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Impact of pulsed electric fields on polyphenols extraction from Norway spruce bark

Meriem Bouras[a,b,*], Nabil Grimi[a], Olivier Bals[a], Eugene Vorobiev[a]

[a] Sorbonne Universités, Université de Technologie de Compiègne, Laboratoire Transformations Intégrées de la Matière Renouvelable (UTC/ESCOM, EA 4297 TIMR), Centre de Recherche Royallieu, CS 60319, 60203 Compiègne Cedex, France
[b] SOFREN, 336 Bureaux de la Coline, Bâtiment A-7ème étage, 92213 Saint-Cloud Cedex, France

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ABSTRACT

The objective of this study is to improve aqueous solid/liquid extraction of the polyphenols contained in Norway spruce (*Picea abies* (L.) Karst.) bark by the use of pulsed electric field (PEF) treatment. The feasibility of PEF treatment was studied through two different PEF protocols with an intensity of $E =$ 20kV/cm. These two PEF protocols are applied at different initial humidity of the bark samples (14% and 21%). PEF treatments protocols were compared to untreated samples (simple diffusion without PEF) and to a diffusion from sawdust of Norway spruce bark. The main parameters for the follow-up of the diffusion kinetics and characterization of extracts were studied: moisture content, pH, electrical conductivity, polyphenols concentration and antioxidant activity. The results of this study showed the positive effect of the pulsed electric fields on intracellular compounds extraction. The PEF treatment enhanced extraction of total phenolic content and antioxidant activity. Indeed, the total phenols content has increased of more than 8 times with the use of PEF treatment. The kinetics of pH and electrical conductivity during treatment were studied. The solid fraction was analyzed and the results showed that PEF treatment does not affect the structure of wood and that there are no modifications of the chemical functions by exposing the samples to PEF treatment.

1. Introduction

Nowadays, the place of renewable energy is increasingly important. Biomass is the primary source of renewable energy in France (Syndicat des energies Renouvelables, 2012). The pulp industry produces large amounts of residues from wood transformation (barks, sawdust, knots...) (Diouf et al., 2009a). For instance, in France there are nearly 275,000 tonnes/year of waste such as bark, sawdust and waste wood yards (Ademe, 2001) which are used as fuel for thermal energy production (burned in boilers) (Diouf et al., 2009a,b; Host and Lowery, 1970; Ku et al., 2007), to produce charcoal (Baker, 1982; Dargan and Smith, 1959) or used as compost for horticulture (Feng et al., 2013). However, these residues constitute a raw material rich in high value added compounds such as bioactive polyphenols. Bark is considered as the richest part of the tree in terms of quantity and complexity of extractives including bioactive polyphenols (Gao et al., 2007; García-Pérez et al., 2012; Harkin and Rowe, 1971; Siddhuraju et al., 2002). Polyphenols are interesting for their abundance in nature and also for their beneficial properties for health (Stevanovic et al., 2009). Indeed, these molecules are powerful antioxidants (García-Pérez et al., 2012; Royer et al., 2010), anti-inflammatory (Stevanovic et al., 2009), antibacterial (Lindberg et al., 2004), antiseptic and antifungal (Amarowicz et al., 2008; Aslam et al., 2009; Bafi-Yeboa et al., 2005; Fukai et al., 2005). They also prevent cardiovascular disease (Manach et al., 2005), osteoporosis (Cassidy et al., 2000), cancer (Ray et al., 2005; Stevanovic et al., 2009), etc. These properties make them highly sought in different industrial fields and also very attractive for consumers. Polyphenols have multiple and varied application fields such as: pharmaceuticals, cosmetics (Moure et al., 2001), medicals, agro-food additives, adhesives (Vázquez et al., 2009, 2008), etc.

The pulp and paper industry in France is a large consumer of Norway spruce and its transformation produces many wastes. Phenolic compounds represent one of the major classes of extractable in spruce tree species (Royer et al., 2010). Manners and Swan

Abbreviations: DM, dry matter; LCV, lower calorific value; PEF, pulsed electric fields; TEAC, trolox equivalent antioxidant capacity; TPC, total phenolic content.

* Corresponding author at: Université de Technologie de Compiègne, Département de Génie des Procédés Industriels, Unité Transformations Intégrées de la Matière Renouvelable (UTC/ESCOM, EA 4297 TIMR), Centre de Recherche de Royallieu, B.P. 20529-60205, Compiègne Cedex, France.
E-mail addresses: meriem.bouras@utc.fr, meriem.bouras@gmail.com (M. Bouras).

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Nomenclature

$d_{electrodes}$	Distance between electrodes (cm)
E	Electric field strength (V/cm)
n	Number of pulses
t_p	Pulse duration (μs)
t_{PEF}	PEF effective time (ms)
σ	Electrical conductivity (mS/cm)

Subscripts

i	Initial
f	Final

(1971) have extracted three stilbenes (astringin, isorhapontin and isorhapontigenin) from the bark of five Canadian spruce species (Engelmann spruce, Sitka spruce, white, black and red spruce). In the case of Norway spruce, several phenolic compounds were extracted: stilbenes and phenylpropanoids (Brignolas et al., 1998, 1995; Latva-Maenpaa et al., 2011; Li et al., 2008; Lieutier et al., 2003; Viiri et al., 2001; Zeneli et al., 2006), condensed tannins (Proanthocyanidins) (Diouf et al., 2009b; Tišler et al., 1986), flavonoids (catechin, epicatechin, and taxifolin) (Royer et al., 2010) and lignans (hydroxymatairesinol) (Pan and Lundgren, 1995). In total, more than 60 compounds have been isolated from various tissues of Norway spruce (Li et al., 2008).

The traditional methods of polyphenols extraction from wood such as maceration (Diouf et al., 2009a; Ku et al., 2007; Vázquez et al., 2001) and soxhlet extraction (Aspé and Fernández, 2011) are time consuming (Kwon et al., 2003), require large amounts of energy and high quantities of solvent (Kaufmann et al., 2001). Moreover, most of these traditional methods are used with the grinded product (Aspé and Fernández, 2011; Diouf et al., 2009a; Royer et al., 2011), which reduces the quality of the extract and complicates its subsequent purification. In addition, the active compounds can be degraded or oxidized (De Castro and Garcia-Ayuso, 1998; Gironi and Piemonte, 2011). New extraction methods have emerged in recent years such as microwave assisted extraction and ultrasound assisted extraction (Aspé and Fernández, 2011; Diouf et al., 2009a). These techniques show a significant decrease of extraction time and solvent consumption and an important increase of the extraction yield (Chemat and Cravotto, 2013; Ghitescu et al., 2015; Mason et al., 2011; Perez-Serradilla and de Castro, 2011) but they are energy consuming (Ghnimi et al., 2011). Currently, pulsed electric fields (PEF) are also used for extraction of molecules of interest from plant materials. PEF treatment leads to electroporation of cell membranes and promotes extraction of intracellular compounds (Vorobiev and Lebovka, 2008). PEF were recently used for polyphenols recovery from grape berries and mash (Boussetta et al., 2012, 2009a,b; Corrales et al., 2008,), flaxseed hulls (Boussetta et al., 2014) and rapeseed stems (Yu et al., 2014).

The aim of the present work is to study the phenolic compounds extraction from Norway spruce bark using pulsed electric fields. The impact of pH, humidity and electrical conductivity on PEF application modes was highlighted. Different extraction protocols have been evaluated: (1) extraction (E), (2) PEF and extraction (PEF&E), (3) extraction + PEF and extraction (E + PEF&E). Each method has been assessed according to total phenols, antioxidant activity, electrical conductivity and pH monitoring, HPLC, FTIR and lower heating value analysis.

2. Materials and methods

2.1. Raw material

This study was conducted with Norway spruce bark. This bark was obtained as the material removed by stem debarking in pulp and paper industry and was provided by Norske Skog Golbey in France. The wood age is estimated at 10–15 years. The bark was sliced into pieces of approximately $10 \times 10 \times 4\,mm^3$ with a miter saw (Metabo Kgs 216, Leroy Merlin, Compiègne, France). The sawdust obtained as residue from the bark preparation with the miter saw was sieved using a vibratory sieving apparatus (Retsch, AS 200). Different sieve mesh sizes were used: (1.6, 1.25, 1.2, 0.5, 0.4, 0.25, 0.1 mm) and the mass retained on each sieve was weighed. The sieve that contains the highest mass was 1.2 mm ($1.2 < d < 1.25$ mm). This particle size was selected for the extraction experimentations. The samples were stored in an oven at 30 °C in order to stabilize the dry matter around 86 ± 2%.

2.2. Pulsed electric field treatment & extraction protocols

The experiments were carried out using a PEF-generator (40 kV-10 kA, Tomsk Polytechnic University Russia) for the treatments with high electric field strength (E = 20 kV/cm).

The PEF apparatus consisted of a pulsed high voltage power generator and a batch one-liter treatment chamber (Fig. 1a). A sample with a total mass of 250 ± 1 g was placed in the cylindrical one-liter treatment chamber. Both of the grounded plate electrodes of this chamber were stainless disk of 11 cm in diameter. The distance between the electrodes ($d_{electrodes}$) was fixed at 2 cm to produce a high PEF intensity of 20 kV/cm.

In all experiments the liquid-to-solid ratio was fixed at 10 (w/w). The PEF generator (40 kV, 10 kA) provided pulses of an exponential form with a pulse repetition rate of 0.5 Hz. The effective time (t_{PEF}) of PEF treatment was calculated:

$$t_{PEF} = n \times t_P \quad (1)$$

Where n (=1–400) is the number of pulses and t_p (≈10 ± 1 μs) is the pulse duration. The pulse width is defined as the time until decay to 37% for exponential decay pulses (Töpfl, 2006).

The temperature before and after the treatment was controlled by K-type thermocouple (±0.1 K), connected to the data logger thermometer Center 305/306 (JDC Electronic SA, Yverdon-les-Bains, Switzerland). The elevation of temperature after treatment never exceeded 6–7 °C.

2.2.1. Preliminary experiments

In preliminary experiments, the PEF treatments of sliced particles were effected in solutions with pH 2 (0.01 M HCL (10%)), pH 7 (water), and 12 (0.01 M NaOH). This study lead to the choice of the solvent. 400 pulses were applied during the treatment process and after each 25, 50, 100, 200, 300 and 400 pulses, samples were taken for phenolic compounds quantification. Extracts were centrifuged for 5 min at 14.000 rpm (MiniSpin plus Centrifuge, Germany) and the supernatants were carefully collected and kept at 4 °C until analyses.

2.2.2. Effect of PEF treatment on the polyphenols extraction

The PEF treatment was applied for the extraction of polyphenols from sliced particles of Norway spruce bark and using a solvent composed of 0.04% of sodium hydroxide (0.01 M NaOH, pH12). The initial electrical conductivity of pH12 solvent was $\sigma \approx 5.74$ mS/cm. The treatment was applied through two protocols:

- Protocol 1: PEF & E. The total quantity of 400 pulses was applied during extraction process. After each 25, 50, 100, 200, 300 and 400