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Quality Preservation and Cost Effectiveness in the Extraction of Nutraceutically-Relevant Fractions from Microbial and Vegetal Matrices

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1. Introduction

Terrestrial and microbial vegetal matrices are a major source of nutraceutically and pharmaceutically relevant chemical compounds of different nature. In several cases the consumption of the raw vegetal or microbial matrix has been part of established diet regimes and has provided the consumers with a host of once unknown dietary benefits. Nowadays, while the consumption of the raw matrix still provides the original functional value, the separation of bioactive-enriched fractions has enabled the production of nutraceuticals, while the addition of nutraceutical fractions to food previously lacking or partially possessing them has lead to the industrial production of functionalised or fortified food respectively.
The separability of nutraceutically relevant fractions depends on the combination of several different features of the carrier matrix and of the fraction to be separated, namely: size, aggregation state and physical (hardness) and chemical (composition) features of the embodying matrix, chemical nature, bonding and degree of dispersion of the fraction of interest in the embodying matrix. Physical, chemical and electrostatic interactions between the embodying matrix, the fractions to be separated and exogenous agents (equipment and process auxiliary substances, and the environment) affect the desired separation; the chemical nature of the solvent (if any), the specific energy applied by the physical agent (if any), the frequency and intensity of the mechanical (e.g., ultrasound) and electromagnetic (e.g., microwave) field (if any), the processing time and temperature and the presence of specific case-by-case unwanted substances (e.g., water, oxygen, metal ions) play a role in the final outcome of the recovery process of the desired fraction.

2. Sub- and supercritical fluid extraction

Sub- and supercritical fluid extraction are promising separation processes aimed at replacing traditional lengthy, laborious, low selectivity and/or low extraction yield, toxic chemical-using separation processes (Herrero et al., 2006).
The low viscosity and (relatively) high diffusivity inherent in the supercritical state confers these solvents better transport properties than liquids solvents have. Furthermore, the "adjustability" of viscosity, density and solvent power (Del Valle and Aguilera, 1999) by acting on fluid density through the change of pressure and/or its temperature make them

amply tunable to the specific separation needs. Last, but not least, using solvents generally recognized as safe (GRAS) helps meet the requirements of food and pharma processes.

Carbon dioxide is the most commonly used because of its moderate critical temperature and pressure (31.1 °C and 7.39 MPa, respectively) and GRAS status. Supercritical CO_2 (Brunner, 2005): dissolves non-polar or slightly polar compounds; has a high solvent power for low molecular weight compounds, which decreases with increasing molecular weight; has high affinity with oxygenated organic compounds of medium molecular weight; has low solvent power for free fatty acids and their glycerides, even lower for pigments, and low solubility for water at temperatures below 100 °C; does not dissolve: proteins, polysaccharides, sugars and mineral salts; exhibits an increasing separating capability as pressure increases for compounds that are scarcely volatile, have a high molecular weight and/or are highly polar. Due to its low polarity, small amounts of "modifiers" (also called co-solvents), in the form of highly polar compounds such as ethanol or water, are often used to improve the separability of polar solutes.

Preparative systems for processing solid or liquid samples have different configurations; basically, they consist of solvent pump(s) deliverying the main solvent and any required modifyer throughout the system, an extraction cell (separation from a liquid) or column (solid), and one or more separators in which the extract is collected when the solvent is expanded.

Extraction from solids is usually carried out discontinuously (batchwise) in a single stage because solids handling in pressurised vessels is troublesome and separation factors are high. Fluid mixtures often suffer from low separation factors and multi-stage contacting (countercurrently for highest effectiveness) becomes a necessity. When separation factors approach unity and many theoretical stages are required for the separation preparative chromatographic systems may be set up on a process scale (Brunner, 2005).

Pressurised liquids (e. g. water and ethanol at an intermediate temperature between their boiling points and their critical temperatures and under the appropriate pressure to maintain them in the liquid state) mitigate the drawbacks of supercritical fluids (e.g. supercritical carbon dioxide): their scarce affinity for polar solutes and extensive capital costs. Subcritical water has been successfully used for the extraction of essential oils, nutraceuticals and bioactives, among which polyphenols (King and Srinivas, 2009).

Water is a highly polar solvent at room temperature and atmospheric pressure due to hydrogen-bonding which reflects in its high dielectric constant. While at room temperature water is unsuitable as a solvent for non-polar compounds, at increased temperature a lowered dielectric constant (from 80 at 25 °C to 27 at 250 °C and 50 bar, intermediate between those of methanol, 33, and ethanol, 24, at 25 °C), viscosity and surface tension and an increased diffusivity confer an increased solvent power for non polar substances. Thermally labile compounds may be degraded at elevated temperatures (Teo et al., 2010) but newly formed bioactives (antioxidants) have also been observed (Plaza et al., 2010).

By adjusting the prevailing temperature and under the required pressure to remain in the liquid state, water may be tuned to the purpose, which may be that of an extraction solvent and/or reaction medium. The main parameters that influence the selectivity and extraction efficiency of pressurised water extraction include temperature, pressure, extraction time, flow rates and concentration of modifiers/additives. The residence time of the solute (reactant) in the aqueous medium becomes a critical parameter in conducting extractions above the boiling point of water and for optimizing reaction conditions (King and Srinivas, 2009).

2.1 Mathematical modelling of Pressurised Water Extraction (PWE)

The very core of PWE extraction may be divided in four sequential steps which take place in the extraction cell filled with sample materials: 1. desorption of solutes from the various active sites in the sample matrix; 2. diffusion of the extraction fluid into the matrix; 3. partitioning of solutes from the sample matrix into the extraction fluid and 4. chromatographic elution of the solutes out of the extraction cell to the collection vial. A two-step, single-site partition-based thermodynamic model is appropriate for PWE extraction (Kubátová et al., 2000 and 2002; Windal et al., 2000): 1. the compound is desorbed from its original binding sites in the sample matrix, under diffusion control but at a sufficiently fast rate to avoid being the overall limiting step; 2. the compound is eluted from the sample under thermodynamic partitioning control (K_D). The shape of an extraction curve would be defined by:

$$\frac{S_b}{S_o} = \frac{\left(1 - \frac{S_a}{S_o}\right)}{\frac{K_D \cdot m}{(V_b - V_a)\cdot \rho} + 1} + \frac{S_a}{S_o}$$

where S_a is the cumulative mass of the analyte extracted after volume V_a (ml), and S_b is the cumulative mass of the analyte extracted after volume V_b (data point b is next to data point a in experimental sequence). S_o is the initial total mass of analyte in the matrix. S_b/S_o and S_a/S_o are the cumulative fractions of the analyte extracted by the extraction fluid of the volume V_b and V_a, respectively. K_D is the distribution coefficient, ρ is the density of extraction fluid at given conditions (g/ml), and m is the mass of the extracted sample (g). The model depends on the extractant volume flowed, but not time.

The experimental device required for PWE is quite simple. Basically, the instrumentation consists of a water reservoir coupled to a high pressure pump to introduce the solvent into the system, a thermostating oven, where the extraction cell is placed and extraction takes place, and a restrictor or valve to maintain the pressure. Extracts are collected in a vial placed at the end of the extraction system. In addition, the system can be equipped with a coolant device for rapid cooling of the resultant extract (Herrero et al., 2006).

2.2 Predicting solubility via the hansen solubility parameter

Predicting solubility parameters capable of telling whether one substance can form a solution by dissolution in another can be done by several thermodynamical methods with a variable degree of theoretical base and reliability. Following the reasoning that dissolution is linked to likeness, i.e. similarity in bonding to itself, a solubility parameter can be defined for every substance as the square root of the cohesive energy density $\delta = (E/v)^{1/2}$ where v is the molar volume of the pure solvent, and E is its (measurable) energy of vaporization (Hildebrand and Scott, 1950). The total energy of vaporization of a liquid consists of multiple individual components, among which (atomic) dispersion forces, (molecular) permanent dipole–permanent dipole forces, and (molecular) hydrogen bonding (electron exchange) (Hansen, 1997). Dividing this by the molar volume gives the square of the total (or Hildebrand) solubility parameter as the sum of the squares of the Hansen D, P, and H components, that is:

$$\delta_T = E/V = E_D/V + E_P/V + E_H/V = \delta_D + \delta_P + \delta_H$$

Hansen parameters can be calculated from literature physical properties and solubility data, molecular structure, or group contribution methods (Hoy 1989; Hoftyzer and van Krevelen 1997; Stefanis and Panayitou 2008, Hansen 2007). These three parameters can be treated as coordinates for a point in the Hansen space; distance in the Hansen space predicts likeliness of reciprocal solubility of the substance pair. To calculate the distance (R_a) between Hansen parameters of substances S_1 and S_2 in Hansen space the following formula is used:

$$\left(R_a\right)^2 = 4 \cdot \left(\delta_{D_2} - \delta_{D_1}\right)^2 + \left(\delta_{P_2} - \delta_{P_1}\right)^2 + \left(\delta_{H_2} - \delta_{H_1}\right)^2$$

which defines the radius of a sphere centered in substance S_1 and identifying the domain of substances which possess equal or better solubility than substance S_2.

An interaction radius (R_o), a characteristic quantity of the substance to be dissolved, can be also defined. The interaction radius effectively defines a metrics for the solubilisation of the substance under concern. Together, R_a and R_o determine whether the pair is within (solubility) range. The ratio R_a/R_o is called the relative energy difference (RED) of the system; if RED < 1 the molecules are alike and will dissolve, if RED = 1 the system will partially dissolve and if RED > 1 the system will not dissolve.

Correlation methods are available for the prediction of the temperature dependence of the solute solubility parameter, such as the Jayasri and Yaseen method (1980). The Hansen solubility prediction method lends itself to a computational identification of the optimal solvent, which corresponds (in principle) to the mixture exhibiting the lowest RED value. Using this approach, minimum diameter Hansen spheres can be obtained by changing the composition and the temperature of the (subcritical) solvent phase and solute-solvent interaction can be predicted and optimised.

Srinivas et al. (2009) studied the solvent characteristics of subcritical fluid solvents at different temperatures interacting with various bioactive compounds from their natural sources; they tested the applicability of this predictive method on valuable nutraceutical solutes extracted from natural sources. The use of this method will be presented with reference to the extraction of flavonoids from grape pomace.

Flavonoids consist of anthocyanins, flavonols, procyanidins with antimicrobial, antiviral and antioxidant as well as food colorant properties. Many investigations (e.g. Cacace and Mazza, 2002) have been carried out on extraction of anthocyanins from berry substrates using subcritical water and water–cosolvent systems, whose results have shown that a maximum extraction yield is obtained in the 120 to 160 °C temperature range. A lower solvent–feed ratio requirement compared to traditional solvent extraction techniques is reported (King et al., 2003). Studies on the effect of temperature and particle size on subcritical water extraction of anthocyanins from grape pomace pointed to an optimal yield at 120 °C using a 150 micron-sized substrate (King et al., 2007). These studies also indicated the possibility of thermal degradation of the target solutes occurring at higher temperatures.

Srinivas et al. (2009) applied the Hansen solubility method to the extraction of malvidin-3,5-diglucoside with water–ethanol mixtures by investigating: 1. solubility in the pure solvents as a function of temperature and 2. solubility in mixtures of the two solvents as a function of concentration. They predicted a slightly higher miscibility with ethanol (radius of 7.74 $MPa^{1/2}$ vs 9.73 $Mpa^{1/2}$) at different temperatures (25 to 75 °C for ethanol; 100 to 200 °C for water): although both ethanol and water can dissolve the target anthocyanins at different

conditions, ethanol would be a preferred solvent for the extraction of anthocyanins. Mixture calculations show a significant decrease in the interaction radius of the Hansen sphere with addition of 10% ethanol. Although the maximum solvent power is predicted at 80% ethanol, the improvement over 10% ethanol is minimal. The optimal solvent temperature using hydroethanolic mixtures is in the range 25 to 75 °C. Results are broadly coherent with data reported by Monrad et al. (2010) and plotted in Figure 1.

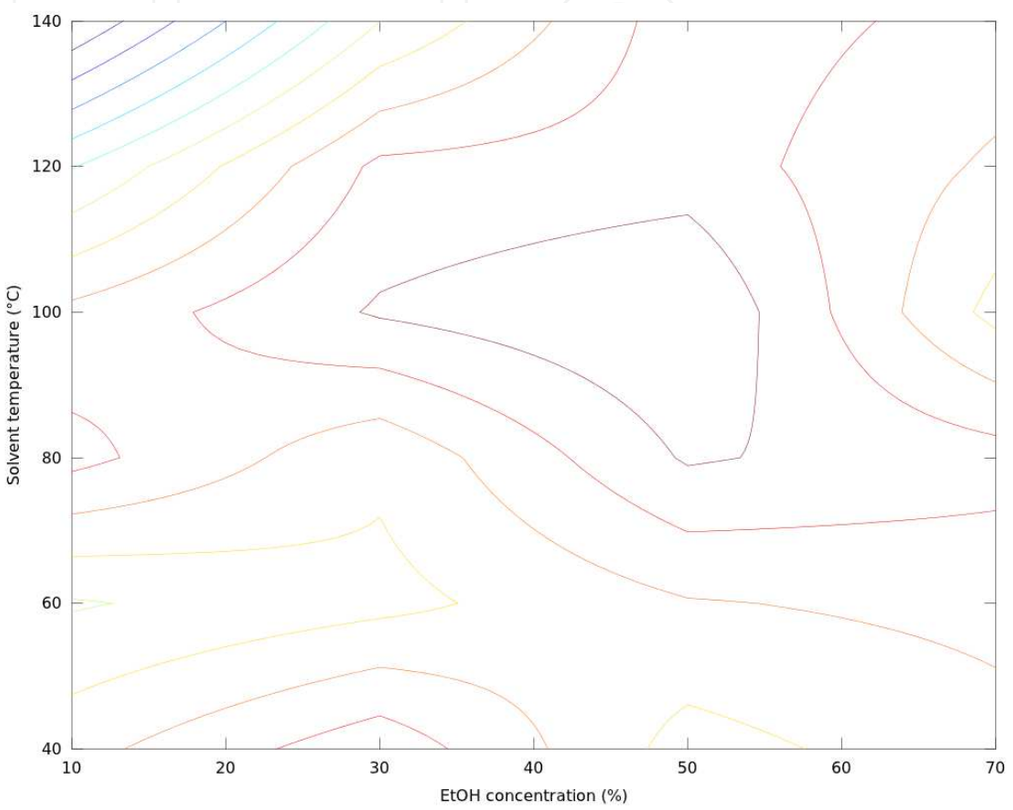

Fig. 1. Concentration of recovered Malvidin-3,5-O-diglucoside in hydroalcoholic extracts from subcritical extraction (Data from Monrad et al., 2010).

2.3 Multiple fluid process tuning for sub/supercritical fluid extraction

Multiple fluid processing involves the integration of two or more fluids held under pressure (above or below their critical temperature and pressure) applied as either mixtures or in a sequential manner for one or more unit processes (King and Srinivas, 2009). Their state may be variable (critical/subcritical/mixed). A large collection of solubility data is available in the literature (Gupta and Shim, 2007) and methods have been developed to predict it (such as the Hansen Solubility Parameter discussed above) to assist broad approaches in fraction separation and chemical transformation choices. Non polar gases can be tuned to the required solute capacity by adjusting their density (pressure) and to (ideally) match the required solubility parameter (polarity) by mixing them with polar modifier substances and possibly adjusting temperature and pressure. Polar substances, in their turn, may serve at one time as solvents and as reactants; water stands out in this respect because of its nil toxicity and cost and because it can potentially simplify process arrangement given that in

most cases it is initially present in the vegetal matrix and its separation may be required for contact with non polar solvents (either organic or inorganic). Added substances may act as solubility enhancers (co-solvent: e.g. polar substances added to supercritical CO_2) or depressors (antisolvent: e.g. gases depending on their critical temperature); again, these added substances may also serve as reactants (e.g. hydrogen for hydrogenation reactions), which may be an atout despite the reduction in solvent power toward the other reactants. On the other hand, gas may be solubilised in subcritical liquid solvents, such as water. In addition to other properties (notably, for water, the dielectric constant) this may serve to tune pH by addition of CO_2, thereby obtaining a versatile medium with respect to acidic-based extraction chemistry and reactions (as in the extraction of flavonoids or in the pretreatment of lignocellulosic biomass) without the burden of a subsequent pH correction.
The change in solvent power which can be obtained by the change of pressure (in the supercritical state) or temperature (in the subcritical state) permits the implementation of multiple unit operations (fractionation of multiple compounds, chemical reaction) with only one running fluid. If pressure change to carry out reciprocal solute/solvent separation and fractionations are deployed for solute separation and energy consumption must be reduced, membrane separations (expecially nanofiltration and reverse osmosis) may be implemented to obtain bulk molecule size-based separation and avoid recompressing the solvent across the full pressure range. Performing a sequential arrangement of the operations may make important savings in equipment capital cost possible. Membrane coupling may be equally useful for compressible fluids (e.g. CO_2) and scarcely compressible fluids (e.g. water or hydroalcoholic solutions); these latter tend to yield dilute solutions that need concentration and membrane processing can obtain that cheaply and neatly.
Antioxidant-containing matrices (e.g. grape and olive waste) are a fruitful area in which to apply combinations of mixed critical and subcritical fluid and unit processing steps. Residence time of the extracted solute in the hot pressurized water must be minimized to prevent degradation of the anthocyanin moieties or their possible reaction with sugars to other products. Some evidence that side reactions in pressurized water could be generating antioxidant moieties has been gathered (King and Srinivas, 2009). An example of pipelined processing is then offered by sunflower oil triglyceride conversion to free fatty acids (in subcritical water) followed by enzymatic esterification of the free fatty acids to FAMES in supercritical CO_2 using lipase catalysis (Baig et al., 2008).

2.4 Cost-effectiveness analysis in supercritical fluid extraction

The extraction of valuable materials from solid substrates by means of SCFs has been carried out on a commercial scale for more than two decades. Large-scale processes are related to the food industry like the decaffeination of coffee beans and black tea leaves and the extraction of bitter flavours (α-acids) from hops. Smaller scale processes comprise the extraction and concentration of essential oils, oleoresins and other high-value flavouring compounds from herbs and spices, and the removal of pesticides from plant material. The extraction of edible oils would be a large-scale process, but, as for all commodity products, the value-added is not high, so the economy of the process is the main problem and must be considered separately for each case.
Oil from oleaginous seeds is traditionally produced by hexane extraction from ground seeds, with a possible thermal degradation of the oil and an incomplete hexane elimination. SC-CO_2 extraction of oil from seeds has been proposed in many cases (Coriander, fennel,

grape, hyprose, sunflower etc--see Reverchon and De Marco, 2006) given that oilseed triglycerides solubilise well in SC-CO_2 at 40 °C and pressure higher than 280 bar. Main parameters of this process are particle size, pressure and residence time. After extraction, the extract solution is sent to a separator working at subcritical conditions so that the reduced solubility enables recovery of oil and elimination of gaseous CO_2 from oil. Alternatively, temperature variations may be used to recover the oil extracted in a process operated at a fixed pressure; energy consumption can be reduced if thermal integration is properly deployed throughout the plant (Reverchon and De Marco, 2006).
While several seed oils have been extracted this way up to the pilot scale, here we take sunflower oil as an example.
The main models of SFE from vegetal matrices are derived from classical models of mass transfer and extraction kinetics.
Several models were already published; they mostly assume plug flow of the fluid through the fixed bed of the solid matrix and mass transfer control located in the fluid phase (as done by Lee et al., 1986), in the solid phase (e.g. Reverchon, 1986), or in both (e.g. Sovová et al., 1994).
Most of them consider only one pseudocomponent, "the solute", characterised by one (averaged) solubility value obtained experimentally. The solid matrix of the seeds is porous, homogeneous, and with constant physical properties during extraction. The physical properties of the fluid are constant as well. Usually, pressure and temperature gradients that appear in the fixed bed are neglected. Perrut et al. (1997) considered one solid phase and one fluid phase, where external mass transfer is the controlling step. Axial dispersion in the bed is neglected and all pores are pre-filled by the solvent during the initial increase of pressure in the extractor until equilibrium is reached between the solid and liquid phase (initial condition). Oil concentration y* at the solid – SCO2 interface depends on an equilibrium relation, function of pressure and temperature y* = f(x, P, T):

$$\rho_f \cdot \varepsilon \cdot \left(\frac{\partial x_f}{\partial t} + v \cdot \frac{\partial x_f}{\partial z} \right) = j_f$$

with

$$v = \frac{Q / \rho_f}{\text{extractor section}} \cdot \frac{1}{\varepsilon}$$

j is the flux of solute that is exchanged between the solid and the fluid phase based on global mass transfer coefficient considered at equilibrium.

$$j_f = a_p \, k_f \, p_f \, (y^* - x_f)$$

Perrut et al. (1997) introduced the notion of "transition concentration" x_t, meaning that oil concentration in SCO_2 is equal to the thermodynamic solubility value y_o regardless of its concentration in the solid concentration as long as this latter is sufficiently rich (above threshold value x_t which is normally in untreated seeds); when the concentration in the solid diminishes oil concentration is determined with a partition coefficient K.

$$x < x_t \quad f(x) = K \cdot x$$
$$x \geq x_t \quad f(x) = y_o$$

Three parameters are adjusted to experimental extraction data (solubility, partition coefficient, transition concentration).

More accurate modelling can be obtained by adding: other dispersion axes (e.g., axial dispersion in the reactor volume and radial dispersion in the seed volume as done by Cocero and García, 2001); volume compartments; a shrinking core model; a dual-zone (intact cells and broken cells; Figure 2) continuous modelling inside the particle (Sovová, 2005). This latter model acknowledges that many cells have been broken by the pre-treatment but, inside the seed, cells are intact so that oil from the broken cells is "free" and can be directly extracted while oil in the intact cells must diffuse through broken cells. Correspondingly one equation must be written for the broken cells and one for the intact cells, where r is the fraction of broken cells in a crushed seed.

$$r \cdot \rho_s \cdot (1-\varepsilon) \cdot \frac{\partial x_1}{\partial t} = j_s - j_f$$

$$(1-r) \cdot \rho_s \cdot (1-\varepsilon) \cdot \frac{\partial x_2}{\partial t} = -j_s$$

The different fluxes between the broken cell and the fluid and between the intact and the broken cells are written (again resorting to the concept of transition concentration):

$$j_f = k_f\, a_p\, \rho_f\, (y^* - x_f)\ ;\ x_1 \neq x_t \text{ and } x_f < K\, x_t$$

$$j_s = k_s\, a_p\, \rho_s\, (x_2 - x_1)$$

The extraction of oil from oleaginous seeds might be one primary target of SFE if the added value of extracted vegetable oils were not very low. However, specialty oils or valuable components co-extracted with common oils could be reasonable target of SFE extraction (Brunner, 2005).

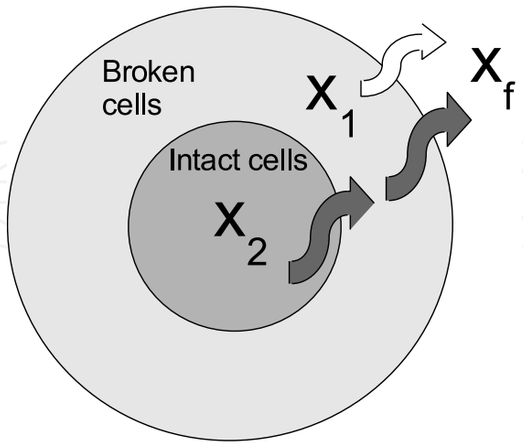

Fig. 2. Illustration of a dual-compartment modelling scheme for crushed seed extraction. (Reprinted from Boutin et al., 2011, with permission from Elsevier)

One drawback to widespread industrial scale adoption of supercritical fluid extraction use is the lack of realistic economical studies. In order to evaluate the feasibility of using SFE in the

extraction of food products or components thereof, an estimate of the production cost must be provided, generally by costing an approximate plant design providing the separation. While the level of accuracy of the modelling (thermodynamics) and costing will affect the reliability of the estimate, a rigorous approach is generally out of the scientific investigator's reach and scope.

The assessment of the industrial economical feasibility of entire supercritical extraction processes has been carried out by a few investigators. Citrus peel oil deterpenation (Diaz et al., 2005), such as essential oil extraction from rosemary, fennel and anise (Pereira and Meireles, 2007) and sunflower oil (Bravi et al., 2002). Then, given that yield and quality of the product are simultaneous targets when investigating an extraction, an example of recovery yield and product quality bridging will be given along the lines of Bravi et al. (2003).

The solid substrate in most cases forms a fixed bed. The SCF flows through the fixed bed and extracts the product components until the substrate is depleted. This extraction from solids consists of two process steps, namely, the extraction, and the separation of the extract from the solvent. During the extraction the supercritical fluid is fed and evenly distributed at one end of the extractor where it flows, upward or downward, through a fixed bed of solid particles of the vegetal/microalgal matrix and dissolves the extractable components. The depletion boundary in the solid matrix will proceed in the direction of flow, as the concentration of the extracted components in the solvent. The shape of the concentration curve depends on the kinetic extraction properties of the solid material and the solvent power of the SCF which, in turn, depend on operating conditions. For the solid as well as for the solvent, the extraction is an unsteady process (Brunner, 1994).

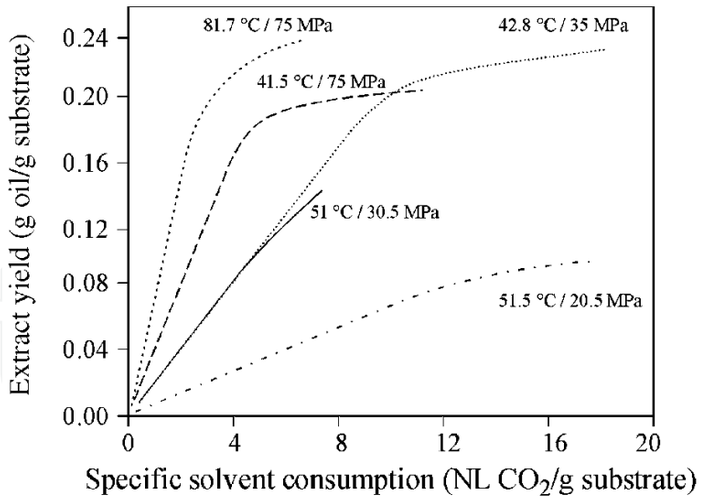

Fig. 3. Extraction yield vs mass of supercritical solvent flowed through the vegetal matrix bed (With kind permission from Springer Science+Business Media: Gas extraction: an introduction to fundamentals of supercritical fluids and the application to separation processes, 1994).

A time profile of the extraction yield will be typically shaped as any one of the curves in Figure 3. The initial part of the curve is a straight line (constant extraction rate). At later times the extraction rate decreases as the total amount of extractible substances in the substrate is

approached. Any extractible component in the solid follows the extraction course pattern. If mass transfer is fluid phase side-limited the extraction profile is represented by a straight line (the slope equalling the extraction rate); however, if mass transfer is split among the contacted phases the extraction has an exponential course. If the solvent enters the extractor free from the extractible compounds and there are no irreversible reactions of the extractible compounds with the substrate, the matrix can be totally depleted; otherwise the extraction curve approaches a non null asymptote (Brunner et al., 2005).

High-pressure processes are particularly energy intensive and economic feasibility depends on energy integration; the solvent cycle scheme plays a central role in this respect (Brunner, 1994). In supercritical fluid processes the solvent can be recirculated either in supercritical or in liquid state; correspondingly, the piece of equipment of choice would be a compressor or a pump. Pumps have a lower capital cost than compressors, but pump-based solvent cycles also require several heat exchangers and condensers and additional heat energy at low extraction pressures. Compressors have a higher capital cost than pumps, but compressor-based solvent cycles require only one heat exchanger and a limited thermal energy supply; at extraction pressures lower than 300 bar, the compressor-based system has higher electrical energy consumption and lower energy consumption compared to the pump cycle (Diaz et al., 2009).

Objective functions have been annualised cost, utility cost (Cygnarowicz and Seider, 1989), energy consumption (Diaz et al., 2000), net profit (Diaz et al, 2003), product cost (Bravi et al, 2002). The approaches range from DAE model resolution (Bravi et al., 2002), nonlinear programming (Cygnarowicz and Seider, 1989), mixed integer nonlinear programming (Diaz et al., 2000, 2003, 2005; Espinosa et al., 2005). Mixed integer programming permits the association of binary variables to design options (e.g. potential process units). Diaz et al. (2005) and Espinosa et al. (2005) performed the optimal design of process and solvent cycle by formulating and solving two nonlinear programming problems.

Rigorous mass transfer models are rare in such models: a dynamic optimization model for an extraction column, also including energy and momentum balances in the packed bed was developed and coded in gPROMS by Fernandes et al. (2007).

Alternative approaches include experimental data-based process optimization, which typically end up in nonlinear correlations among process variables (Létisse et al., 2007 adopted extraction temperature, solvent flow rate and operating time).

As an example here we discuss the optimisation approach followed by Bravi et al. (2002 and 2003). The optimisation requires 1. devising an industrially-feasible process layout and 2. identify optimal operating conditions and assess SFE-extracted sunflower oil economic economic acceptability in the food market. This latter aim requires the setup of a comprehensive mathematical model of the whole process (extraction section and recovery section).

The adopted process included multiple batch extractors in parallel, each containing a fixed bed of seeds, and multiple CO_2 recovery stages operating at decreasing pressures (Figure 4). The optimisation problem size was reduced by adopting: 1. Perrut's (1997) non-continuous and piecewise-linear solid-fluid equilibrium mathematical model of the extraction phase and extraction conditions (40 °C and 280 bar); 2. oil approximation as a single component; 3. mass transfer resistance occurring only in the solvent phase; 4. negligibility of in-extraction enthalpy variations; 5. requirement of constant extract flow rate.

A comprehensive model for the entire process describing extractors, expansion valve, separator, compressors, solvent recovery vessels, heat exchangers, pipe union joints and

branches was set up. The BWR equation of state was chosen as a trade-off between accuracy and computational weight.

The performance criterion (objective function) adopted for the optimisation was the unit oil production cost, estimated by rigorously accounting the operating costs directly referenced by the process design (e.g., compression costs and duty requirements) and applying short-cut techniques for all the remaining operating costs (e.g., manpower) and for the equipment cost estimates (by the cost index method). Aim was finding the minimum unit oil production cost as a function of the time allotted for the extraction phase on each seed batch and of the prevailing pressure in the oil separator at constant: size of each extraction vessel, number of simultaneously flowed extraction vessels and circulating solvent flow rate.

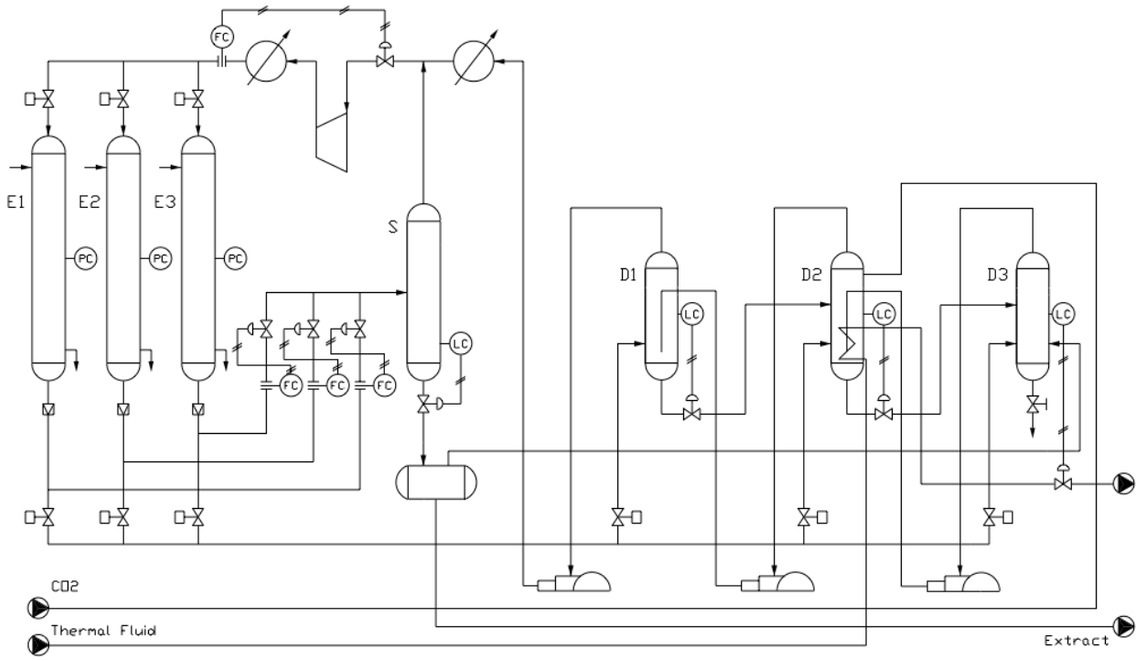

Fig. 4. Process scheme for contnuous sunflower oil extraction with supercritical CO_2 (Reprinted from Bravi et al., 2002, with permission from Elsevier).

A reduction of the required power expenditure for solvent recompression can be obtained by increasing the operating pressure of the oil separator but this reduces oil recovery. Cost minimisation led to optimal pressure in the separator of a single-extractor plant (100 bar). Furthermore, under the adopted equilibrium model, productivity collapses over time, when most of the seed bed has a very low oil concentration (Figure 5); from an economic standpoint product return decreases while the operating cost remains unchanged, thus leading to an increase of the average oil production cost. On the other hand, at the end of the extraction cycle the seed bed still contains an oil residue that cannot be exhausted by using re-circulated solvent but can be exhausted with hexane, yielding a lower-class product.

Reducing extraction time increases oil production rate at the expense of a lower recovery. In the example, extraction times of 20, 10, 5 minutes makes the best possible use of a plant with 3, 4, or 5 extractors. The authors found product cost to be minimum cost for a 4-extractor plant (0.67 Euro/kg)

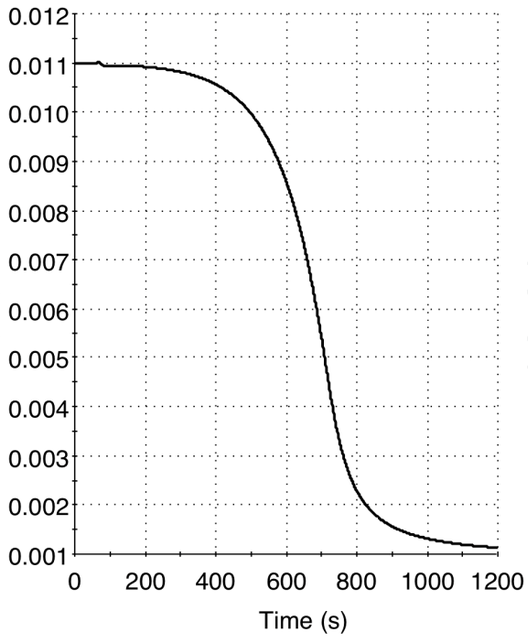

Fig. 5. Oil content in the supercritical phase leaving from a single extractor during a single extraction phase (Reprinted from Bravi et al., 2002, with permission from Elsevier)

Quality optimisation in extraction by SC-CO_2 can be obtained by suitably modelling the adopted quality parameters, which can be done relatively easily if these latter can be related to components whose concentration can be modelled by resorting to mass balances, mass transfer coefficients, and thermodynamic equilibria. Sunflower oil acidity modelling performed by Bravi et al. (2003) will be reported here as an example; another target may be the content of lipid-soluble alpha-tocopherol.

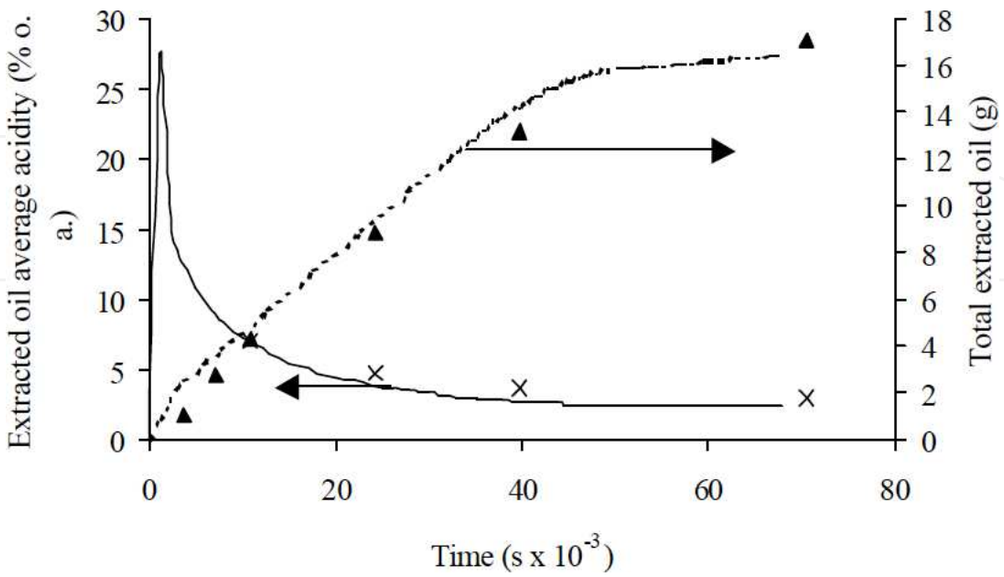

Fig. 6. Model prediction of instantaneous sunflower oil acidity and total extracted sunflower oil mass as a function of extraction time (From Bravi et al., 2003)

The mathematical model of this process takes into account the mass and enthalpy balances of carbon dioxide and oil in the extractor, the expansion valve and the separator. Oil is considered as being made of two components: free oleic acid (as acidity is customarily reported), and a triacyglyceridic pseudo-component. Perrut's et al. (1997) mathematical model of the oil extraction was rewritten to account for two simultaneously extracted components. The parameter estimation was carried out by optimising the model fit on the data obtained by means of a suitable oil quality-oriented experimentation.

Batch extraction runs were carried out on ground sunflower seeds in Perrut's conditions at different CO_2 flow rates and the composition was recorded on the collected oil in four well-mixed batches per extraction run. The results shows an initial constant-rate extraction phase after which productivity declines; the combined time profile of the collected oil acidity as a function of the amount of oil extracted clearly shows that the acid component tends to concentrate in the first oil fraction (Figure 6).

From the point of view of process optimisation an improvement of the quality of oil can be obtained by two means: 1. by eliminating the first portion of the extracted oil, which gives the largest contribution to acidity and/or 2. by extending the oil extraction degree; in order to keep some more oil extracted in the initial stages of the operation, much more must also be extracted in the final stages to ensure that the overall acidity is within the limits. Both of these measures increase the oil production cost because the oil extraction rate is maximum at the beginning of the operation and then decreases continuously and asymptotically to zero. As suggested by Bravi et al. (2002) the only partially exhausted oil matrix can be treated in a conventional extraction plant using hexane; we add here that the first extracted oil, which features an excessive acidity but is hexane-free, could be de-acidified and sold as a different product.

When optimising SFE yield in a stream concentrated in some desired component, identifying yield and extraction kinetics as a function of temperature, solids particle size distribution and CO_2-to-solids mass ratio are a key step before modelling can be applied. However, industrially-relevant practices may reduce yield compared to lab vales.

As an example, based on Molero Gómez's et al. (1996) set of oil yield-relevant set of optimal extraction conditions from grape seeds (pressure 200 bar, temperature 40°C, seed fragment size 0.35 mm, seed moisture content up to 6.5% and processing time 2 h), Bravi et al. (2007) carried out a study aimed at investigate the yield and extraction kinetics of α-tocopherol-enriched grape seed oil as a function of temperature, solids particle size distribution and CO_2-to-solids mass ratio (hereinafter denoted as CSMR) and identifying the optimal extraction conditions defined as those ensuring a high yield in α-tocopherol-concentrated oil. Their experimental procedure did not include pre-soaking (elsewhere denoted as 'equilibration') of the seed matrix to better reflect the prospective process conditions in a forthcoming industrial use (From Bravi et al., 2003, with kind permission from AIDIC Servizi s.r.l.).

They confirmed that oil yield with CO_2 (14.4%) is slightly below that with hexane (15.4%) when the vegetal matrix is treated at a moderately low temperature and observed that temperature effects on yields must be analysed with care, as they may be the result of complex interactions between oil solubility in CO_2 and mass transfer coefficients, arguing that this may be due to a soaking degree which changes during the extraction itself. As Bravi et al. (2007) pointed out, this may lead to unexpected inverted yield vs temperature relationships.

The effect of fragment size is that of increasing the oil yield at any tested CSMR; this effect can be explained with the increase in the available surface area for mass transfer and with the reduction of the time required for the initial soaking of the vegetal matrix. From the practical point of view, the results of their work suggest that the grape seeds should be milled to a maximum size of 425 mm.
As far as α-tocopherol in the extract is concerned, its concentration in the oil extracted with SC-CO_2 increases with the extraction temperature; this result is coherent with the higher solubility of α-tocopherol in SC-CO_2 at 80°C than at 40°C measured by Chrastil (1982).

3. Optimisation of enzyme-assisted extraction

Whatever the solvent, purely solvent-based extraction (i.e., without the intervention of synergic agents) of bioactive compounds often suffers from low extraction yields, and requires long extraction times and leaves traces of the organic solvent used, generally toxic to some extent. When solvent extraction is carried out, resistance to solute migration to the bulk of the solvent may be controlled within the solid phase, within the liquid phase, or be split among the two. When the first, or even the last is true, non polar solvents may be able to overcome cellulosic barriers but may be unable to solubilise the desired compounds; in turn, polar solvents may be a suitable solvent for the desired compounds but may be unable to reach the solute location site inside the matrix. In both cases, reducing the mechanical hindrance to solute migration may make the operation significantly faster and reduce solvent requirements; as a side gain, it also reduces any degradation reaction that may affect the desired product during the extraction itself.
Enzymes, derived from bacteria, fungi, animal organs or vegetable/fruit extracts, have been used particularly for the treatment of plant material prior to conventional methods for extraction. Plant materials like vanilla, pepper, mace, mustard, fenugreek, rose, and citrus peel which have potential as a rich source of flavor have been studied for enzyme-assisted extraction of flavors. Similarly enzyme-assisted extraction of color has been studied in plant materials like marigold, safflower, grapes, paprika, tomato, alfalfa, and cherries (Sowbhagya and Chitra, 2010). Winemakers may make use of pectolytic enzymes to break down the middle lamella between the pulp cells and the pulp and skin cell walls releasing pigments, improving both juice yields and colour extraction (Ducruet et al., 1997). A list of some commercially relevant products recently obtained using enzyme-assisted extraction is reported by Puri et al. (2011).
Enzymes have been used to enhance extraction (e.g. flavonoids from plant material as by Kaur et al., 2010) while minimizing the use of solvents and heat and disrupt the pectin–cellulose complex (e.g. in citrus peel to enhance flavonoid production by Puri et al., 2011). A digestion step prior to extraction by solvents was found to be necessary to efficiently carry out the extraction from the raw material (Dheghan-Shoar et al., 2011).
Various enzymes such as cellulases, pectinases and hemicellulases are often required to disrupt the structural integrity of the plant cell wall; these enzymes hydrolyze cell wall components and degrade the pectin-cellulose complex in fruits, thereby increasing cell wall permeability, which results in higher extraction yields of bioactives.
The enzymatic reactions are usually conducted at low temperature (15 °C to 45 °C), the actual operating temperature being dictated by the trade-off between the two controlling phenomena (mass transfer enhancement by increased temperature which reduces required

extraction time and thermal degradation of any thermolabile extracted compound). Above ~60 °C, heat alters the enzyme molecule irreversibly.

In order to carry perform cost-effective enzyme-assisted extractions the features and subtleties of enzyme catalysis must be considered and the appropriate enzyme or enzyme combination for the plant material selected must be identified.

Cost effectiveness and optimisation of enzyme-assisted extraction is obtained by identifying the optimal extraction conditions, aimed at maximising process profitability, which entails maximising the recovery rate of the target bioactive(s) while minimising their in-process degradation. Optimisation parameters, therefore, belong to two sets relevant to the enzymatic pretreatment and to the extraction phase respectively. The pretreatment should be optimised with regard to prevailing temperature and pH, pretreatment time, enzyme solution-to-solid ratio, solid particle size (distribution), enzyme load and enzyme composition; the extraction should be optimised with regard to prevailing temperature and pH, time, and solvent system deployed.

Synergism is due a special consideration when optimising enzyme-assisted extractions as it is for any enzyme-assisted hydrolysis. Considering the example of lycopene extraction from tomato waste, enzyme synergism may show up when they are acting simultaneously (e.g. Zuorro et al., 2011) or sequentially (Ruiz Teran et al., 2001). In the former case, enzyme preparations containing 50:50 pectinase and cellulase were found to have a significant synergistic effect (rate x18 w.r.t untreated material) with respect to simple enzymes (rate x3); in the latter, it was observed that cellulase (from *Trichoderma reesei*) and mixed enzyme cocktail (a mixture of arabinases, cellulases, hemicelullases, xylanases, and pectinases from *Aspergillus niger*) do not work efficiently when used together. Furthermore, results obtained using cellulase after the enzyme cocktail are similar to those observed when the cocktail is used alone. However, when cellulase was used first the extractive reaction proceeded with the highest efficiency (Table 1).

Enzymes used for pretreatment	Vanillin g/100 g
Water (control)	1.07 ± 0.08*
Cellulase + water	1.17 ± 0.06*
Cellulase + ethanol	2.70 ± 0.17*
Viscozyme + cellulase + water	1.17± 0.11
Viscozyme + cellulase + ethanol	2.66 ± 0.07
Cellulase + viscozyme + water	2.30 ± 0.10
Cellulase + viscozyme + ethanol	3.66 ± 0.04
Viscozyme + water	1.17 ± 0.05*
Viscozyme + ethanol	2.45 ± 0.21*

Table 1. Effect of enzyme treatment on vanillin extraction from vanilla beans. Viscozyme L. (Novozymes) is a mixture of arabinases, cellulases, hemicelullases, xylanases, and pectinases (Adapted from Ruiz Teran et al., 2001).

Enzyme-assisted extraction of bioactive compounds from plants has potential commercial and technical limitations: 1. the cost of enzymes (although this is going to decrease thanks to biofuel research); 2. inability of currently available enzyme preparations to hydrolyze some fractions of plant cell walls, limiting extraction yields of some compounds; 3. scale up to

industrial scale may be troublesome because local process conditions in the equipment may be difficult to maintain at the larger scale.

Enzyme-assisted extraction optimization by traditional methods is time consuming and can ignore the interactions among various factors. The response surface method enables the evaluation of several process parameters simultaneously (along with their interactions up to the desired order) such as: time, temperature, pH, enzyme type and concentration. During cell wall degradation the polysaccharide–protein colloid can be degraded thereby creating an emulsion that interferes with the extraction. Therefore, non-aqueous systems are preferable for some materials because they minimize the formation of polysaccharide–protein colloid emulsions (Puri et al., 2011). Prior knowledge of the cell wall composition of the raw materials helps in the selection of an enzyme or enzymes useful for pretreatment; predictive methods would be useful to speed up the optimisation or keep up with product property changes, but nothing covering this gap has reached the open literature yet.

4. Optimisation of Microwave-Assisted Extraction (MAE)

Microwaves are electromagnetic waves in the frequency range 300 MHz to 300 GHz, that is between wavelengths of 1 cm and 1 m. Most small size microwave instruments operate at 2450 MHz and have an energy output between 600-700 W**.** At this frequency, the electric field changes the orientation of water molecules 2.45 x 109 times every second, while thermal agitation tends to restore the chaos inherent to the system. Thus creating an intense heat that can escalate as quickly as several degrees per second (depending on frequency and sample size, it has been estimated up to 100 °C/s at 4.9 GHz by Lew et al., 2002). The heating phenomenon is based on the interaction of the electrical field with the individual compounds of a material (possibly characterised by an inhomogeneous macrostructure). The transformation of electromagnetic energy in thermal energy occurs by two mechanisms: ionic conduction and dipole rotation. The ionic conduction generates heat due to the resistance of medium to ion flow. The migration of dissolved ions causes collisions between ions and molecules because the direction of the ions changes every time the electromagnetic field changes its orientation. Dipole rotation is related to the alternating movement of polar molecules, which try to line up with the electric field; thus only selective and targeted materials warm-up based on their dielectric constant.

The efficiency of the microwave heating depends on the dissipation factor of the material, tan δ, which measures the ability of the sample to absorb microwave energy and dissipate heat to the surrounding molecules as given by:

$$\varepsilon'' \tan\delta = \varepsilon'$$

where ε″ is the dielectric loss which indicates the efficiency of converting microwave energy into heat while ε′ is the dielectric constant which measures the ability of the material to absorb microwave energy. The rate of conversion of electrical energy into thermal energy in the material is described by:

$$P = K f \varepsilon' E^2 \tan\delta$$

where P is the microwave power dissipation per unit volume, K is a constant, f is the applied frequency, ε′ is the material's absolute dielectric constant, E is the electric field strength and tan ı is the dielectric loss tangent.

Multiple collisions from this agitation of molecules generate energy dissipation and therefore a temperature increase whose value depends on the local intensity of energy dissipation, on the local specific heat, and on the local conductivity by which heat diffuses to or from neighbouring areas of the material when these latter are cooler or hotter.

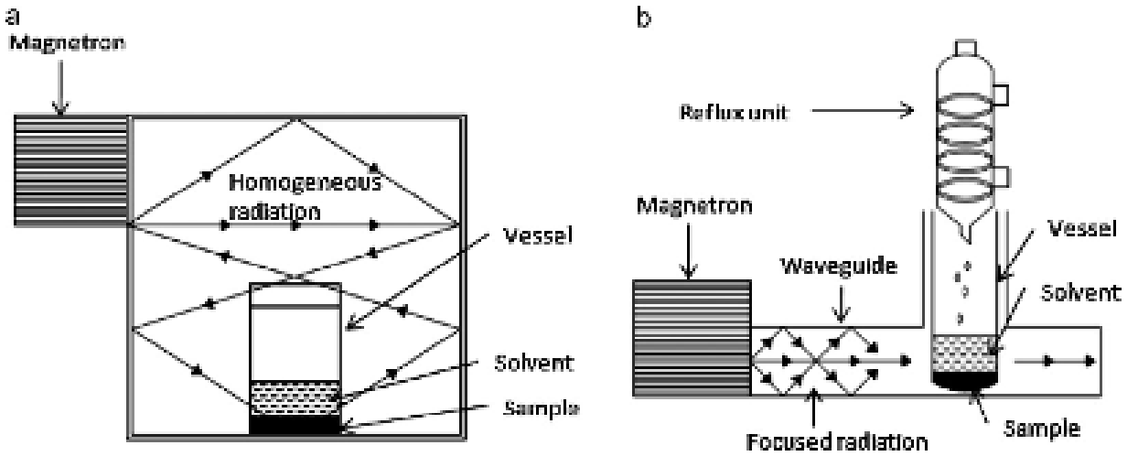

Fig. 7. (a) Closed type microwave system and (b) open type microwave system. (Source: Mandal et al., 2007)

Microwave-assisted extraction aims at supplying heating locally where the solvent or soaking medium is present, thus speeding up heating, with the aim of reducing bioactives degradation reactions and cause a discregation of the vegetal matrix structure. This latter aim requires that the basic structure of the extracted particles be stiff. Vegetal cells generally feature a stiff structure; microalgae lacking frustule or thick outer exopolysaccharide envelope may not benefit from microwave-assisted extraction (Pasquet et al., 2011), while wall-possessing microalgae benefit greatly from it (yields 3x-5x in a fraction of the time; Cravotto et al., 2008).

Microwave assisted extraction may be carried out in closed and open systems (Figure 7). Modifications of the basic scheme include operation under reduced pressure (for operation at reduced temperature and suction-enhanced migration of solutes); under nitrogen blanket (for increased protection against oxydation); without added solvent (resorting to constitutive/hydration water); with simultaneous microwave and ultrasound field (to facilitate the formation of cracks in the solid matrix, change the perceived polarity of the solvent and enhance the mass transfer) (Chan et al., in press).

Microwave-assisted extraction effectiveness strongly depends on: solvent choice, solvent to feed ratio, extraction time, microwave power, temperature, sample characteristic, effect of stirring.

Solvents which are suitable for conventional extraction techniques may not be suitable for microwave assisted extraction. Ethanol (used in the 40% to 100% concentration range) is a good microwave converter (good absorber as a dipole with a low specific heat) and is by itself suitable for extracting many active compounds from plants. However, modifiers can be added to solvents which are unsuitable per se to microwave capture in order to enhance their overall performance. Water was added as modifier to diethyl ether and ethanol or water can be added into poor microwave absorber such as hexane to enhance microwave

heating efficiency (Alfaro et al., 2003; Ku et al., 2007). Room temperature ionic liquids interest lies in their negligible vapor pressure, wide thermal range in the liquid state, good thermal stability, tunable viscosity, miscibility with water and organic solvents, good solubility and extractability for various organic compounds (Du et al., 2007). Mixing carbonyl iron powders with the moist sample may increase absorption of microwave energy, particularly where solvent is limited (such as in SFMAE by Wang et al., 2006).

An optimum ratio of solvent to solid ratio ensures homogeneous and effective heating; above, heating may be insufficient and below mass transfer barrier may establish as the distribution of active compounds is concentrated in certain regions and their displacement out of cell matrix may be impaired (Mandal et al., 2010). The optimum ratio of solvent to solid ratio normally is in the interval 10--50 ml per g of solid matrix (Chan et al., in press).

Microwaves heat the sample locally and act as a driving force for damaging the plant matrix so that analytes can diffuse out and reach the solvent. Furthermore, the solvent viscosity and surface tension decrease improves mass transfer. Therefore, increasing the power will generally improve the extraction yield and result in shorter extraction time; however, the gain may be offset by thermal degradation of the solute, so that an optimal temperature (and microwave power) exist for each application (Chan et al, in press).

Process time (regardless of power) is from a few minutes to more than 1 h. Extending process time has been found to decrease the extraction yield due to thermal degradation, oxidation, or hydrolysis. The optimum microwave application mode should be adapted to the type of solid matrix and may be power-regulating (continuous medium power or pulsed high-power microwaves), for optimised degradation of the solid matrix; temperature-regulating (power is dependent variable), for optimised preservation of the bioactive extracts.

Prior to treatment the vegetal matrix is usually dried, powdered and sieved, avoiding too a small particle size which would be difficult to separate and would require equipment clean-up procedures later. Pretreating with water the vegetal matrix after drying may increase bioactives release thanks to the localised heating of the trapped soaking water which gives rise to cracks. The modified water content of the vegetal matrix also alters the balance between hydrolyzation (favoured) and oxidation of active compounds (Wang et al., 2006).

Stirring mitigates the negative effect of low solvent to feed ratio on extraction yield by improving mass transfer and thus avoiding the buildup of concentration gradients that impair the dissolution of bioactives bound to the sample matrix.

The determination of optimum MAE operating conditions is usually carried out through statistical optimization studies. A collection of optimised extraction conditions for a number of plant-sourced vegetal matrices is reported by Chan et al. (in press)..

A variation of MAE involves the deployment of microwaves without any added solvent. Historically, dry distillation was used by alchemists for sublimation and extraction. Solvent free microwave-assisted extraction (SFME) was conceived for laboratory scale applications in the extraction of essential oils from different kinds of aromatic plants. SFME is neither a modified microwave assisted extraction (MAE) which use organic solvents, nor a modified hydro-distillation which use a large quantity of water, both of which are more energy intensive due to the larger heat requirements to evaporate and condense the added solvents. Based on a relatively simple principle, this method involves placing plant material in a microwave reactor, without any added solvent or water. The heating undergone by

constitutive water within the plant material expands the plant cells and leads to rupture of the glands and oleiferous receptacles, whereby essential oil is first freed and then evaporated together with the in situ water of the plant material. The vapours are continously condensed by a cooling system located outside the microwave oven. The water excess is refluxed to the extraction vessel in order to restore the in situ water to the plant material. A large number of different essential oils have been extracted by SFME (Lucchesi et al., 2004).

5. Optimisation of ultrasound-assisted extraction

Extraction enhancement by ultrasound is attributed to the propagation of ultrasound pressure waves, And to the resulting cavitation phenomena. The implosion of cavitation bubbles generates turbulence which accelerates diffusion and impingements and collisions which result in surface peeling, erosion, punching (Ugarte-Romero et al., 2006) and particle breakdown. This effect provides exposure of new surfaces further increasing mass transfer (Vilkhu et al., 2008).

Dry materials may swell, hydrate and increase their pore size under ultrasound treatment. Furthermore the particle size distribution of the vegetal matrix is shifted toward the smaller sizes, so that the cell surface directly exposed to extraction increases (Vinatoru, 2001).

Solvent selection is usually based on achieving high molecular affinity between the solvent and solute. When cavitation bubbles are generated in the bulk of the solvent phase by the ultrasound field, their hydrophobic surfaces increase the net hydrophobic character of the extraction medium (Vilkhu et al., 2008) so that its affinity toward non polar components is increased. Cavitation being initiated by drop of the total pressure below the saturation pressure of the solvent, ultrasound assistance in extraction with a given solvent will be affected by the physical properties of this latter (cavitation intensity decreases as vapour pressure and surface tension increase). When a supercritical solvent is used, cavitational events are impossible, since there is no liquid/gas phase boundary. However, other mechanisms are postulated, such as acoustic streaming and the presence of gas pockets in the solid causing cavitational collapse (Patist and Bates, 2008).

Ultrasound may also give rise to multiple simultaneous processes, such as extraction and (sono)chemical modification, whereby the food product may be modified by physical and chemical mechanisms. Cravotto et al. (2004), for instance, reported wax conversion to policosanol (common name for a mixture of C_{24}–C_{34} linear saturated fatty alcohols, a rich source of nutrients and pharmacologically active compounds) during rice bran extraction by using ultrasounds.

Although it is relatively easy to perform an ultrasound-assisted extraction at the laboratory scale, designing it for industrial scale is quite demanding. Some of the issues that need consideration when attempting to design an optimal ultrasound-assisted process at a significant scale have been reported by Vilkhu et al. (2008): 1. the nature of the tissue being extracted and the location of the components to be extracted with respect to tissue structures; 2. pretreatment of the tissue prior to extraction; 3. the nature of the component being extracted; 4. the effects of ultrasonics primarily involve superficial tissue disruption; 5. increasing surface mass transfer; 6. intra-particle diffusion; 7. loading of the extraction chamber with substrate; 8. increased yield of extracted components; 9. increased rate of extraction, particularly early in the extraction cycle enabling major reduction in extraction time and higher processing throughput.

Apparently, there is potential for ultrasonic cavitation to propagate free radicals (hydroxyl). Radical production should be quenched by the addition of small amounts of ethanol to cool cavitation bubbles and slow any radical-involving reactions (Vilkhu et al., 2008).

6. Optimisation for novel sources of bioactives: Microalgae production

The microalgae have in practice an interesting composition in regard to main components such as protein, polyunsaturated fatty acids (PUFA), pigments, and carbohydrates (Doucha 2009). The protein content is consistently high in micro algae. Some cyanobacteria (blue-green algae) are characterised by a high protein content (60-65%), not commonly found among higher plants. But, for full utilisation of the protein, special treatment of the microalgae is generally necessary. Moreover, microalgae are excellent producers of essential amino acids. However, until to date only three species are cultivated on industrial scale level: these are the cyanobacterium *Arthrospira,* the green algae *Chlorella* and *Dunaliella.* Their biomass is used for production of a rather limited range of products, most of them directed to the nutraceutical market. The success of these three species is due to the fact that they can be grown in a very selective medium (*Arthrospira* and *Dunaliella*), therefore contamination of parasites or competing organisms (microalgae, fungi, and others) is naturally prevented even in open reactors where it is possible to ensure a low cost of production for the biomass (Boussiba and Affalo, 2005), while *Chlorella* is endowed with a remarkably high growth rate sustained by organic acid addition in fermentor.

6.1 *Chlorella vulgaris*

Chlorella vulgaris cells contain β-1,3-glucan, polysaccharides and also a rich source of proteins, 8 essential amino acids, vitamins (B-complex, ascorbic acid), minerals (potassium, sodium, magnesium, iron, and calcium), β-carotene, chlorophyll, ''CGF" (Chlorella growth factor), as well as other health-promoting substances (Hac´on-Lee et. al 2010). β-1,3-glucan is an active immunostimulator, a free- radical scavenger and a reducer of blood lipids (Ryll et al., 2003). However, various other health-promoting effects have been clarified (efficacy on gastric ulcers, wounds, and constipation; preventive action against atherosclerosis and hypercholesterolemia and antitumor action). *Chlorella vulgaris* biomass has colouring properties and has been tested with success as a pigment source for farmed products with functional activity (e.g. as antioxidants). The total annual production of *Chlorella* is estimated to be about 2000 t. The production process is based on the mixotrophic nature of the *Chlorella* strains and uses acetic acid as a carbon source. The production cost of biomass is not clear. However, based on claims that in those systems a high biomass concentration is achieved (more than 10 g/l) one may reach the conclusion that the production cost may be a figure close to those of open pond systems (10 -15 US$/Kg). However, the cost can raise to up to 30 US$/Kg, for example in the Central part of Europe where the adverse climatic conditions do not allow to grow the alga all the year around. Up to about 10 years ago most of the production took place in Taiwan and only 10-15% of the total production was carried out in green houses in Japan. The market for *Chlorella* products is limited to the Far East, mainly Japan. In some early works it was reported that *Chlorella* extracts may have affect the growth and production of lactic acid by lactic bacteria. The growth facilities are based on round concrete ponds mixed by a rotating arm which also provides CO_2 and acetic acid

supply. Another development that should be mentioned is the attempt to set up new facilities for culturing *Chlorella* in Europe. One of them is located in Czech Republic (2 tons a year production capacity, Kopecky personal communication) which is based on inclined reactors that allow to maintain a fast flow rate of thin layer culture and as a result enables maintenance of high biomass concentrations and high volumetric productivity (Masojidek et al. 2010). The second facility is located in Germany, and uses tubular photobioreactors made with glass tubes arranged on a vertical fence and placed in a greenhouse. The total annual capacity is claimed to reach 150 tons per year (Pulz 2001). Although *Chlorella* market is still the largest one in term of gross revenue US$, one can expect that without developing new products and reducing the production cost *a sine qua non condition* for use of *Chlorella* biomass as feed additive in the animal feed market, it is difficult to expect an expansion of the production capacity.

6.2 *Arthrospira platensis*

At present *Arthrospira* (commercially indicated as *Spirulina*) represents the second most important commercial microalga in term of total market value US$ (after *Chlorella*), while in terms of total biomass produced, the *Arthrospira* market is twice or more of that occupied by *Chlorella* (Torzillo and Vonshak 2003). The major producers of *Arthrospira* are the DIC group of companies, Earthrise in California, USA, Hainan DIC Marketing in Hainan Island, China. On the whole these facilities produce about 1000 metric tons of *Arthrospira* annually (Belay et al. 2008; Sili et al. 2011). An other important *Arthrospira* producer is Cyanotech Corporation of Hawaii with an annual production of 300 tons. Other producers are located mainly in the Asia-Pacific region, particularly in China and India (Lee et. al 1997). The highest production capacity of *Arthrospira* biomass takes place in China. Recent estimates are that the total potential of the different sites of this country may exceed 2000 t. Production is carried out in raceway ponds of 2000-5000 m^2 in size and may contain between 400 and 1000 m^3 of culture according to the dept adopted which can vary between 15 and 40 cm depending on season, desired algal density and, to a certain extent, the desired biochemical composition of the final product (Belay et. al 2008). The major share of the market for this organism is for health food involving crude biomass production. This has the advantages of simple processing (harvest and rudimentary handling) keeping production costs reasonably low, and of eluding the competition of the chemical industry which cannot match the wealth in nutritional bioactive components and attractiveness of natural products (Boussiba and Affalo 2005). *Arthrospira platensis* (Soletto et. al 2008, Harun et. al 2010) has commercialized as nutraceutical food, also a strong immune-stimulated molecule, Immulina®, can be extracted from it (Grzanna et. al 2006).

6.3 Dunaliella

This species is being grown as a source of beta-carotene. this carotenoid can accumulate in the cells grown under nutrient limitation and high sun light up to 12% of the dry weight (Ben-Amoz and Avron 1973). This pro-vitamin A product is widely used in the feed and food industry. Today, the product is available mainly in two forms: dried or extracted. Dried *Dunaliella*, in powder or pill form, is considered to be highest quality. The product is harvested by means of concentration and centrifugation, and thereafter dried by spray-drying. In this form, the product is mainly addressed to health food market for direct

human consumption. The price is based on the beta-carotene content, and can reach about 2000 US$ per Kg of beta-carotene. The second kind of product is beta-carotene extracted into a vegetal oil. This product can be applied as a food colorant and a pro-vitamin additive for human consumption, for fish and poultry feed, or in the cosmetic industry as an additive to sunscreen products. From few reports it is estimated that the price, on the basis of beta-carotene content, varies in the range of US$ 500-600 per Kg of beta-carotene.

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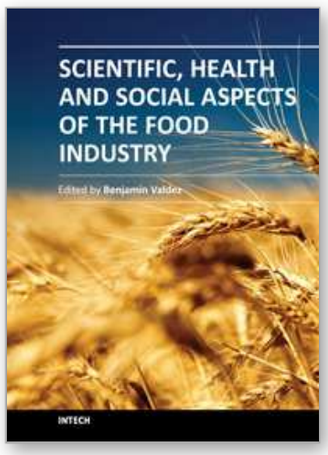

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This book presents the wisdom, knowledge and expertise of the food industry that ensures the supply of food to maintain the health, comfort, and wellbeing of humankind. The global food industry has the largest market: the world population of seven billion people. The book pioneers life-saving innovations and assists in the fight against world hunger and food shortages that threaten human essentials such as water and energy supply. Floods, droughts, fires, storms, climate change, global warming and greenhouse gas emissions can be devastating, altering the environment and, ultimately, the production of foods. Experts from industry and academia, as well as food producers, designers of food processing equipment, and corrosion practitioners have written special chapters for this rich compendium based on their encyclopedic knowledge and practical experience. This is a multi-authored book. The writers, who come from diverse areas of food science and technology, enrich this volume by presenting different approaches and orientations.

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