Greener Solvents for Old Challenges

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ABSTRACT

At present, there is a clear need for alternative non-toxic solvents able to meet the Principles of Green Chemistry and Green Engineering and preferentially obtained from renewable sources. In the present work, three green solvents have been used, namely ethanol, ethyl lactate and limonene, to obtain high value lipids from the cyanobacteria Spirulina (Arthrospira platensis). Among the lipids found in Spirulina, γ-linolenic acid (GLA) is the most valuable, and is found at high concentration. Moreover, considering that Spirulina is easy to grow, and that the syntheses of different compounds mainly depend on growing conditions, the interest of obtaining lipids from algae and microalgae is increasing every day.

Ethyl lactate and limonene are from natural origin and easily biodegradable; they can be found traditionally in consumed foodstuff like wine or beer (ethyl lactate) or fruits (limonene). These solvents have polarities in the range of acetonitrile and hexane, respectively. Therefore, these solvents, together with ethanol, can be used to cover a wide range of polarities.

In order to achieve greener extractions, pressurized liquid extraction (PLE) with ethanol, ethyl lactate, limonene and their binary mixtures, together with ethanol-expanded CO_2 (gas-expanded liquid; GXL) extraction were selected. Advantages of PLE are mainly the low solvent consumption, short extraction times and improved kinetics. On the other hand, the use of GXLs has, over SFE, the advantage of milder pressures and can be considered as an extraction liquid media ranging from the neat organic solvent to supercritical-CO_2 (SC-CO_2).

Optimization of extraction conditions was carried out using chemometric approaches, namely: a Taguchi L9 design for the gas expanded liquid extractions (ethanol:CO_2) and a central composite (4^3) for PLE experiments.

The responses elected for the optimization were: total yield, fatty acid content and γ-linolenic acid in extracts. For its quantification GC-MS was used previous derivatization by ethylation.

INTRODUCTION

Spirulina *(Arthrospira platensis*) lipids have been extracted using supercritical CO_2 as a green alternative to hexane [1], but extraction yields are lower than hexane extractions.

Gas Expanded Liquids (GXLs) are emerging as a promising media to perform extractions, reactions, and separations. A GXL is a liquid whose volume is increased when pressurized with a condensible gas such as carbon dioxide. CO_2-expanded liquids are the most commonly used classes of GXLs representing liquid media ranging from the neat organic solvent to supercritical-CO_2 (SC-CO_2) [2]. However, the possible use of GXLs for the extraction of highly valuable lipid components in foods or natural products has not been explored yet although the milder pressures required for working with GXL, as compared to traditional systems, make the industrial applications of GXLs commercially attractive [2].

Pressurized liquid extraction (PLE) is an extraction method alternative to supercritical fluid extraction (SFE). Compared to SFE, in PLE the solvent is kept below its critical conditions. The use of liquid solvents heated above their normal boiling points, but pressurized to keep them in the liquid state, favours the use of shorter extraction times and reduces solvent consumption compared to traditional Soxhlet extraction [3].

Despite all the well-known advantages of supercritical CO_2 as an effective and environmentally friendly solvent to obtain valuable products from natural sources [4,5], its low polarity could be stated as its major disadvantage for its use in extraction processes dealing with polar/semipolar compounds. Lactate esters have demonstrated excellent solvent properties and low toxicity and are potential alternatives to many halogenated solvents. Lactate esters such as ethyl lactate have the ability to dissolve a wide range of chemicals; ethyl lactate has been approved by FDA for use in food industries [6]. Ethyl lactate is a colorless solvent produced from the fermentation of carbohydrate feedstock available from the corn and soybean industries. On the other hand, the use of limonene as non-polar extracting agent to replace hexane has been slightly studied and only few examples can be found in the literature, to extract rice bran oil [7], or carotenoid from tomatoes [8].

The aim of the present work was to evaluate the use of novel extraction techniques and solvents to obtain γ-linolenic acid (GLnA)-enriched fractions from Spirulina microalgae. A first approach consisted on chemometric optimization of ethyl lactate combined with ethanol under pressurized conditions (using pressurized liquid extraction, PLE) and GXLs (mixtures of CO_2:ethanol under near critical conditions). A second optimization was also studied under the best PLE conditions but using limonene as extracting solvent. The main goal was to compare two green solvents of different polarity: ethyl lactate (semi-polar) and limonene (non-polar) towards the extraction of a valuable fatty acid, GLnA.

MATERIALS AND METHODS

Samples and reagents

Spray-dried Spirulina was purchased from Algamar S.A. (Pontevedra, Spain) and stored under dry and dark conditions until use. Ethanol and washed sea sand (0.25-0.30 mm diameter) were supplied by Panreac Quimica S.A. (Barcelona, Spain). Ethyl lactate, d-limonene (food grade, kosher), acetyl chloride, GLnA, heptadecanoic acid, butylated hydroxytoluene (BHT) and polyunsaturated fatty acid (PUFA) standards of marine source (PUFA No. 1) were purchased from Sigma-Aldrich (St. Louis, MO). n-Hexane was purchased from Labscan (Dublin, Ireland). He, CO_2 (both Premier quality) and N_2 (Technical quality) was obtained from Carburos Metalicos (Air Products Group, Madrid, Spain). Deionized water was obtained using a Milli-Q system from Millipore (Molsheim, France).

Pressurized liquid extraction (PLE)

PLE extraction experiments were performed using an Accelerated Solvent Extractor™ system (ASE 200, Dionex Corporation, Sunnyvale, CA). 2.0 g of Spirulina and 4.0 g of sea sand were mixed and loaded into an 11 mL volume extraction cell. The extraction cell was fitted with cellulose filter at the inlet and outlet. In the first step, the extraction cell was filled with the selected solvent and the pressure was increased to the desired level. Initial heat-up time was then applied depending on the extraction temperature. After the static stage of extraction, the cell and the tubing were rinsed using fresh solvent. Then, all the solvent present in the system was purged using N_2 gas. The solvent from this stage was collected in a vial and pressure of the unit was released. The extracts were subjected to solvent removal with vacuum for ethanol and hexane, while N_2 stream at 100 °C for ethyl lactate and limonene removal.

Gas Expanded Liquid Extractions

A Suprex PrepMaster (Suprex, Pittsburgh, PA) supercritical fluid extractor was used for the GXL extractions; this extractor was equipped with a dual piston pump for CO_2. Two g of Spirulina was mixed with 4.0 g of sea sand and the mixture was loaded into a 20 ml stainless-steel extraction cell. The extraction cell was fitted with glass wool at the inlet and outlet. Ethanol was pumped using a Jasco PU2080 HPLC pump (Jasco Inc., Easton, PA) and mixed at high pressure with supercritical CO_2. Compressed fluid mixture was fed to the heater prior to entering the extraction cell. The flow rate was controlled using a needle valve as variable restrictor. Extracts were collected

in a glass vessel cooled by ice. To avoid sample degradation, the extracts were stored at -20 ºC protected from light until drying step, using a rotary evaporator.

Chemometric optimization

The GXL experiments were designed using Taguchi's L9 orthogonal array that can deal with four factors at three levels each by performing nine experiments [9]. Design-Expert version 8.0.3 (Stat-Ease Inc., Minneapolis, MN) was employed for analyzing the relationship between the responses and process factors and also for the prediction and verification of model equation. Factors elected were: temperature, pressure, dynamic extraction time and % ethanol. Those factors ranged between: 40-80 ºC, 10-30 MPa, 30-90 min and 10-50% ethanol respectively.

To optimize PLE experiments a four-factorial (extraction temperature, extraction pressure, extraction time and solvent composition), three-level central composite design consisting of 30 experimental runs was performed. Experimental values ranged from 60 to 180 ºC for temperature, from 3.4 to 20.7 MPa for pressure, from 5 to 15 min (one cycle) for time and from 100% ethanol to 100% ethyl lactate for the solvent composition.

The rest of the PLE experiments, were carried out using n-hexane, n-hexane:ethanol (1:1, v/v), limonene, and limonene:ethanol (1:1, v/v) as solvent, at fixed temperature, pressure, and extraction time of 180 ºC, 20.7 MPa, and 15 min, respectively.

Fatty acid analysis

To determine the fatty acid content in extracts, GC-MS analysis was performed. Previous to qualitative and quantitative determination, an esterification step was performed using ethanol-acetyl chloride (95:5, v/v). Then ethylated samples were injected in a Shimadzu QP 2010 Chromatograph equipped with a 007-CW Carbowax, 12 m × 0.1 mm × 0.1 μm column (Quadrex, Woodbridge, CT, USA).

RESULTS

The different responses used for optimization were:

Total yield (%, w/w) = ((Weight of the Extract (g) / Weight of Spirulina (g)) x 100 *(1)*
Lipid % in the extract = ((Weight of the Extracted Lipids (g) / Weight of the Extract (g)) x 100 *(2)*
GLnA % in the extract = ((Weight of GLnA (g) / Weight of Extract (g)) x 100 (*3)*
GLnA recovery= ((GLnA % in the extract /Total yield (%, w/w))/ Total GLnA % in Spirulina) x 100 (*4)*

These responses can be grouped in two types, those related to the amount of material extracted (total yield and GLnA recovery %) and those accounting for the selectivity of the process (% of lipid and GLnA in extract).

Total yields (%, w/w) were up to 3 times higher in PLE (ethanol:ethyl lactate) than in GXL (ethanol:CO_2). The use of one extracting phase seems to favor the recovery of a higher amount of extract, in contrast to the use of two phases in GXL experiments ($SCCO_2$ and expanded liquid ethanol). Since ethanol is used at a level higher than that of its solubility level, the extra amount will be present as a second (liquid) phase. Critical conditions of ethanol (6.1 MPa and 240 °C) are always higher than the maximum conditions of pressure and temperature (30.0 MPa and 80 °C) applied in the $SCCO_2$-expanded ethanol extractions of this study and as a result the additional ethanol in the system can be considered in its subcritical state [10].These results are in agreement with those obtained previously in our research group concerning Spirulina extraction using PLE and supercritical fluid extraction, SFE [1,11]. Although the average GLnA level in the extracts was a bit higher when using GXLs for the extraction (8.8% vs. 7.3% for PLE), the higher yields obtained in PLE resulted in higher recoveries of GLnA when using this extraction technique.

By using a factorial experimental design, it is possible to obtain information not only on individual factors but also on possible interactions among the various factors of the study. Such data can provide clearer and more complete understanding of the processes taking place during the extraction. Moreover, an experimental design can lead to an equation (a model) to help predict the future responses by changing the value of the factors in the model [1].

For all responses considered, temperature was the main factor in the case of PLE (ethyl lactate:ethanol), in fact, the most significant one (Figure 1a). This can be explained by an increase in the solubility of the components at higher temperatures and also by a decrease in the liquid (solvent) viscosity favoring the mass transport diffusion from the matrix to the liquid solvent. On the contrary, the effect of temperature on the GLnA fraction in the extract is negative. Such reduction in the GLnA fraction due to higher temperature is also expected by considering the increased number of the compounds (other than lipids) extracted at higher temperatures.

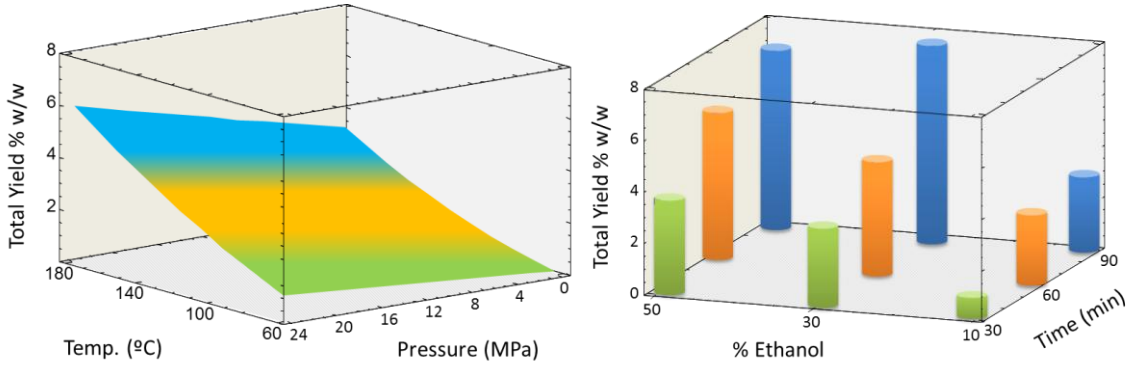

Figure 1.- Effect of significant factors (temperature and pressure) on the total yield (%, w/w) of pressurized liquid extraction using ethyl lactate:ethanol (a). Effect of significant factors (time and ethanol (%)) on the total yield (%, w/w) of gas expanded liquid (GXL) extraction (b).

Considering the GXL experiments, temperature was found the less significant factor. On the other hand, the fraction of ethanol and also the extraction time were the main parameters. Figure 1b shows the effects of the main factors of this study on the total yield (%, w/w) of the extract. Total extraction yield (%, w/w) for GXL increased by increasing both extraction time and ethanol fraction (%) in the solvent. Such behavior in the extraction with GXL is quite different from that of the traditional SFE. In a GXL, the physical behavior resembles that of a pressurized liquid, as can be seen when approaching levels up to 50%. In fact, Figure 2 shows the important increase in the total extraction yield (%, w/w) when increasing ethanol contribution from 10 to 30%, where the changes in the physical behavior of ethanol from a supercritical (or near-critical) fluid to a GXL occurs.

The use of experimental designs can be helpful for optimizing the conditions to achieve the best results. The optimal conditions predicted by the model are shown in Table 1. As expected, maximum temperature was proposed as the optimum conditions in PLE while maximum time and ethanol (%) was suggested as optimum conditions for GXL. As can be seen in Table 1, the prediction is pretty close to the mean real value proving the fitting of the mathematical models to the real extraction processes.

Under the optimized PLE conditions, limonene was tested as an alternative solvent to substitute non-polar toxic solvents like hexane. In this case, pure limonene and limonene:ethanol (1:1) were employed together with hexane, that was used for comparative purposes. As mentioned, the optimized PLE conditions were the following: 180 °C; 20.7 MPa; 15 min. Extractions using pure limonene provided twice as much yield than those using pure hexane, and around half of the yield obtained when ethanol was used as cosolvent; this fact could be due to the high amount of polar lipids in Spirulina, namely, phospholipids, glycolipids and other cell membrane lipids. Compared to previous works where the fatty acid profile of Spirulina extracts was studied by using non polar extraction solvents (namely Soxhlet hexane extraction or supercritical CO_2) [1, 12], pressurized limonene extractions provided similar results in terms of fatty acid compositions. But PLE provided, compared to Sohxlet

extraction [13], higher GLnA content with shorter extraction times and lower volume consumption. Therefore, it can be concluded that hexane can easily be replaced by limonene and that the use of this solvent in PLE has several advantages in terms of yields, recoveries and environmental impact.

Table 1. Conditions for the optimized pressurized liquid extraction (PLE) and gas expanded liquid (GXL) predicted by software and experimental responses obtained for the extraction of γ-linolenic acid (GLnA)

Extraction methods	Optimized conditions				Predicted responses		Experimental responses	
	T† (°C)	P‡ (MPa)	Extraction time (min)	Ethanol (%)	Total yield of extract (%, w/w)	GLnA recovery (%)	Total yield (%, w/w)	GLnA recovery (%)
PLE (ethyl lactate)	180	20.7	15	50	21.5	63.9	20.7	68.3
GXL (CO_2)	40	30.0	90	50	7.0	28.0	6.7	24.7

†Temperature; ‡Pressure

GLnA recovery: Total amount of extracted GLnA to the total amount of GLnA present in Spirulina

CONCLUSION

In the present work the ability of two alternative extraction techniques (GXLs and PLE with ethyl lactate and limonene) to obtain high-value lipids from natural products has been demonstrated. By using statistical models, it was possible to describe and predict future responses such as total yield and GLnA recovery. GXLs have been used in this work for the first time in food products and have demonstrated its performance as an intermediate between PLE and supercritical fluids for the extraction of medium-polar compounds.

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REFERENCES

[1] MENDIOLA J. A., Jaime L., Santoyo S., Reglero G., Cifuentes A., Ibañez E., Señoráns F. J, Food Chem., 102, (**2007**), 1357-1367

[2] SCURTO AARON M., Hutchenson K., Subramaniam B., *Gas-Expanded Liquids: Fundamentals and Applications*, in: Gas-Expanded Liquids and Near-Critical Media, American Chemical Society, **2009**, pp. 3-37.

[3] WANG L., Weller C.L., Tr. Food Sci. Technol., 17 (**2006**) 300-312.

[4] HERRERO M., Mendiola J.A., Cifuentes A., Ibáñez E., J. Chromatogr A, 1217 (**2010**) 2495-2511.

[5] PEREIRA C.G., Meireles M.A.A., Food Bioproc. Technol., 3 (**2010**) 340-372

[6] VU D.T., Lira C.T., Asthana N.S., Kolah A.K., Miller D.J., J Chem Eng Data, 51 (**2006**) 1220-1225.

[7] MAMIDIPALLY P. K., Liu S. X., Eur. J. Lipid Sci. Technol., 106, (**2004**) 122-125.

[8] CHEMAT-DJENNI Z., Ferhat M. A., Tomao V., Chemat F., J. Essent. Oil Bear. Plants, 13, (**2010**) 139-147.

[9] BAHRAMIFAR N., Yamini Y., Shamsipur M., J Supercrit Fluids, 35 (**2005**) 205-211.

[10] JOUNG S.N., Yoo C.W., Shin H.Y., Kim S.Y., Yoo K.P., Lee C.S., Huh W.S., Fluid Phase Equil, 185 (**2001**) 219-230.

[11] HERRERO M., Martín-Álvarez P.J., Señoráns F.J., Cifuentes A., Ibáñez E., Food Chem, 93 (**2005**) 417-423.

[12] TURNER C., IBÁÑEZ E., *Pressurized Hot Water Extraction and Processing*, in: N. Lebovka, E. Vorobiev, F. Chemat (Eds.) Enhancing Extraction Processes in the Food Industry CRC Press (Taylor & Francis Inc), Boca Ratón (FL), USA, **2011**, pp. 223-254.

[13] GOUVEIA L., Oliveira A., J. Ind. Microbiol. Biotechnol. 36, (**2009**) 269-274.

Comparative Life Cycle Assessment Study of Green Extraction Processes to Obtain Antioxidants from Rosemary Leaves.

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ABSTRACT

Rosemary is one of the most appreciated natural sources for bioactive compounds with different activities such as antioxidant, antimicrobial or anticarcinogenic. Antioxidant activity has been associated to some of its components, among them, phenolic diterpenes such as carnosic acid, carnosol and rosmarinic acid.

A careful selection of the extraction process together with the optimization of the extraction conditions, are of high importance to obtain rosemary extracts with high bioactivity. Among the different extraction processes, supercritical fluid extraction (SFE) and pressurized hot water extraction (PHWE) have demonstrated to be the most selective and environmental friendly techniques. However, extracts obtained by these processes usually require a drying step (freeze or hot drying step) which is both energy and time consuming.

In this work, a new process combining PHWE and powder formation on-line (water extraction and particle formation on-line process, WEPO®) has been developed to obtain dry antioxidant powder from rosemary leaves in one step. In this process, parameters related to the extraction efficiency and selectivity (water flow rate and temperature) as well as parameters involving spray stability and powder formation have been considered at the same time. The obtained extracts have been evaluated in terms of their antioxidant activity using the DPPH method.

Finally, in order to assess the viability and environmental impact of the new process, a comparison with other green processes used for antioxidant extraction from rosemary leaves such as SFE and PHWE (both followed by a freeze drying step) has been performed in terms of Life Cycle Assessment (LCA). Moreover, a sensitivity analysis of the LCA has been carried out to study the different environmental impact between the processes whether they are employed in different countries.

INTRODUCTION

Rosemary has been extensively studied for its beneficial properties such as antioxidant [1, 2], antimicrobial [3] or anticarcinogenic [4]. Antioxidant activity has been mainly attributed to the presence of phenolic diterpenes such as carnosic acid, carnosol and rosmarinic acid [5]. To selectively extract antioxidant compounds from rosemary, several processes have been optimized, such as pressurized hot water extraction (PHWE) and supercritical fluid extraction (SFE) [6-9]. However, depending on the type of process and compounds of interest, either water or a polar organic solvent are needed and therefore, extracts obtained usually require a drying step which is both energy and time consuming.

One of the most promising ways to dry compounds from organic solutions is the use of particle formation processes based on supercritical fluids; these processes involve different solvent-antisolvent steps [10, 11]; in the case of aqueous solution, these processes are not suitable for drying due to the low solubility of supercritical carbon dioxide ($scCO_2$) in water. In 2009, we patented a new process combining PHWE plus particle formation on-line (WEPO, Water Extraction and Particle formation On-line) as a novel way to obtain dried complex extracts from rosemary leaves in one step [12]. Recently, the WEPO process has been described and studied for the production of antioxidant powders from fresh onion as well [13]. In the present work we present the WEPO