Industrial Crops and Products 74 (2015) 309–318

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

# A new way for the oil plant biomass valorization: Polyphenols and proteins extraction from rapeseed stems and leaves assisted by pulsed electric fields

X. Yu, O. Bals, N. Grimi*, E. Vorobiev

*Sorbonne Universités, Université de Technologie de Compiègne, Département de Génie des Procédés Industriels, Unité Transformations Intégrées de la Matière Renouvelable (UTC/ESCOM, EA 4297 TIMR), Center de Recherche de Royallieu, B.P. 20529–60205 Compiègne, Cedex, France*

ARTICLE INFO

*Article history:*
Received 17 December 2014
Received in revised form 25 February 2015
Accepted 14 March 2015

*Keywords:*
Rapeseed
Extraction of polyphenols and proteins
Pulsed electric field
Selective extraction

ABSTRACT

This work aims at investigating the effects of pulsed electric field (PEF) on the extraction of polyphenols and proteins from rapeseed (*Brassica napus* L.) stems and leaves. The PEF (0.2–20 kV/cm) was applied on fresh rapeseed stems (diameter, $d$ = 6 mm; thickness, $h$ = 10 mm) and leaves (surface, $S = 10 \times 10\ mm^2$). The PEF-induced cell membrane damage ($Z$) of rapeseed tissue was studied. The PEF (5–20 kV/cm) increased the extraction of total polyphenols from rapeseed stems and leaves. Treatment at 5 kV/cm resulted in the highest polyphenols purity. In addition, the data evidence that the polyphenols content in rapeseed stems and leaves decreased (≈74%) as plant maturity advanced while the change for protein content is more gentle (≈35.6% decrease). Results of the maturation effect may be useful to determine the optimum harvest time for the rapeseed by-products valorization.



## 1. Introduction

Rapeseed (*Brassica napus* L.), a winter or spring annual oil crop in the Brassica family, currently occupies third place in the world production of vegetable oil (after palm and soybean oil) (Thiyam et al., 2004). According to Food & Agriculture Organization (FAO), worldwide production of rapeseed was 62.5 million tons in 2011. France is the fifth rapeseed producing country (after China, Canada, India, and Germany), producing 5.4 million tons, and one of the top producers of rapeseed biodiesel (De Fraiture et al., 2008). After oil extraction, large amounts of rapeseed residues (stems mainly) are left behind in the field. Chemical analysis showed that these residues contain proteins, polyphenols, and some desirable amino acids (arginine, methionine, lysine etc.) (Ucar and Ozkan, 2008; Rezvani et al., 2012). Thus, the valorization of these renewable by-products would have a large contribution to the sustainable development in the oil industry and biomass transformation.

Recent studies on the rapeseed residue valorization focus on the rapeseed meal (oil cake) obtained after oil extraction due to its relatively high proteins content (around 38% w/w). Therefore, it has been widely used in animal feed (Pustjens et al., 2014). The surface functional properties of rapeseed meal proteins are also of special interest for many non-food applications, such as coatings, films, and emulsions (Krause and Schwenke, 2001). Other prospects for the rapeseed meal valorization include production of pectin in the food industry due to its high carbohydrate content (30–60%) (Jeong et al., 2013). Besides rapeseed meal, the rest of the plant (stems mainly) is usually beneficial as a cover crop. It provides good soil cover over winter to prevent soil erosion and can improve soil tilth with its root system. In the last years, the lignocellulosic nature of rapeseed straw has been used for the bioethanol production by a biochemical process including pretreatment, enzymatic hydrolysis, and fermentation (Karagöz et al., 2012; López-Linares et al., 2013). As a source of lignocellulosic fibrous material, rapeseed straw also motivated interest in pulping and papermaking industries compared to clear-cutting rainforests (Mazhari Mousavi et al., 2013).

Chemical analysis showed that the crude proteins content in rapeseed stems and leaves was 4% and 10%, respectively (Sincik et al., 2007). In addition, organic acids, fatty acids, and polyphenols were also detected in the organic solvent extractives (Farag et al., 2013). The concentration of intracellular compounds in rapeseed varies by its growth stage. Sincik et al. (2007) showed that the crude proteins content in rapeseed decreased as the plant moved from

* 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), Center de Recherche de Royallieu, B.P. 20529–60205 Compiègne Cedex, France. Tel.: +33 3 44 23 44 42.
*E-mail address:* nabil.grimi@utc.fr (N. Grimi).

http://dx.doi.org/10.1016/j.indcrop.2015.03.045
0926-6690/

the full flowering stages toward the full podding stages. However, reports dealing with the recovery of these valuable compounds are really rare.

Nowadays, the recovery of valuable compounds from food residue comprises principally five stages including macroscopic pretreatment, macro- and micro-molecules separation, extraction, purification, and nutraceuticals formation (Galanakis, 2012). Emerging technologies based on non-thermal concepts, such as pulsed electric field (PEF) has received increased interest in the development of green and sustainable extraction techniques for natural products (Boussetta et al., 2014; Puértolas et al., 2010). PEF processing involves treating plant materials or biosuspensions between two electrodes by voltage pulses in the order of 0.1–80 kV/cm. When critical electrical potential is reached, electrical breakdown and local structural changes of cell membranes occur, which increase the permeability that facilitate passage of intracellular compounds to the surrounding solution. The treatment is applied for a very short time ($10^{-4}$ and $10^{-2}$ s), so there is little heating effect of the sample ($\Delta T < 10\,^{\circ}C$) and the energy consumption is low. It should also be noted that no solvent or only distilled water is used during PEF-treatment, which makes this procedure a real green extraction method (Chemat et al., 2012; Rombaut et al., 2014). Depending on electric conditions, such as electric field strength and number of pulses, PEF-treatment could also control the selectivity of extraction by regulating the degree of membrane destruction (De Vito et al., 2008). Ohshima et al. (1995) showed that with electric field strength ranging from 0 to 18 kV/cm and 0 to 500 pulses (pulse duration $t_i = 0.7\ \mu s$) the intracellular compounds from yeast cells can be extracted while keeping the cell wall intact. Thus, the co-release of impurities due to the cell rupture can be reduced compared to conventional methods, such as high pressure and thermal extraction. Besides, the PEF technology has been referred to require additional energy consumption during scale up to obtain equivalent recovery yield of valuable compounds. Future development of cost-effective impulse generation systems should be considered (Galanakis, 2013).

On our knowledge, there exists only one published study concerning the PEF application on the rapeseed. In that study, Guderjan et al. (2007) concluded that PEF pretreatment (5–7 kV/cm and 1.8–3.6 ms) permitted to increase the rapeseed oil extraction yield and quality. More valuable compounds, such as tocopherols, polyphenols, and phytosterols were extracted. PEF-treatment was proved to be a promising non-thermal method in many fields (extraction, pasteurization, compression, etc.). However, no research work was found in the literature concerning the feasibility of PEF application on other parts of rapeseed, such as stems and leaves. The rapeseed stem has a complex structure, which highly depends on the maturity state. The prediction of the efficiency of PEF-treatment is much difficult. The presence of epicuticular wax on rapeseed leaves may reduce the efficiency of PEF-treatment.

The main objective of this study was to investigate PEF-treatment efficiency on the selective extraction of polyphenol and protein from rapeseed stems and leaves. The effect of PEF-treatment on the tissue damage and the improvement of polyphenols extraction yield are discussed and the results are compared to grinding (non-selective method). In addition, a study aimed to evaluate the relationship between rapeseed maturation and polyphenols and proteins contents was also conducted.

## 2. Materials and methods

### 2.1. Plant materials

Rapeseed samples (cultivar Quartz) were collected every week from a local field in Chevrière, France, from early February to late June in 2013. Immediately following the collection, all samples were brought to the laboratory and were stored in a cold room at 4 °C until required.

### 2.2. Estimation of plant maturity

Before measurement of maturity degree, the rapeseed samples were cleaned to remove dirt and foreign particles from the surfaces. Ten main stems and ten leaves of rapeseed were selected randomly to measure plant height and leaf length. Three complete rapeseed samples were hand-separated into leaves and stems components and weighed to determine leaf/stem mass ratio.

### 2.3. Analysis of moisture content

The moisture content of fresh rapeseed stems and leaves ranged from 84–89%; it was measured by drying 2 g of fresh tissue at 130 °C to constant weight in a moisture analyzer (Scaltec SMO 01, France).

### 2.4. Extraction experiments with grinded samples

Forty grams of fresh rapeseed stems or leaves were grinded by electric grinder (Bamix M140, Switzerland) for total polyphenols and total proteins extraction. To determine the optimal extraction conditions, the effect of four experimental parameters (time, temperature, solvent, and pH) most believed to affect the extraction process was studied by the method of control variants. The solid to liquid ratio was 1:8 for stems and 1:13 for leaves in all experiments. Agitation at 500 rpm was provided by using a mechanical stirrer (VELP Scientifica, Italy).

### 2.5. Electrical conductivity disintegration index Z and energy input for tissue damage

The effect of PEF-treatment on the tissue damage was estimated by electrical conductivity disintegration index $Z$ (Vorobiev and Lebovka, 2009):

$$Z = \frac{(\sigma - \sigma_i)}{(\sigma_d - \sigma_i)} \quad (1)$$

where $\sigma$ is the measured electrical conductivity value and the subscripts $i$ and $d$ refer to the conductivities of the untreated and completely damaged tissue, respectively. Application of the above equation gives $Z = 0$ for an intact tissue and $Z = 1$ for completely damaged tissue obtained by combined electroporation and ohmic heating. After such treatment, the tissue was heated to 60 °C and the electrical conductivity of the tissue attained its maximal value $\sigma_d$ (Loginova et al., 2010). To determine $Z$-values, cylinder-shaped samples (diameter $d = 6$ mm, height $h = 5$ mm for stems; $d = 28$ mm, $h = 5$ mm for folded leaves) were placed between two stainless electrodes. PEF-treatment was carried out using a laboratory generator, 400 V–38 A (Service Electronique University of Technology of Compiegne (UTC), France). This PEF generator provides bipolar pulses of near-rectangular shape. Different electric field strengths $E$ (200 V/cm, 400 V/cm, and 800 V/cm) were applied to determine the values of $Z$. The PEF-treatment conditions consisted on a series of $n$ (=2) pulses, with pulse duration $t_i$ (=1 ms), and time duration between pulses $\Delta t$ (=0.1 ms). A series of pulses $N$ (=200) were repeated each $\Delta t_t$ (=10 s). The total time of the PEF-treatment was calculated by using the following formula:

$$t_{PEF} = n \times t_i \times N \quad (2)$$

The electrical conductivity of samples was measured during the pause time, between series of pulses, at the frequency of 0.5 kHz, selected as optimal for removing polarization effects on the electrodes and the tissue sample (Grimi et al., 2010). The temperature