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

María Castro-Puyana[1], Jose A. Mendiola[1], Irene Rodríguez-Meizoso[2], Charlotta Turner[2], Elena Ibáñez*[1]

[1] Laboratory of Foodomics. Bioactivity and Food Analysis Department. Institute of Food Science Research (CIAL-CSIC); Nicolás Cabrera 9, Campus UAM Cantoblanco, 28049 Madrid, Spain.
[2] Lund University; Department of Chemistry, Center for Analysis and Synthesis; SE-22100 Lund, Sweden.
Corresponding author: elena@ifi.csic.es; Phone: (+34) 91 001 7956; Fax: (+34) 91 001 7905

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 process applied to rosemary leaves and a comparison with SFE and PHWE [14] in terms of environmental impact of producing dry rosemary extracts using the different processes.

MATERIALS AND METHODS

Samples and reagents

Rosemary (*Rosmarinus officinalis* L.) sample, consisted of dried rosemary leaves obtained from Murciana de Herboristeria (Murcia, Spain). Cryogenic grinding of the sample was performed under carbon dioxide. Particle size (500-1000 µm) was determined by passing the ground plant material through sieves of appropriate size. The whole sample was stored in amber flasks at - 20 ºC until use.

1,1-diphenyl-2-picrylhydrazyl (DPPH, 95% purity) was from Sigma-Aldrich (Madrid, Spain), methanol (HPLC grade) from Lab Scan (Dublin, Ireland) and ethanol (99.5%) from Panreac (Barcelona, Spain). Milli-Q water was obtained using a purification system (Millipore Corporation, Billerica, MA, USA) and deoxygenated in an ultrasound bath for 15 min before its use. CO_2 (N38 quality) and N_2 (Technical quality) were obtained from Praxair (Madrid, Spain).

Experimental procedure of WEPO

Figure 1 shows a scheme of the home-built equipment to carry out the water extraction and particle formation on-line process (WEPO). The extraction cell was filled with a mixture of rosemary leaves (1 g) plus washed sea sand (2 g). The process starts by filling the cell with water at room temperature and high flow rate (0.5 mL/min). Then the water flow is stopped and the CO_2 flow and the heating systems (oven and heating tape) are started. When the conditions are reached (80 bar and 2-3 mL/min for the CO_2, 200 ºC for the oven), N_2 flow is started and water is pumped in continuous flow through the extraction cell at a chosen flow rate. The water extract is mixed under pressure with the supercritical CO_2 in a low dead volume tee-type connection, forming a gas expanded liquid that flows through a restrictor and reaches the expansion-drying chamber. In this chamber, the pressure and temperature are below the critical point of the CO_2, so the solution is rapidly expanded because the CO_2 becomes a gas. The aerosol formed in the expansion-dyring chamber is dried by the hot N_2 current. After the selected extraction time, valve V1 is closed and the water flow is stopped while CO_2 and N_2 flows continue for 10 more minutes. Thus, the entrance of extract droplets in the expansion-drying chamber due to a possible residual pressure in the extract line is avoided. Particles are collected from the walls of the expansion-drying chamber.

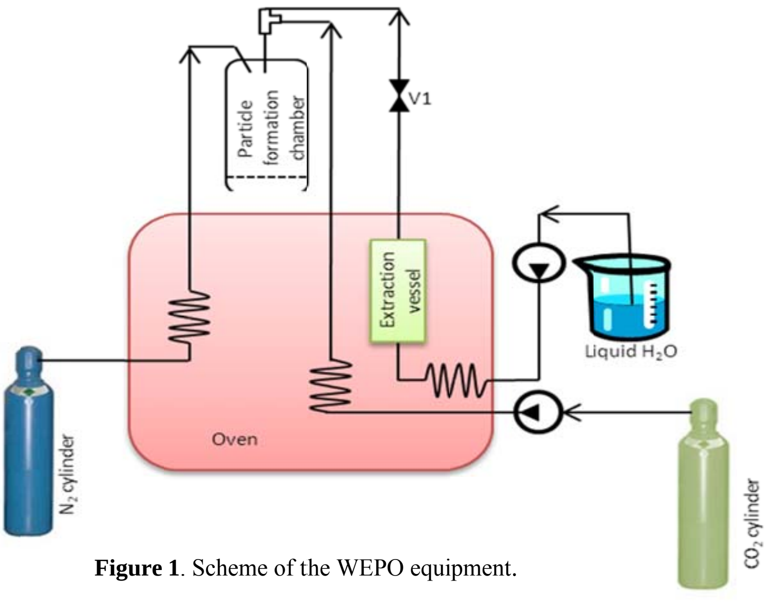


Figure 1. Scheme of the WEPO equipment.

Antioxidant capacity analysis

The antioxidant capacity of the different extracts was determined by the DPPH radicals capture method, by using the following procedure [15]: 23.5 mg of DPPH were dissolved in 100 mL of methanol. This stock solution was diluted 1:10 with methanol. Then 0.1 mL of rosemary extracts at different concentrations and 3.9 mL of DPPH diluted solution were placed in test tubes to complete the final reaction media (4.0 mL). Reaction was completed after 4 h at room temperature and absorbance was measured at 516 nm in a UV/VIS Lambda 2 Perkin Elmer Inc. spectrophotometer (Wellesley, MA, USA). Methanol was used to adjust zero and DPPH-methanol solution as a reference sample.

The DPPH concentration in the reaction medium was calculated from the following calibration curve, determined by linear regression (n= 7; r= 0.9999) as [DPPH] = (Abs + 0.0029) / 0.0247. The percentage of remaining DPPH against the extract concentration was then plotted to obtain the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% or EC_{50}.

Life cycle assessment (LCA)

The SimaPro software PRé 7.3 was used to perform LCA calculations. Thus, the environmental aspects of SFE, PHWE and WEPO processes were compared. The key inventory data along with the database sources for the three extraction processes are showed in **Table 1.** Besides**,** system boundaries considered in the LCA analysis were established. The steps previous to the extraction and those after production stage are not included since these are assumed to be identical for all the processes studied. For SFE and PHWE, a drying step (vacuum drying for SFE and freeze drying for PHWE) has been included. Solid and liquid disposal and emissions were also considered in the forms they are produced, i.e. composting, incineration, wastewater treatment or emission to air. The energy consumption of each component employed in the extraction process (oven, pump, freezer, freeze dryer, rotavapor) was calculated based on their specification and uptime.

Table 1. Key inventory data for production of rosemary extracts (1 kg) by PHWE, SFE and WEPO

	PHWE	**SFE**	**WEPO**	**Data Source**
		Products		
Rosemary extract	1 kg	1 kg	1 kg	-
		Inputs		
From nature				
Rosemary	2.6 kg	15.4 kg	3.6 kg	-
From technosphere				
Water	47.5 kg	-	23.5 kg	Ecoinvent
Nitrogen	738.8 kg	-	3.6 kg	ELCD
Carbon dioxide	-	526.1* kg	26.1 kg	Ecoinvent
Ethanol	-	38.8 kg	-	Ecoinvent
Electricity	4.07 kWh	127 kWh	0.737 kWh	Ecoinvent
		Outputs		
Emissions to air				
Water	-	-	23.5 kg	-
Nitrogen	-	-	3.6 kg	-
Carbon dioxide	-	27.7* kg	26.1 kg	-
Waste to treatment				
Solid waste	1.6 kg	14.4 kg	2.6 kg	Ecoinvent
Waste water	47.5 kg	-	-	LCA Food DK
Solvents mixture	-	38.8 kg	-	Ecoinvent

*The amount of CO2 corresponded with the net value used taking into account a recycling of 95 % and a loss of 5 % from the initial amount (553.8 kg)

The characterization method used in the study was CML 2 baseline 2000 V2.05 (available in the SimaPro software) which include ten impact categories (abiotic depletion, acidification, eutrophication, global warming, ozone layer depletion, human toxicity, fresh water aquatic ecotoxicity, marine aquatic ecotoxicity, terrestrial ecotoxicity, and photochemical oxidation). Besides, the cost derived by the energy employed in each process was added as impact category taking into account the energy price for industrial consumers published for by the Europe´s Energy Portal (€ per kWh for a consumption of 1 GWh/year) [16].

RESULTS

WEPO process set-up

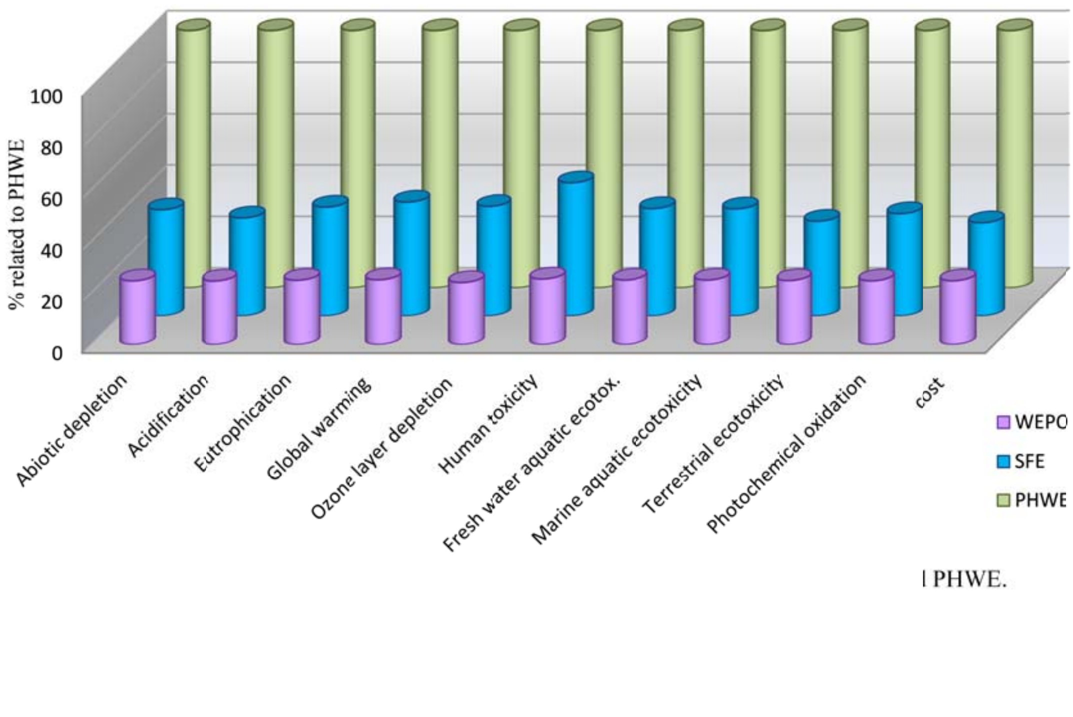
The equipment shown in **Figure 1** combines two processes: the continuous flow PHWE of rosemary leaves and the continuous production of an aerosol from the extract assisted by a supercritical CO_2 nebulization system, which is instantaneously dried by a hot N_2 current. Thus, the extraction and precipitation takes place in the same system with a small time delay between these two processes. A temperature of 200 °C was selected to carry out the experiments, based on previous works from our research group, since it enables to extract the maximum amount of carnosic acid, and therefore the maximum antioxidant activity from rosemary [17]. It is important to consider that in the step of drying extracts, there is no influence on the solubility parameters of the different compounds and therefore, all the compounds extracted will precipitate in the expansion-drying chamber. CO_2 and N_2 temperatures were also 200 °C. The entrance of these hot currents inside the expansion-drying chamber provides a temperature of 70 °C in the expansion-drying chamber. The CO_2 pressure was set at 80 bar to ensure that it is in its supercritical state. N_2 and CO_2 flow rates (0.6 and 2.5 mL/min respectively) were selected in order to obtain a proper aerosol from the tip of the restrictor that reaches the expansion-drying chamber (spray formation was visually evaluated). The whole process was performed using mild conditions and in absence of oxygen and light; therefore, it is expected to obtain particles with intact biological activities. The parameters chosen made it possible to carry out the process with three different water flow rates, 0.1, 0.2 and 0.3 mL/min.

The antioxidant capacity of the dried powders obtained was determined by the DPPH radical scavenging method, which provided values of EC50 around 10.5 $\mu g \cdot mL^{-1}$ in all the conditions tested. These antioxidant capacity values were comparable to those obtained for rosemary with a commercial PHWE extractor plus a freeze-drying process [14, 17].

Comparative Life Cycle Assessment

Once the WEPO process was set-up, its environmental impacts were compared with SFE and PHWE in terms of LCA for the production of 1 kg of dry rosemary extract with high antioxidant capacity.

Figure 2 shows the environmental impacts in the different categories considered by the LCA approach for the three extraction processes. As can be seen, the WEPO process provided the lowest environmental impacts in all the categories, being around one fourth of the impacts produced by PHWE+freeze drying. By analyzing all the categories shown in **Figure 2,** it is clear that extraction solvents have no important impact mainly because they are green solvents and they are used in very low volumes.



l PHWE.

The high significance of electricity consumption in the three production processes compared in this study is clear in the environmental impact categories considered as well as for associated costs. In fact, even those processes using CO_2 (SFE and WEPO), which expected impact in terms of global warming could be stronger, are more influenced by the amount of CO_2 necessary to take electricity to the production facilities than on the amount of CO_2 used for the production of antioxidant rosemary extract. As a consequence, the WEPO process could be outlined as the greenest way to obtain high quality antioxidants from natural origin compared to other well-established green production ways.

Due to the large impact of electricity, a sensitivity assessment of the LCA was carried out to study the different environmental impact among the three processes in different countries. This study demonstrated that the lowest environmental impact in all the categories is obtained for the WEPO process, independently of the country.

CONCLUSION

The WEPO process can be considered a suitable and promising process to obtain, in only one step, dry rosemary extracts with high antioxidant capacity from rosemary leaves. Besides, the viability and environmental impact of the WEPO process has been assessed, in comparison with other green extraction processes (SFE+vacuum drying, PHWE+freeze-drying and WEPO), in terms of LCA. The results obtained demonstrated that the lowest environmental impacts in all the categories were achieved using the WEPO process.

ACKNOWLEDGMENTS

This work was supported by AGL2008-05108-C03-01 (Ministerio de Educación y Ciencia), CSD2007-00063 FUN-CFOOD (Programa CONSOLIDER-INGENIO 2010), and S-0505/AGR/000153 (Comunidad Autónoma de Madrid) projects. C.T. acknowledges the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS, 2006-1346); the Swedish Research Council (VR, 2006-4084); and the Swedish Foundation for Strategic Research (SSF, 2005:0073/13, RMA08-0044). M.C-P. thanks MICINN for her "Juan de la Cierva" contract.

REFERENCES

[1] CUVELIER, M.E., RICHARD, H., BERSET, C., J. Am. Oil Chem. Soc., Vol. 73, **1996**, p. 645.
[2] QUIRIN, K.W., Innovations Food Technol., Vol. 18, **2003**, p. 186.
[3] ROMANO, C.S., ABADI, K., REPETTO, V., VOJNOV, A.A., MORENO, S., Food Chem., Vol. 115, **2009**, p. 456.
[4] VISANJI, J.M., THOMPSON, D.G., PADFIELD, P.J., Cancer Lett., Vol. 237, **2006**, p. 130.
[5] THORSEN, M.A., HILDEBRANDT, K.S., J. Chromatogr. A, Vol. 995, **2003**, p. 119.
[6] IBAÑEZ, E., LÓPEZ-SABASTIÁN, S., FERNÁNDEZ, J., TABERA, J., BUENO, J.M., BALLESTER, L., REGLERO, G., Food Sci. Technol. Int., Vol. 7, **2001**, p. 177.
[7] BENSEBIA, O., BARTH, D., BENSIA, B., DAHMANI, A., J. Supercrit. Fluids, Vol. 49, **2009**, p. 161.
[8] LINDAHL, S., EKMAN, A., KHAN, S., WENNERBERG, C., BÖRJESSON, P., SJÖBERG, P., NORDBERG KARLSSON E., TURNER, C., Green Chem., Vol. 12, **2010**, p. 159.
[9] TURNER, C., IBAÑEZ, E., Pressurized hot water extraction and processing *in* Enhancing Extraction processes in the food industry-Contemporary food engineering, LEBOVKA, N., VOROBIEV, E., CHEMAT, F., (Ed.), CRC press, Boca Raton, **2011**, p. 223.
[10] KNEZ, Z., WEIDNER, E., Curr. Opin. Solid State Mater. Sci., Vol. 7, **2003**, p. 353.
[11] IMSANGUAN, P., PONGAMPHAI, S., DOUGLAS, S., TEPPAITOON, W., DOUGLAS, P.L., Power Technol., Vol. 200, **2010**, p. 246.
[12] IBÁÑEZ, E., CIFUENTES, A., RODRÍGUEZ-MEIZOSO, I., MENDIOLA, J.A., REGLERO, G., SEÑORÁNS, F.J., TURNER, C., Spain patent P200900164. **2009** (http://hdl.handle.net/10261/32687).
[13] ANDERSSON, J., LINDAHL, S., TURNER, C., RODRÍGUEZ-MEIZOSO, I., Food Chem., *Submitted*.
[14] HERRERO, M., PLAZA, M., CIFUENTES, A., IBÁÑEZ, E., J. Chromatogr A, Vol. 1217, **2010**, p. 2512.
[15] BRAND-WILLIAMS, W., CUVELIER, M.E., BERSET, C., Lebensmittel-Wissenschaft und-Technologie, Vol. 28, **1995**, p. 25.
[16] Europe´s Energy Portal. http://www.energy.eu/#Industrial-Elec (Checked on December **2011**)
[17] IBAÑEZ, E., KUBÁTOVÁ, A., SEÑORÁNS, F.J., CAVERO, S., REGLERO G., HAWTHORNE, S.B., J. Agric. Food Chem., Vol. 51, **2003**, p. 375.