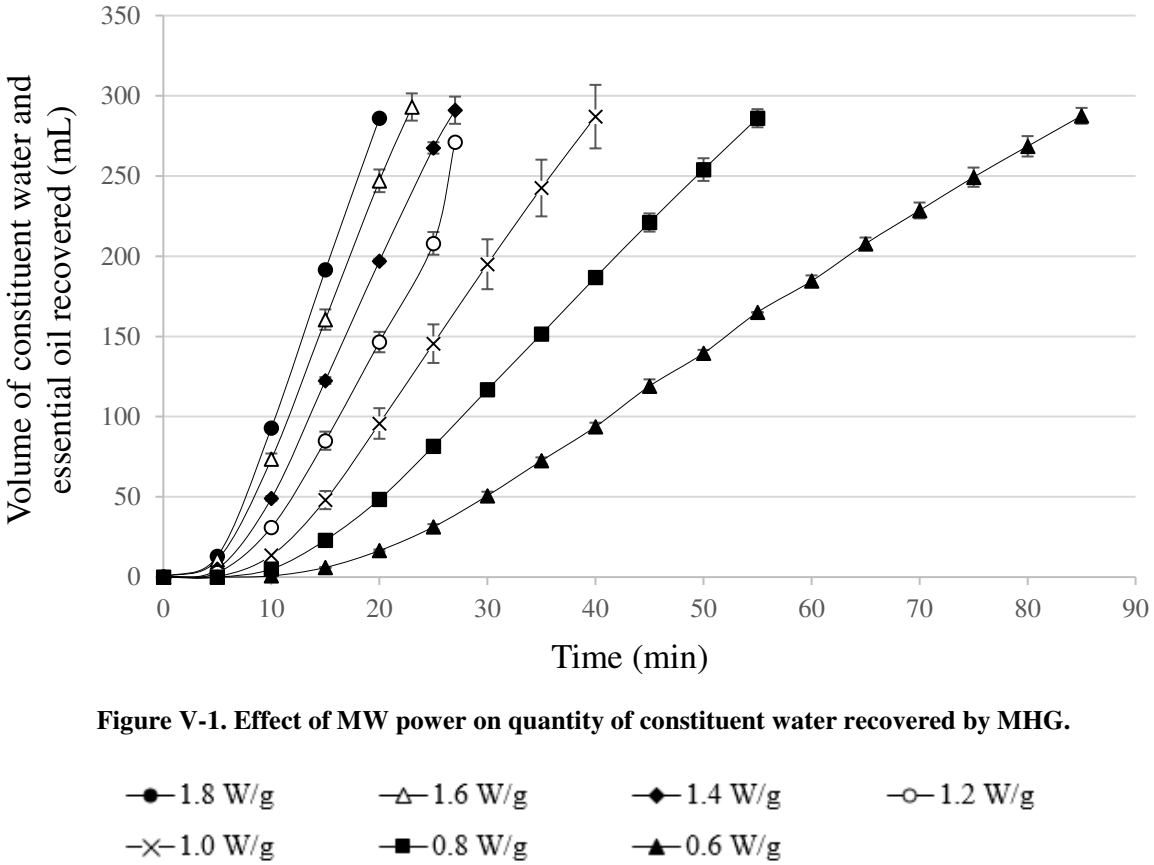


Figure V-1. Effect of MW power on quantity of constituent water recovered by MHG.

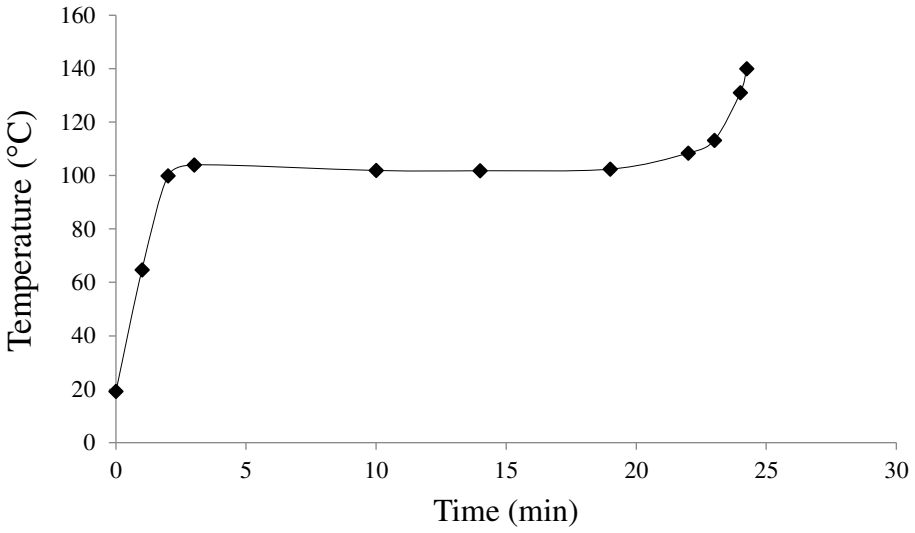


Figure V-2. Evolution of temperature in the matrix submitted to microwaves (1.6 W/g).

V.3.1.2 Evaluation of essential oil quality

Table V-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 V-1**, first and second columns), and EO obtained by MHG treatment of GP at different powers (**Table V-1**, third to ninth columns). First of all, extraction of EO from GP by HD was more efficient than extraction of EO from GR (0.3 g EO/100 g GP and 0.2 g EO/100 g GR). Previous industrial pressing of GR may have caused the de-structuration of rhizomes and therefore may have improved EO availability. It can be noticed as well that MW power had not any effect on EO extraction yields as 0.2 g EO/100 g GP were recovered in all cases. MHG extraction of EO from GP appeared as less efficient than HD of GP, however this result is not very accurate since the design of glassware in the MW laboratory oven provided to recover totally the EO extracted (EO drops remained on reactor walls). To prevent this loss of yield, we could imagine the use of a double layer extractor to limit the phenomenon of condensation on glassware walls.

Considering composition, zingiberene is generally considered as a characteristic compound in ginger EO. In literature, it is mainly found between 20 % and 30 % in EO [260,261]. GC-FID analysis of EO obtained by HD was in accordance with literature as zingiberene content was 25.2 %. In EO obtained by MHG, zingiberene percentage was

constant for powers from 0.8 W/g to 1.6 W/g (medium powers) with a content of 23 to 25 %. However, for extreme powers (0.6 W/g and 1.8 W/g), zingiberene content decreased significantly (both 18.4 %).

The differences of aromatic profiles between EO obtained by the reference process and MHG indicate that the extraction process impacts EO quality. This result has already been shown and explained in previous works. For example, it is reported that the contact between a plant material and the solvent during the process can lead to EO degradation [28]. From our results, it can be concluded that aromatic profiles of EO were similar for MHG extraction conditions except for 0.6 W/g and 1.8 W/g experiments. For these last powers, long extraction time (90 min) and intense MW irradiation (1.8 W/g) respectively could induce a degradation of some compounds in EO [262].

			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

DW: Dry weight

Table V-1. Volatile compounds and antioxidants extracted from ginger plant material.

V.3.1.3 Impact of microwave pretreatment on phenolics extraction efficiency

Constituent water recovered after MHG was analyzed by HPLC to determine its content in gingerols (6-gingerol, 8-gingerol and 10-gingerol) and 6-shogaol. Those compounds are specific phenolics of ginger, 6-shogaol being a degradation product of 6-gingerol by dehydration [76]. They were not detected in the constituent water so we admitted that all phenolics remained into GPMHG. GPMHG obtained after MHG process at different powers and initial GP were characterized as described in section II.4.1.2.2 in order to show a potential effect of MHG treatment on phenolics content, particularly a potential degradation of these compounds. A characteristic HPLC chromatogram of extracts is illustrated in **Figure V-3**. The results are presented in **Table V-1**. It can be noticed that generally, MHG treatment did not cause the degradation of gingerols and 6-shogaol when comparing results obtained for GP and GPMHG. They were even better extracted when MHG treatment was performed (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 *et al.* [172,263], MW seem to alter cell walls of ginger, so gingerols and 6-shogaol were more available in GPMHG than in GR and GP for extraction. It can be underlined that 6-shogaol content in GPMHG was higher than in GP (0.08-0.11 % against 0.02 % respectively), certainly due to high temperature associated to MHG process. However these amounts of 6-shogaol were insignificant compared to contents in gingerols recovered (for GPMHG at 1.6 W/g: 0.08 % of 6-shogaol and 1.37 % of gingerols in plant material). Extraction at power beyond 1 W/g did not involve the degradation of phenolic compounds in GPMHG as described for onion polyphenols in previous work [172].

The previous results enabled to select a MHG power of 1.6 W/g as optimal for the second step of our DBR **(Figure II-6)** since this power enabled to recover total removable water and EO in 20 min, 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.

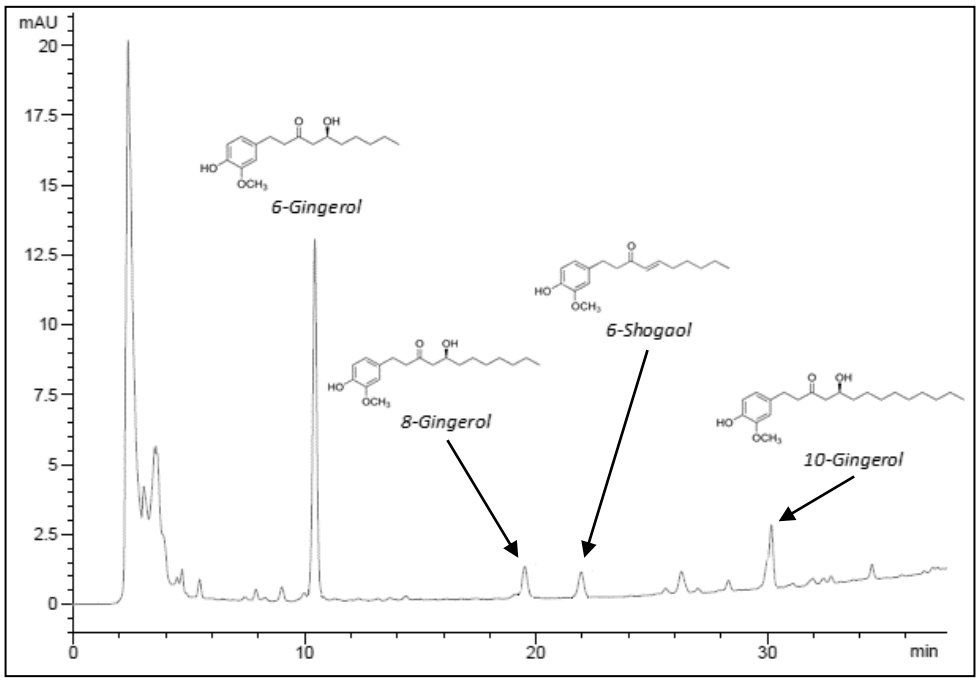


Figure V-3. Specific HPLC-DAD chromatogram of a ginger extract at 282 nm.

V.3.2 Ultrasound assisted extraction of gingerols and 6-shogaol from GPMHG

Phenolic compounds are conventionally extracted from ginger with 75 % ethanol [163]. 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 II-6**). 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. We are aware that is it is not the solvent of choice according to gingerols and 6-shogaol solubility, but it fits perfectly with the concept of bio-refinery developed in the study. Moreover, solubilization performance towards the compounds of interest could be improved since it is not simple water but constituent water of ginger.

UAE is a process which is used to increase extraction yield of various phytochemicals [3,264]. US emitted by probe or in bath generate microbubbles which alter vegetal cells by cavitation phenomenon enhancing extraction of targeted compounds [265]. 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 V-4**.

Until 25 min of extraction, dry matter evolution followed the same trend for each UI assessed. Beyond 25 min 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 min. A significant increase was observed for UAE (13.4 W/cm^2; 0.242 W/cm^3) after 90 mins 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 **Figure V-5**. 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 [266–269]. 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.

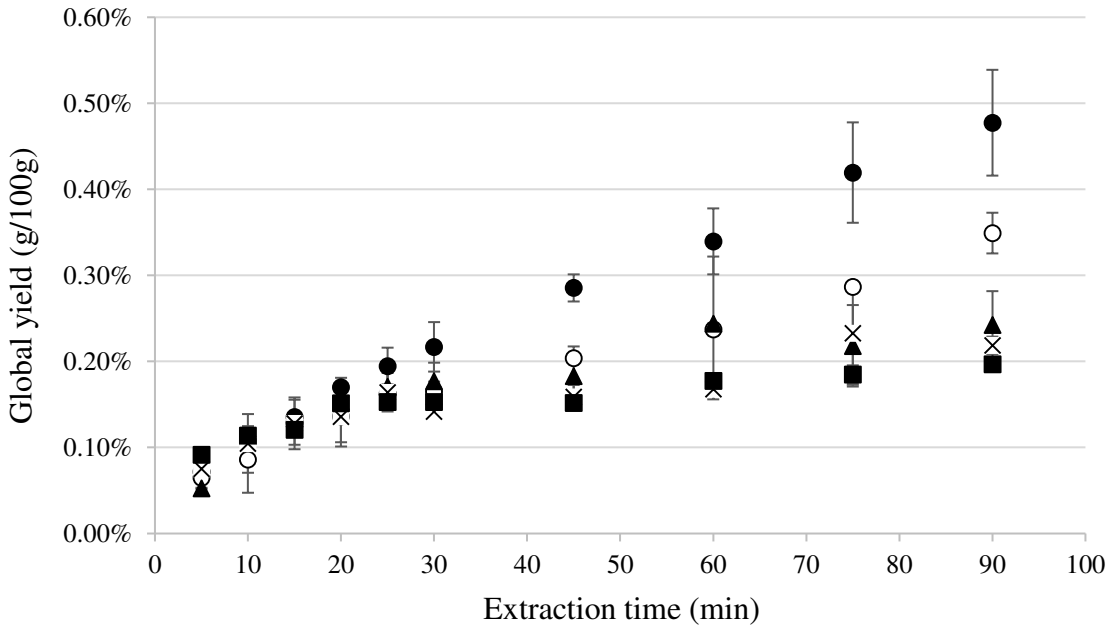


Figure V-4. Evolution of extract's dry weight as a function of ultrasonic intensity (and power density).

On **Figure V-5**, quantities of phenolics extracted from plant material are also reported. We were aware that gingerols solubility into pure water at ambient temperature is quite limited, but we wanted US to intensify as far as possible their extraction in this solvent. 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 V-1**. This value was determined as 1.37 % of GPMHG. It appeared difficult to extract completely this amount of compounds in water since it exceeds the solubility limit, however it was possible to reach almost 80 % recovery. As shown in **Figure V-5**, 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.

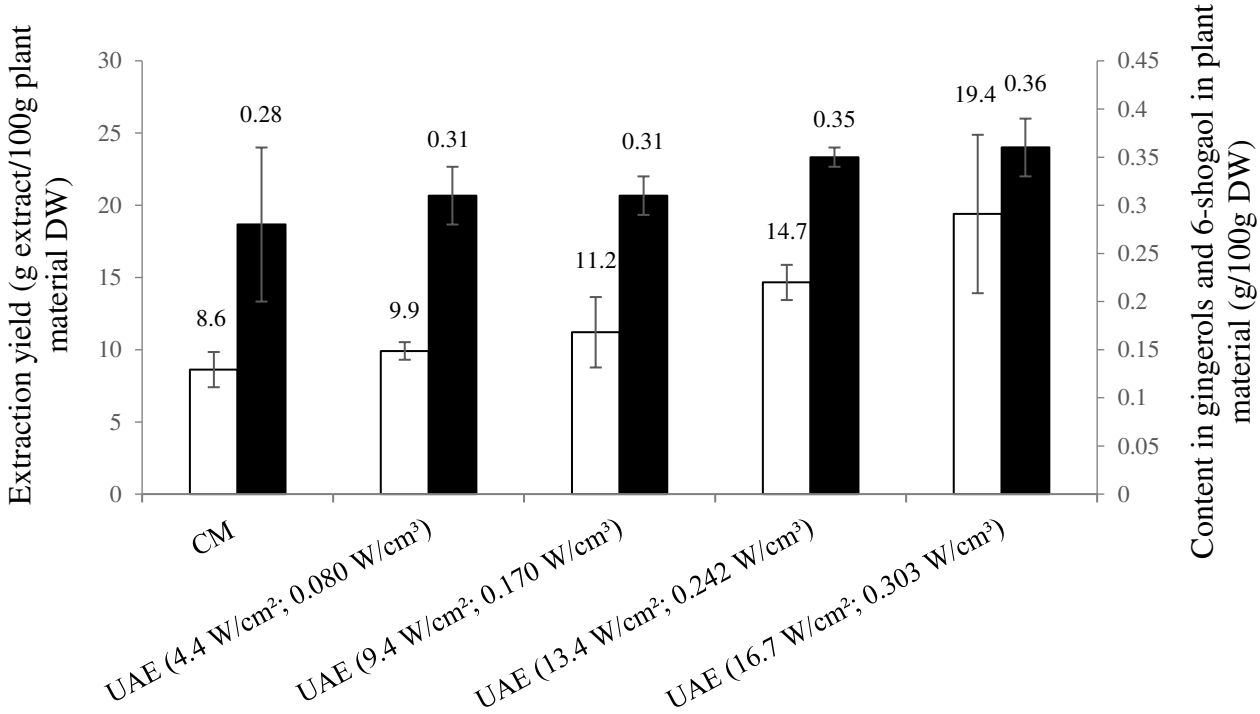


Figure V-5. Effect of US on extraction yield and gingerols and 6-shogaol content in the extracts.

V.3.3 Large scale microwave and ultrasound assisted extraction

Pilot scale experiments were performed for MHG using the MAC-75 equipment **(Figure II-7 (b))**. MAC-75 apparatus is a multimode MW 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 MW 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 MW power were at most 75 L and 6 kW respectively. Approximately 4 kg of press cake were therefore submitted to MW during 25 min and condensate (EO and constituent water) was recovered at

the end of experiment as it was done at laboratory scale. Several food by-products (garlic, onion and GP) 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 MW technology for by-products valorization. For UAE, a 30 L extraction tank from REUS company can be used to up-scale laboratory experiments **(Figure II-8 (b))**. The reactor is composed of a quadruple output of US 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 UAE is a promising technique that can be considered at industrial scale, especially when water is chosen as solvent [270].

V.3.4 Process assessment according to the six principles of green extraction

A process assessment of the DBR developed in this work was performed and compared with a CBR composed of an HD step for the recovery of EO and an ethanolic extraction step for the extraction of antioxidants from ginger **(Figure V-6)**. The bio-refineries were evaluated according to the six principles of green extraction developed by Chemat *et al.* [14]. 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 [271]. In this study, a simplified view of the bio-refineries was assessed. The six parameters considered were defined and calculated as follows:

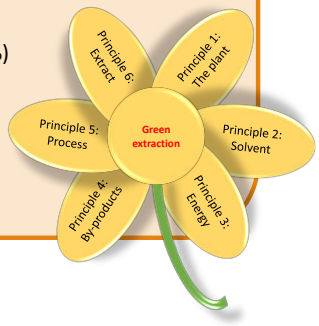
- **Raw material** (Principle 1): percentage of valorized raw material from food processing industry (in %)
- **Solvent** (Principle 2): (mass of ethanol) / (total mass of solvent used for the bio-refinery) (in %)
- **Energy** (Principle 3): energy consumption for the bio-refinery of 1.150 kg of raw material considering extraction and evaporation steps based on the energy transfer equation [177] (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 min)
- **Product recovery** (Principle 6): (mass of final product recovered) / (mass of available product in the plant material)

On **Figure V-6**, 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 min to 110 min. Moreover, in the DBR, no waste was generated as illustrated in **Figure II-6**, 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 DBR method in terms of time and energy.

- **Raw material** (*principle 1*): % of valorized raw material from food processing industry
- **Solvent** (*principle 2*): $\frac{\text{mass ethanol}}{\text{total mass of solvent used in the bio-refinery}}$ (%)
- **Energy** (*principle 3*): energy consumption for the bio-refinery of 1.150 kg of raw material (extraction and evaporation steps) (kWh)
- **Waste** (*principle 4*): $\frac{\text{mass waste}}{\text{total mass of solvent +raw material used in the process}}$ (%)
- **Process** (*principle 5*): extraction duration for the bio-refinery (min)
- **Product recovery** (*Principle 6*): $\frac{\text{mass of final product recovered}}{\text{mass of available product in the plant material}}$



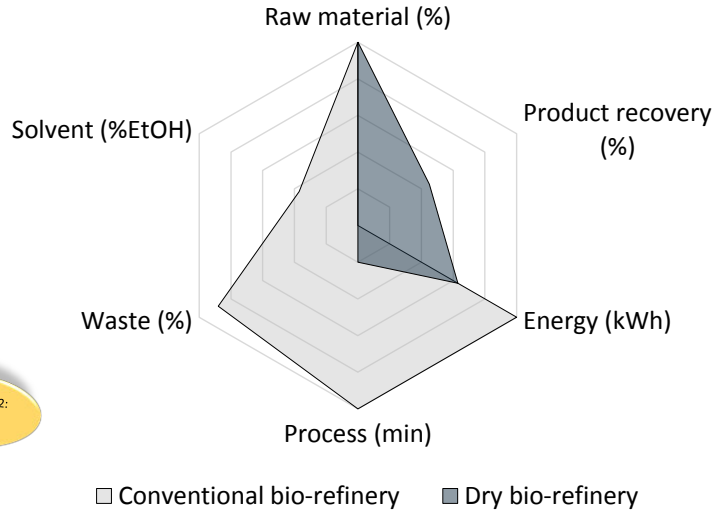


Figure V-6. Process assessment of "dry" bio-refinery and conventional bio-refinery according to the six principles of green extraction.

V.4 CONCLUSION

This study aims at total valorization of ginger by-products moving towards developing an original concept of 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 **(Figure V-7)**. 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 CM, 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 EO, an extract rich in phenolics, and a solid residue rich in fibers and phenolic acids, which can be thereafter incorporated in food formulations.

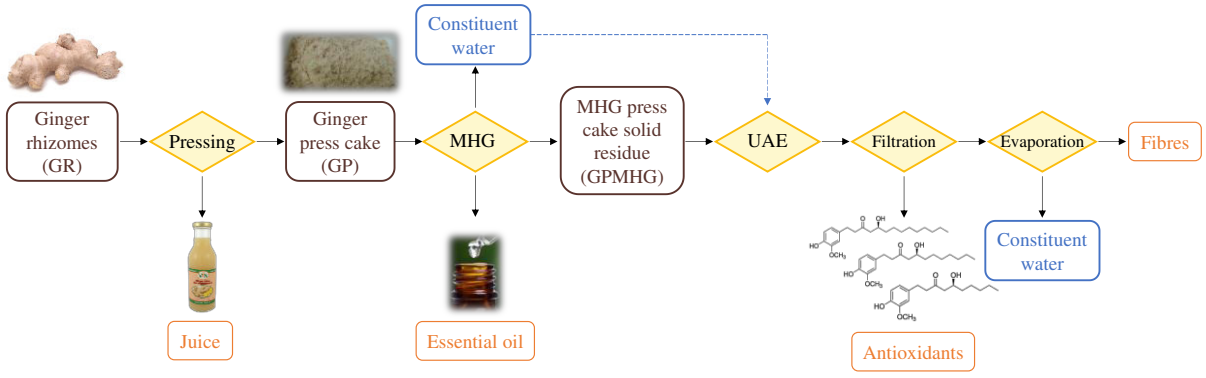


Figure V-7. Total valorization of ginger by-products using a bio-refinery concept.

CONCLUSION GENERALE

Les principaux objectifs de ce travail de thèse ont été de mettre en œuvre des procédés d'extraction alternatifs pour améliorer le taux d'extraction et les interactions solutés/solvants des procédés industriels existants tout en diminuant la durée totale, le nombre d'opérations unitaires, la consommation énergétique et l'impact global du procédé sur l'environnement ; de valoriser les co-produits d'extraction en produits à haute valeur ajoutée au vu des quantités conséquentes générées ; de remplacer les solvants pétroliers et dangereux pour les utilisateurs et l'environnement par des solvants alternatifs plus « verts » ; et enfin d'accompagner scientifiquement l'implémentation industrielle des améliorations de procédés définies précédemment. Afin d'apporter des réponses à ces problématiques, cette thèse a proposé trois principaux projets traitant de l'intensification de procédés par des technologies innovantes, la substitution de solvants nocifs pour l'Homme et l'environnement par des alternatives plus « vertes » et la valorisation de co-produits de fruits et légumes en produits à haute valeur ajoutée.

En premier lieu, le Chapitre I a présenté une étude bibliographique sur l'éco-extraction des produits naturels. Ses six principes se référant à la matière première, au solvant, à la consommation énergétique, aux co-produits d'extraction, au procédé et à l'extrait final ont été mis en relation avec le principe d'intensification et pour chacun d'entre eux, un constat de la situation actuelle a été dressé. Ce chapitre a permis d'identifier les bonnes pratiques à mettre en œuvre pour s'inscrire dans une démarche d'éco-extraction et a montré que l'adoption d'une telle démarche présentait un intérêt environnemental, économique et social. De plus, les différentes « success stories » développées pour illustrer chaque principe ont montré que l'éco-extraction ne se limitait pas à un seul type de matière première ou d'extrait mais pouvait se retrouver à la fois pour des applications agro-alimentaire, cosmétique et pharmaceutique. Enfin, cette partie a également souligné que certains aspects sont très matures et aboutis, comparés à d'autres qui restent encore au stade de recherche et développement. Cependant, cela permet de mieux appréhender de potentielles évolutions de la réglementation, notamment en ce qui concerne les solvants autorisés pour l'extraction.

Le Chapitre II décrit l'intégralité des méthodologies expérimentales utilisées dans cette thèse, incluant les protocoles d'extraction ainsi que les méthodes d'analyse quantitative et qualitative.

Dans la première partie de cette thèse (Chapitre III), le procédé d'extraction des acides rosmarinique, carnosique et ursolique à partir du romarin a été intensifié par l'usage d'ultrasons et de micro-ondes dans l'éthanol 90 %. Plusieurs procédés ultrasons et micro-

ondes ont été étudiés et comparés aux reflux et macération conventionnels en termes d'efficacité et de sélectivité d'extraction, ainsi qu'en termes de consommation énergétique. Les résultats ont montré que l'usage de ces technologies alternatives permettait d'obtenir des résultats similaires ou améliorés comparé aux procédés conventionnels et qu'il était possible d'avoir une sélectivité d'extraction en fonction de la technologie d'intensification utilisée. En effet, l'usage des ultrasons à basse température (40 °C) a mené à une extraction préférentielle des acides carnosique et ursolique tandis que l'usage des micro-ondes à haute température a permis d'obtenir l'acide rosmarinique. De plus, l'utilisation des ultrasons et des micro-ondes pour l'extraction est apparu comme une alternative verte puisque la consommation énergétique de tels procédés a été réduite par rapport à celle des procédés conventionnels.

Dans la deuxième partie de ce travail, l'effet du ratio en éthanol dans le solvant d'extraction a été évalué pour l'obtention d'extraits de romarin. Plus particulièrement, l'effet sur le rendement massique d'extraction, la composition (teneur en acides rosmarinique et carnosique) et les capacités réductrice, anti-radicalaire et antioxydante des extraits ont été étudiés. De plus, afin de mieux comprendre le phénomène de solubilisation intervenant lors de l'extraction, les solubilités des acides rosmarinique et carnosique ont été évaluées dans les différents mélanges éthanol:eau en utilisant un logiciel de prédiction (COSMO-RS) et ont été comparées aux solubilités expérimentales. Les résultats obtenus ont permis de conclure que le choix du solvant était crucial pour orienter l'extraction puisque le solvant pour lequel le rendement massique est optimal (éthanol 50-80 %) n'est pas le même que celui optimisant le rendement en actif (éthanol 30 % pour l'acide rosmarinique et éthanol 100 % pour l'acide carnosique), ni que celui optimisant l'activité de l'extrait (éthanol 30 %). Avant de choisir le solvant, il est donc nécessaire de déterminer quel paramètre on souhaite optimiser. Cette étude a également montré que l'éthanol 100 % était le meilleur solvant pour solubiliser à la fois l'acide rosmarinique et l'acide carnosique, ce qui démontre que l'extraction n'est pas qu'un phénomène de solubilisation. En effet, si la solubilité et la sélectivité d'extraction sont corrélées pour l'acide carnosique, ce n'est pas le cas pour l'acide rosmarinique qui est mieux extrait avec de l'éthanol 30 %. Ce résultat suggère donc que l'histolocalisation des composés d'intérêt dans la plante influence grandement leur taux d'extraction. L'étude a aussi montré que la teneur en acide rosmarinique et l'activité de l'extrait étaient corrélées, ce qui fait de ce composé un déterminant majeur de l'activité. La présence d'autres composés actifs affectant positivement l'activité globale de l'extrait a également été démontrée. Enfin, au vu des résultats obtenus dans l'étude, il a été possible de développer un concept de bio-raffinerie du

romarin pour extraire successivement l'acide carnosique peu polaire et l'acide rosmarinique polaire en faisant varier le ratio en éthanol. Ce procédé a permis d'avoir des recouvrements en actifs de 82 % et de 71 % pour l'acide carnosique et l'acide rosmarinique respectivement. Au-delà des aspects plus fondamentaux qu'elle évoque, cette étude a donc permis de montrer qu'il était possible d'obtenir séparément les deux principaux actifs du romarin en un seul procédé, tout en utilisant un solvant « vert » pour l'extraction. Ce résultat est positif puisqu'actuellement, deux procédés indépendants, dont l'un mettant en œuvre de l'acétone, sont réalisés pour obtenir les acides rosmarinique et carnosique.

Enfin, une troisième partie de cette thèse a été consacrée à la valorisation de co-produits de gingembre par un procédé inspiré de la bio-raffinerie. Une bio-raffinerie « sèche » sans solvant ni eau ajoutés et utilisant des technologies vertes a été développé et son bilan environnementale a été dressé. A partir du press cake de gingembre qui est un déchet de l'industrie des jus de fruits ont été obtenus une huile essentielle, un extrait riche en composés phénoliques et un résidu solide riche en fibres et en antioxydants. L'huile essentielle a été extraite par MHG (Microwave Hydrodiffusion and Gravity) et l'effet de la puissance micro-ondes sur l'efficacité d'extraction et sur la qualité de l'extrait a été étudié. La puissance optimale a également été déterminée comme 1,6 W/g de matière première. Puis, le résidu solide de gingembre après traitement micro-ondes a été soumis aux ultrasons dans son eau de constitution pour extraire les composés phénoliques (gingerols et 6-shogaol). L'intensité ultrasonore (ou densité de puissance) a été optimisée pour maximiser les rendements d'extraction. L'usage des ultrasons à 16,7 W/cm^2 (ou 0,303 W/cm^3) a permis d'augmenter significativement le rendement massique d'extraction, cependant il n'a pas permis d'atteindre un taux de recouvrement d'actifs similaire à celui obtenu par une procédé conventionnel dans l'éthanol 75 %. La solubilité limitée des actifs dans l'eau a été un facteur limitant. Par contre, le résidu solide trouve une utilité pour sa teneur résiduelle en composés phénoliques et en fibres. Enfin, les résultats ont montré que l'empreinte environnementale de la bio-raffinerie « sèche » développée dans l'étude a été réduite par rapport à celle d'une bio-raffinerie mettant en œuvre des procédés conventionnels.

En résumé, ces travaux de thèse ont permis de montrer qu'il été possible :

- D'intensifier le procédé d'extraction des composés d'intérêt du romarin par l'usage d'ultrasons et de micro-ondes, et d'obtenir une sélectivité d'extraction en fonction de la technologie utilisée ;

- D’obtenir les deux principaux antioxydants du romarin en un seul procédé en utilisant des mélanges hydro-alcooliques plutôt que des solvants plus nocifs tels que l’acétone ;
- De valoriser des co-produits alimentaires en produits à haute valeur ajoutée par un procédé inspiré de la bio-raffinerie sans solvant ni eau ajoutés.

De manière plus générale, cette thèse a démontré que l’éco-extraction est un concept qui peut aisément être implémenté en industrie et qu’il incarne l’avenir du domaine de l’extraction végétale, auquel chaque acteur industriel devra adhérer à plus ou moins court terme.

Les différents résultats obtenus dans cette thèse soulèvent cependant des questions qui pourraient faire l’objet de futures recherches et de perspectives pour ce travail:

- La prise en compte de l’extraction comme un procédé complet (et non comme une opération unitaire) et par conséquent élargir la démarche de chimie verte aux étapes de purification d’actifs et de formulation de l’extrait ;
- L’évaluation du potentiel des co-produits alimentaires et leur intégration dans un système d’économie circulaire ;
- La mise en place d’un outil d’Analyse de cycle de vie spécifique à l’extraction pour quantifier et qualifier de manière plus standardisée l’impact environnemental global d’un procédé d’extraction.

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RESUME

Avec les préoccupations environnementales et sociétales inhérentes au secteur industriel d'aujourd'hui, il est devenu nécessaire d'inventer et développer de nouveaux procédés d'extraction œuvrant pour une chimie verte plus propre vis-à-vis de l'environnement, et plus sûre vis-à-vis des utilisateurs. C'est dans ce contexte qu'ont été imaginés et définis les six principes de l'éco-extraction présentés dans la première partie de ce manuscrit. Cette démarche a totalement inspiré cette thèse puisqu'elle est constituée de trois études directement reliées aux principes de l'éco-extraction : l'intensification de procédés par des technologies alternatives, la substitution de solvants toxiques par des homologues plus « verts » et la valorisation de co-produits d'extraction. Ce travail a permis de montrer qu'il était possible d'intensifier le procédé d'extraction des composés d'intérêt du romarin par l'usage d'ultrasons et de micro-ondes, et d'obtenir une sélectivité d'extraction en fonction de la technologie utilisée ; d'obtenir les deux principaux antioxydants du romarin en un seul procédé en utilisant des mélanges hydro-alcooliques plutôt que des solvants plus nocifs tels que l'acétone ; et de valoriser des co-produits alimentaires en produits à haute valeur ajoutée par un procédé inspiré de la bio-raffinerie sans solvant ni eau ajoutés.

Mots-clés: éco-extraction, intensification, substitution du solvant, valorisation de co-produits, antioxydants.

ABSTRACT

With the current environmental and social concerns of industrial sector, it has become necessary to invent and develop new extraction processes with green chemistry values, more environmentally friendly and safer for users. In this context were imagined and defined the six principles of green extraction presented in the first part of this manuscript. This demarche inspired completely this thesis since it is composed of three studies directly related to the principles of green extraction: process intensification using alternative technologies; replacement of harmful solvents by greener ones; and valorization of extraction by-products. Therefore, it has been shown in this work that the extraction of compounds of interest from rosemary could be intensified using microwaves or ultrasound, and that an extraction selectivity could be achieved according to the technology used; that the two main antioxidants of rosemary could be recovered in a single process using hydro-alcoholic mixtures rather than more harmful solvents such as acetone; and finally that food by-products could be valorized into high value products using a process inspired rom the bio-refinery, without solvent nor added water.

Keywords: green extraction, intensification, solvent substitution, by-products valorization, antioxidant.