

## Yield of Polyphenolic Substances Extracted From Spruce (*Picea abies*) Bark by Microwave-Assisted Extraction

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Closed-system microwave-assisted extraction was applied to extract total phenolics from spruce (*Picea abies*) bark, using 96.6% ethanol as an extractant. The influence of particle size (0.3; 1.0; 2.5 mm), time (3 to 20 min), and temperature (60; 80; 100 °C) on polyphenol recovery was also studied. Higher extraction temperature and smaller particle size resulted in a higher yield of extracted polyphenols. However, the effect of extraction time on yield was more complicated. The effect of all three factors is tentatively explained.

*Keywords:* Extraction; Microwave extraction; Spruce bark; Folin-Ciocalteu reagent

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### INTRODUCTION

Bark is an attractive renewable raw material, comprised of all types of silviculture vegetation. This renewable resource is a major alternative raw material for the chemical industry. Valorisation is a key component of an economic lignocellulosic biorefinery (Jablonsky *et al.* 2015a; Surina *et al.* 2015). For many years, research has tried to find applications for extractives other than their use as fuel. Larger proportions of extractives could be used for industrial and commercial processes if an economical method could convert substances into a marketable product with sufficient profit margins. Most bark is burned as fuel; however, bark contains hundreds of natural products, some of which have cytotoxic, antioxidant, antifungal, antibacterial, antimycotic, cytotoxic, antiviral, antitumor, antimalarial, insecticidal, antimutagenic, tumorigenic, and antimycotic properties. They can act as repellents, antifeedants, and growth inhibitors. They can also increase the activity of pheromones and behave as pheromones. Therefore, many treatment strategies have been developed to isolate extractives with a high yield. The extraction and purification or fractionation processes of these active or bioactive substances are essential because they are used in the preparation of fine chemicals, dietary supplements, nutraceuticals, functional food ingredients, food additives, and pharmaceutical and cosmetic products. Several papers describe extraction by an agent in differing conditions (Song *et al.* 2012; Spigno and Faveri 2009; Jablonsky *et al.* 2015b; Ház *et al.* 2016; Kreps *et al.* 2016; Jablonský *et al.* 2016; Sládková *et al.* 2016).

Closed-system microwave-assisted extraction (MAE) is an important technique for extracting valuable compounds from lignocellulosic materials (Guo *et al.* 2001; Hao *et al.* 2002; Gao *et al.* 2006; Ghaly and Adams 2007; Hemwimon *et al.* 2007; Ghani *et al.* 2008; Yeoh *et al.* 2008; Gujar *et al.* 2009; Spigno and Faveri 2009; Michel *et al.* 2011; Zhang *et al.* 2011). MAE of extractive substances may be affected by factors such as frequency and power of the microwave, time of applying microwave radiation, moisture content, particle size, ratio of solid to liquid, type and composition of solvent, temperature, pressure, and number of extraction cycles (Wang and Weller 2006; Yang and Zhang 2011; Hadkar *et al.* 2013). MAE is an improved extraction method with high efficiency with regard to duration time and environmental friendliness (Wang and Weller 2006). Microwave heating is also effective for the extraction of alkaloids (Ganzler *et al.* 1990), terpenes (Carro *et al.* 1997), polynuclear aromatic hydrocarbons (Tomaniová *et al.* 1998), and phenolic compounds (Spigno and De Faveri 2009; Dahmoune 2014; Simić *et al.* 2016, Krishnan *et al.* 2016).

The present work studied the impact of temperature, particle size, and time on the yield of extractives from spruce (*Picea abies*) bark using MAE. Based on the known ability of ethanol to absorb microwave radiation and act as excellent extractant (Gabriel *et al.* 1998; Japón-Luján and Luque de Castro 2006; Luque-Rodríguez *et al.* 2006), a water-ethanol extractant was applied to samples in this research.

## EXPERIMENTAL

### Materials

#### *Spruce bark characterisation*

Spruce (*Picea abies*) bark was kindly supplied by Bioenergo Ltd. (Ruzomberok, Slovakia). The bark was air-dried, ground, and separated into three fractions of different particle sizes (0.3, 1.0, and 2.5 mm) using sieves. The spruce (*Picea abies*) bark was extracted using the accelerated solvent extraction method (Sluiter *et al.* 2008), weighed, dried, and analyzed to determine the content of lignin, ash, and holocellulose (Table 1). The residual lignin content was determined as Klason lignin (TAPPI T222 1998), and the extractive content was determined according to Sluiter *et al.* (2008). Ash was determined using TAPPI T211 (1998), and holocellulose was quantified with sodium chlorite treatment following the procedure of Wise *et al.* (1946).

**Table 1.** Composition of Spruce (*Picea abies*) Bark

Spruce Bark Component	Composition (%)
Holocellulose	52.0 ± 0.2
Lignin	26.4 ± 1.3
Ash	3.6 ± 0.4
Extractives	18.0 ± 1.6

Note: Values represent the average of six replicates ± standard deviation

### Methods

#### *Closed-system microwave-assisted extraction*

MAE was performed using a MicroSYNTH Labstation (maximum output 1.5 kW, 2.45 GHz, maximum temperature 250 °C, maximum pressure 100 bar; Milestone Inc., Shelton, CT, USA.), with an HPR 100 (high-pressure 100 mL) reactor. A known amount

of particles (~ 2 g) was suspended in 20 mL of 96.6% ethanol, followed by microwave irradiation at 60, 80, and 100 °C for 1, 2, 3, 5, 10, 15, and 20 min in the reactor. Three minutes of heat-up time was applied to reach the desired temperature. After MAE, the extracted liquors were cooled to room temperature (maximum cooling time < 15 min) and immediately filtered through No. 1 filter paper to separate the extract and the residue.

#### *Yield of extractives*

The yield of extractives ( $Y$ , %) was determined after each experiment by drying the samples at 105 °C to a constant weight. The results are expressed on the basis of the dry matter before and after extraction as shown in Eq. 1,

$$Y (\%) = 100 \times (m_i - m_{\text{extr}})/m_i \quad (1)$$

where  $m_i$  is the mass (g) of the bark before extraction and  $m_{\text{extr}}$  is the mass (g) of the bark after extraction and drying, respectively.

#### *Total polyphenols content*

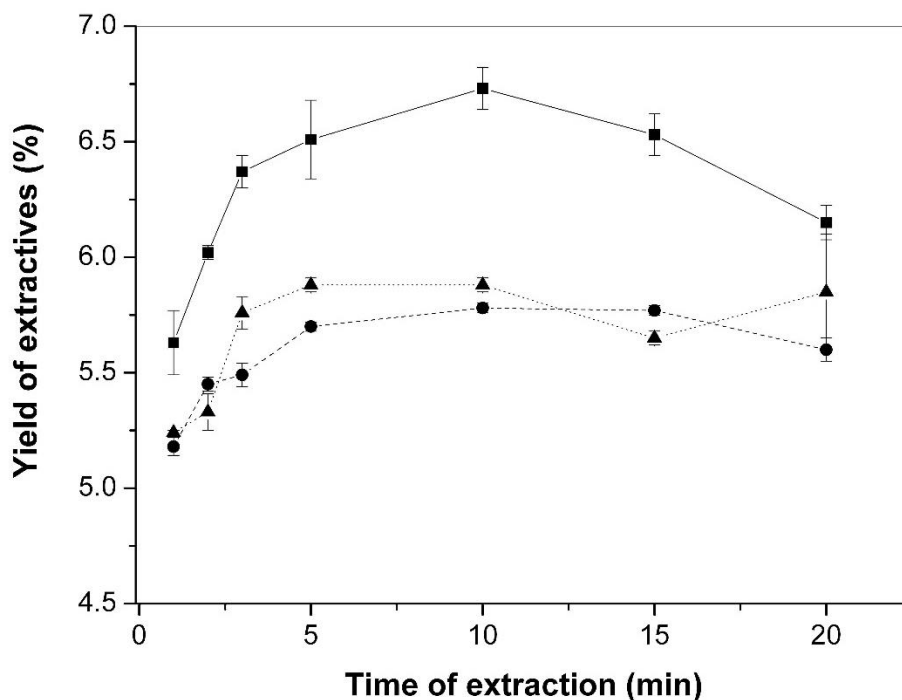
The total polyphenols content (TPC) was determined by the Folin-Ciocalteu method based on redox reactions of phenols (Singleton *et al.* 1998). Briefly, according to this method 1000 mg of standard gallic acid was dissolved in 100 mL of distilled water in a volumetric flask (10 mg/mL of stock solution). Folin-Ciocalteu reagent (0.5 mL) (Fisher Scientific Chemicals, Illkirch, Slovakia), 0.1 mL of the standard solution of gallic acid or an extract, and 3 mL of distilled water were pipetted into a test tube. After 5 min, 1.5 mL of 20% sodium carbonate solution and 4.9 mL of distilled water was added. After stirring, the mixture was incubated at room temperature for 60 min in a dark enclosed flask, and the absorbance of the solution was recorded at 765 nm. A calibration curve determined that the concentration of gallic acid ranged from 0.05 to 7 mg/mL. The TPC in extracts was determined using the calibration curve based on the absorbance at 765 nm and expressed as gallic acid equivalents (GAE) in 100 g of dry bark.

## RESULTS AND DISCUSSION

Extraction curves that show the effect of particle size on the yield of extractives at a constant temperature (60 °C) are depicted in Fig. 1. These findings are in agreement with previous investigations (Wang and Weller 2006; Yang and Zhang 2011; Hadkar *et al.* 2013; Baldosano *et al.* 2015). The yield of extractives from the spruce (*Picea abies*) bark was between 5.18 and 6.73%. Extracting smaller particle sizes produced a higher yield (Fig. 1). For larger particle sizes (greater than 0.3 mm), the yields were lower. This can be attributed to the reduced intrinsic capacity for diffusion of a solvent (Baldosano *et al.* 2015). The size of particle played a crucial role in this process. Smaller particle sizes offer a greater surface area for mass transfer. Finer particles, however, are more prone to agglomeration. Particles with a size < 0.3 mm have a lower extraction efficiency (Baldosano *et al.* 2015). This effect can be interpreted as a consequence of the lower ability of the solvent to pass through the sample due to its aggregation. Small particles such as this were not investigated in this work.

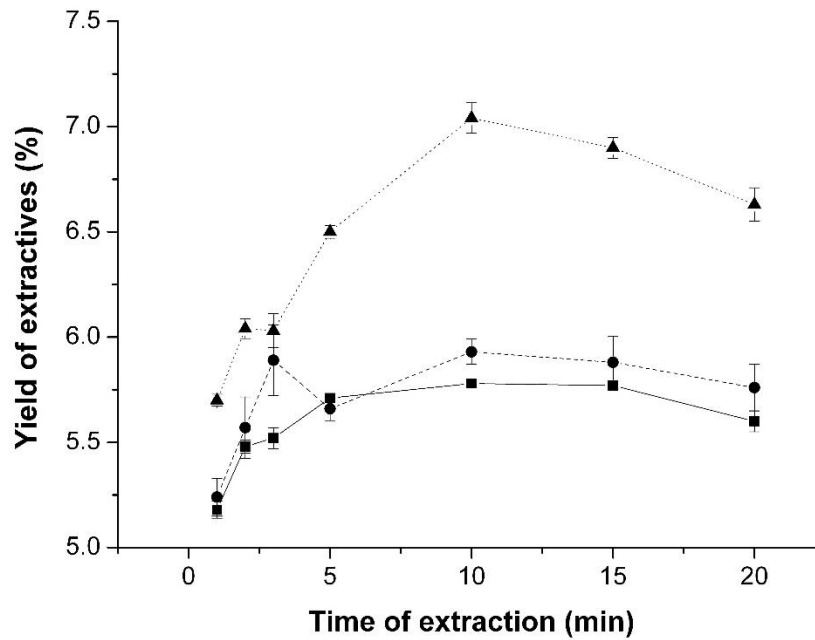
Figure 1 also documents the impact of time on the yields. Increasing time was found to be favorable for the extraction efficiency, but only for 10 min after the start of extraction. At 15 min, the yields were lower than those gained during the first 10 min of extraction. An important aspect of particle size in industrial applications is not only the efficiency of the extraction, but also the handling of the input material. When processing samples with 0.3 mm particles, the bark stuck to the container and caused big problems, especially in the filtration of liquid extract from the solids. These technical problems were not observed when processing particles with a larger diameter. For this reason, it was necessary to choose the optimal particle size on the basis of efficiency and technical handling, which was 1 mm. The observed trend could be explained by the temperature evolution of the mixture (Fig. 2).

The rapid increase in temperature might enhance the extraction through increased diffusivity (Hiranvarachat and Devahastin 2014). Higher temperatures might also help loosen components of the cell wall, or even disrupt cell structure, resulting in an enhanced release of extractives into the solvent. However, prolonged extraction time decreased the extractives contents. The results were in agreement with those of Hiranvarachat *et al.* (2013). The extractives yield from spruce (*Picea abies*) bark ranged from 5.18 to 7.04% at different temperatures with a particle size of 1.0 mm. The largest yields were obtained at 100 °C. These experiments showed that the increase in temperature had a positive effect on the efficiency of MAE.

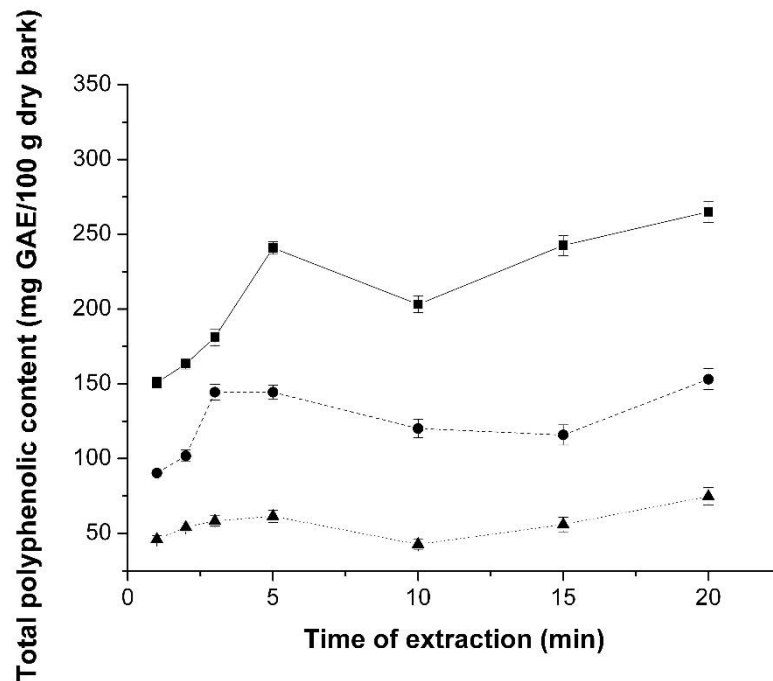


**Fig. 1.** Impact of particle size on the yield of extractives (in %) on spruce bark. The temperature of extraction was 60 °C, and the moisture content of particles before microwave extraction were 0.3 mm =  $8.02 \pm 0.16\%$ ; 1 mm =  $9.56 \pm 0.28\%$ ; 2.5 mm =  $8.62 \pm 0.35\%$ . ■ – particle size 0.3 mm, temperature of extraction 60 °C; ● – particle size 1 mm, temperature of extraction 60 °C; ▲ – particle size 2.5 mm, temperature of extraction 60 °C.

In the case of 1 mm particles it was found (Fig. 2) that the yield of extractives is time dependent. Up to 10 min the yield increases with time of extraction, later it slowly decreases.

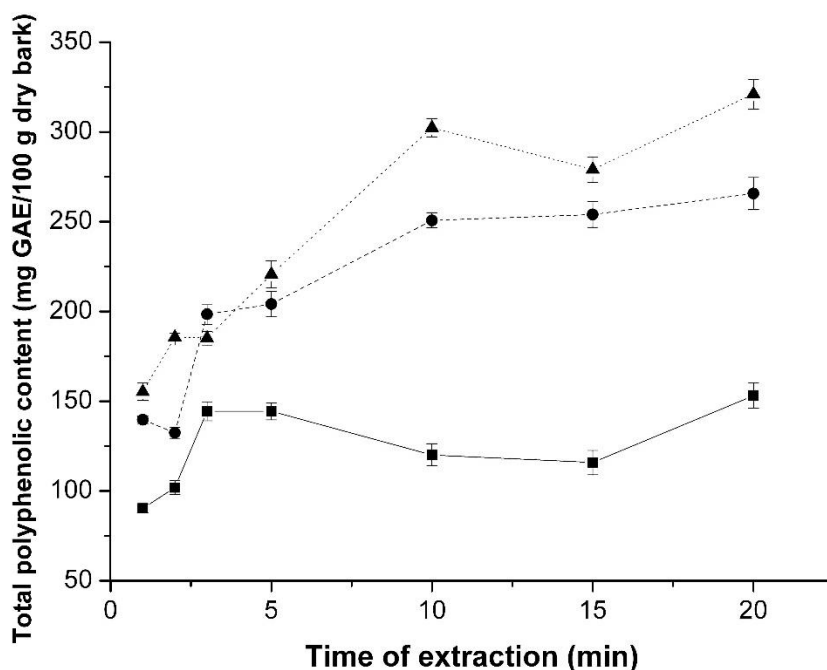


**Fig. 2.** Impact of temperature on extractives yield from spruce bark with a particle size of 1 mm ■ – temperature of extraction 60 °C; ● – temperature of extraction 80 °C; ▲ – temperature of extraction 100 °C



**Fig. 3.** Impact of particle size on the total polyphenolic content extracted from spruce bark by MAE at 60 °C. ■ – particle size 0.3 mm, ● – particle size 1 mm; ▲ – particle size 2.5 mm

The total extracted polyphenolics, as assessed by Folin-Ciocalteu assay, varied between 42.7 and 265.0 mg GAE per 100 g of dry bark for different particles at a temperature extraction of 60 °C (Fig. 3). The yield reached 90.3 and 321.1 mg GAE per 100 g dry bark at 60 °C and 100 °C, respectively, when extracting 1 mm particles (Fig. 4). Other studies on European softwood bark extracts (Jerez *et al.* 2007; Yesil-Celiktas *et al.* 2009; Legault *et al.* 2013) reported values in the same range as determined in this work. As shown in Fig. 3, smaller particles produced higher concentrations of polyphenols in extracts. This trend is consistent with the pattern of increased total yield of extractives with decreased particle size. The extraction time had a positive impact on the proportion of polyphenols in a liquid extract, but there was not a reduction in the polyphenol yield at 15 min, in contrast to the total extract. In Fig. 4 it is clear that higher yields of polyphenols were achieved at higher temperatures. The same trend was also measured in determining yields of extractives.



**Fig. 4.** Impact of temperature on the total polyphenolic content extracted from spruce bark by MAE, with particle size 1 mm. ■ – temperature of extraction 60 °C; ● – temperature of extraction 80 °C; ▲ – temperature of extraction 100 °C

## CONCLUSIONS

1. The particle size influenced the yield of extractives and content of the active substances expressed as the content of total polyphenolic compounds determined by the Folin-Ciocalteu reagent.
2. At 60 °C, the highest yield of extractives (6.73%) was reached when extracting the 0.3 mm fraction for 10 min. However, the yield of total polyphenolic substances did not follow identical time dependence as the yield of extractives reached the maximum value at 20 min extraction.

3. The highest yield of extractives (7.04 %) from a 1 mm fraction was reached at 10 min; the polyphenolics yield from these particles was highest after 20 min extraction.
4. The optimum particle size is dictated by economics: a balance between recovery or maintenance, processing cost, solid to liquid separation efficiency, and the cost of a given processing method. The total extraction yield, as well as the yield of the total polyphenolic substances, was the greatest when processing the finest (0.3 mm) bark fractions at 60 °C. However, with subsequent handling, processing is more appropriate for bark fraction of size 1 mm.

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