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Kinetic study of polyphenols extraction from olive (*Olea europaea* L.) leaves using instant controlled pressure drop texturing

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ABSTRACT

The aim of this investigation is to study the impact of the technology of instant controlled pressure drop: "DIC" on the kinetics extraction of olive leaf (*Olea europaea* L.) polyphenols. The conditions of extraction were: ethanol 95%, temperature: 55 °C and ratio $r = 40\ g\ g^{-1}$ dry basis for 3 h. The extraction kinetics of total polyphenols content (TPC) of DIC-treated and untreated leaves were performed according to the method of Folin–Ciocalteau. The ultra-performance liquid chromatography (UPLC) was used to study the extraction kinetics of seven phenolic compounds of olive leaves: apigenin-7-glucoside, hydroxytyrosol, luteolin-7-glucoside, oleuropein, tyrosol, vanillic acid, and verbascoside. DIC-assisted solvent extraction allowed reducing the extraction time from 120 to 15 min while increasing the extracted yields. Phenomenological analysis of extraction kinetics was determined through the two-stage Coupled Washing/Diffusion CWD kinetic model. DIC texturing could, in favorable cases, increase the starting accessibility and systematically the internal effective diffusivity for phenolic compounds.

1. Introduction

Phenolic compounds are secondary plant metabolites which provide defense to plants against oxidizing agents and free radicals [1]. In the olive plant (*Olea europaea* L.), the main active compounds are: oleuropein, hydroxytyrosol and tyrosol [2]. Oleuropein is the major phenolic compound in olives and leaves; it belongs to the group of secoiridoids, as well as verbascoside, which are abundant in Oleaceas, Gentianales and Cornales [3,4]. Hydroxytyrosol has the same structure as tyrosol, but it possesses an extra hydroxy group in the meta position [5]. Other phenolic compounds have been identified in olive leaf extract such as: caffeic acid, p-coumaric acid, vanillic acid, vanillin, luteolin, diosmetin, rutin, luteolin-7-glucoside, apigenin-7-glucoside, and diosmetin-7-glucoside [2,6–9]. These phenolic compounds provide several biological activities such as: antioxidant [6,10–12], antimicrobial [9,13,14], anti-inflammatory [15], and anti-HIV [16] activities. These polyphenols, such as hydroxytyrosol and vitamin E, have been also reported to be potent inhibitors of LDL oxidation in vitro [17].

In general, phenolic compounds in plant cells are accumulated in two sites: (i) the cell wall for flavonoids, esterified ferrulic acids and lignins, and (ii) the vacuole for the soluble phenolic compounds and their derivates [18]. According to the localization of these phenolic compounds, conventional [19–21] and assisted solvent extractions [22–24] were used to recover these phenolic compounds.

Instant controlled pressure drop DIC treatment is a pretreatment usually used to assist and intensify solvent extraction of flavonoids [25], essential oils [26,27] and polyphenols [2]. In fact, it induces expansion of the vegetal material to become more adapted to mass transfer. Due to this expansion, the microstructure of plant materials is modified [2,27,28], thus allowing active compounds to be more available, and the effective diffusivity of solvent to be higher.

The extraction kinetic modeling is a useful tool, which facilitates the optimization, the control of processes, and the determination of kinetic parameters. Different models were used to describe solid–liquid extraction kinetic of plant-based active compounds: (i) empirical models including Naik's model [29,30], sorption/desorption-similar Peleg's model [29,31], two-parametric models such as parabolic diffusion model and power law model [32], and

Abbreviations: CWD, Coupled Washing/Diffusion model; DIC, instant controlled pressure drop (Détente Instantanée Contrôlée); EtOH, ethanol solvent; TPC, total phenolic compounds; UPLC, ultra-performance liquid chromatography.

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Ponomaryov empirical equation [33], and (ii) phenomenological models including the second Fick's law of diffusion [34], and the models derivated from the modification of Fick's law such as the unsteady state diffusion through plant material and film theory [33]. Whilst empirical models are simple, they only permitted to scaling-up. However, the phenomenological models allow predicting and controlling the behavior of the different parameters that influence the extraction process.

A specific fundamental study of the solvent extraction kinetics was defined by Ben Amor and Allaf [25] in order to identify the impact of the expansion of granules by DIC texturing. The authors recommended the two-stage (Coupled Washing/Diffusion CWD) kinetic model [25,35] to study both external (agitation) and internal (diffusivity) effects, separately.

In the present case, the impact of DIC texturing on the polyphenol extraction from olive leaves is usually observed in terms of comparative yields and kinetics. The experimental results were used to identify the main parameters of CWD kinetic model: effective diffusivity and starting accessibility.

2. Materials and methods

2.1. Raw material

Olive leaves (*O. europaea* L. var Chemlali) were harvested from the arboterum of "Ecole Nationale d'Ingénieurs de Sfax" in Mai 2013, cleaned with distilled water. Untreated olive leaves were dried in an airflow oven at 40 °C until constant weight. After drying, olive leaves were ground in a grinder (Retsch Grindomix, GM 200) at 10,000 rpm for 1 min. The obtained powder were stored for later analysis.

2.2. Chemicals and samples

All the solvents used for chromatographic purposes were of HPLC grade. Absolute Ethanol was of analytical grade and purchased from Carlo Erba (Val de Reuil, France). Folin–Ciocalteu phenol reagent and Sodium Carbonate were purchased from Sigma–Aldrich (Buchs, Switzerland). Oleuropein, hydroxytyrosol, tyrosol, vanillic acid, apigenin-7-glucoside, luteolin-7-glucoside and verbascoside were obtained from Sigma–Aldrich (Steinheim, Germany).

2.3. DIC treatment

DIC treatment of olive leaves was previously described [2]. Fresh olive leaves were placed in DIC treatment vessel where a vacuum is established. Then, a thermal treatment (high temperature–short time) stage is achieved using saturated steam. The thermal treatment is followed by an abrupt pressure drop toward a vacuum, which induced an instant autovaporization resulting in an "instant" cooling of the solid material. After DIC texturing, olive leaves were immediately dried in an oven at 40 °C at a rate of 1 m s^{-1}. The obtained powder were stored for later analysis.

2.4. Solvent extraction kinetics of DIC treated and non-treated leaves

Solvent extraction kinetics of untreated and DIC-treated leaves were carried out using an oil batch extractor system (Memmert, Germany) with a three-necked top round bottomed flask and an electric stirrer. An amount of approximately 5 g of olive leaf powder was placed in the batch extractor with 250 ml of 95% EtOH concentration at 55 °C for untreated and DIC-textured olive leaves (P = 0.58 MPa; number of cycles = 1; total time = 22 s). Extraction kinetics were monitored by taking samples of 1 ml at predetermined time (5, 10, 20, 30, 40, 60, 90, 120, 180 and 240 min). Samples were then filtered with 0.2 μm filter. The extraction kinetics of total polyphenols content (TPC) of DIC-treated and untreated leaves were performed according to the method of Folin–Ciocalteau. The ultra-performance liquid chromatography (UPLC) was used to study the extraction kinetics of phenolic compounds.

2.5. Determination of total phenolic compounds (TPC)

Total phenolic compounds (TPC) in the untreated and DIC-treated extracts was determined according to the Folin–Ciocalteau method [2].

2.6. UPLC analyses

Ultra Performance Liquid Chromatography UPLC (Waters Instruments, Acquity UPLC H-CLASS, Singapore) coupled to a diode array detector (PDA Waters UPLC LG 500 nm) was used to analyze the phenolic compounds of olive leaf extracts according to Mkaouar et al. [2]. The column was an HSS T3 (2.1 × 100 mm, 1.8 μm). The flow-rate was 0.65 mL min^{-1} at 45 °C. The wavelength was 280 nm and 350 nm. A ternary gradient elution was used: acetic acid–water (solvent A); acetonitrile (solvent B) and water (solvent D). The solvent mixture A was isocratic with 1%. The gradient was isocratic during 1 min; 30% B at 10 min; 95% B at 12 min; 1% B at 12.1 min.

2.7. Kinetic modeling

The fundamental phenomenological study of polyphenols solvent extraction process was achieved through the two-stage (Coupled Washing/Diffusion CWD) kinetic model, which implies both "starting accessibility" and "effective diffusion" of the plant material [36].

The extraction process kinetics could be analyzed through two stages:

- The "washing" stage; described by the starting accessibility δX_s (expressed in kg of extract per kg of dry material): it is the amount of extracted solute in a very short time (t near to 0) from the material surface. The intensification of this stage implies a dynamic convection [25] instead of external diffusion.
- The second extraction stage is diffusion: it is the movement of the molecules that assures the transfer with the gradient of concentration. This process, taking place within the solid matrix, cannot be intensified through any external thermal or mechanical modification. This is similar to 2nd Fick's law with an effective diffusivity D_{eff} (m^2 s^{-1}) [35,37].

2.7.1. Washing stage: starting accessibility

At a first time, the intensification of solvent extraction is usually performed through increasing the external interaction between solvent and matrix surface (washing) which induces removing surface solute. The amount of extracted solute m_s depends on the nature of solvent and solute, the exchange surface, and the temperature.

The extraction rate of solute from surface is:

$$\frac{dm_s}{dt} = k_e S(\varpi_s - \varpi_{solvent}) \quad (1)$$

where

m_s: mass of the extracted solute (kg of solute).
k_e: interaction coefficient between the solvent and the surface (kg of solvent m^{-2} s^{-1}).