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Green extraction procedures of lipids from Tunisian date palm seeds

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ABSTRACT

This study was performed to compare, in term of quality and quantity, conventional and green lipid extraction procedures of three Tunisian date palm (*Phoenix dactylifera* L.) seeds cultivars (Deglet Nour, Allig and Belah). Extractions were performed using *n*-hexane and 2-methyltetrahydrofuran (MeTHF) as solvents. Identification and quantification of extracted fats and lipids were carried out by gas chromatography coupled with a flame ionization detector and high performance thin-layer chromatography.

Results showed that Deglet Nour presents the highest lipids extraction yield (7.24–5.97%) using the both solvents. Hansen solubility simulation demonstrated that MeTHF is a good alternative solvent for lipid date seeds extraction. In addition, extraction of oils from date pits with ultrasound and micro-wave were better in terms of extraction time (30 min versus 8 h) comparing to soxhlet procedure and of extraction yield comparing to maceration (about 6% versus 4%). Triglycerides represent almost the whole recovered oils composition with 99% and the fatty acids were mainly oleic (44.02–46.9%), lauric (20.00–23.10%), myristic (8.88–11.26%), palmitic (9.00–10.73%) and linoleic (6.13–9.21%) acids.

1. Introduction

The date palm tree (*Phoenix dactylifera* L.) is grown widely in arid and semiarid regions of the world. Date palm is the major fruit tree; it constitutes the basis of economy and the principal source of remuneration for the Tunisian people living in Sahara (Besbes et al., 2004a,b). Tunisia is the 10th producer and the first exporter of dates in the world. During five years, Tunisian production has attain about 127,000 tons per year with Deglet Nour variety constitute about 60% of the total production due to her good sensory quality and high commercial value (Besbes et al., 2009). In Tunisia, varieties of Deglet Nour, Allig and Bellah are much consumed. The large quantity of date seeds could be easily collected from the date processing industries or from the waste products (represent about 30% of the production in Tunisia) coming either directly from the gap-conditioning or from the stations palm grove. On an average, weight of date seeds is about 10–15% of date mass (Hussein et al., 1998) and contains between 4 and 13% crude oil (Biglar et al., 2012; Al-Farsi et al., 2007; Besbes et al., 2005). Though, these by-products are generally consumed either as a conventional soil fertilizer or as complementary feed materials for animals and poultry (Vandepopuliere et al., 1995). Also, they were used to make coffee in the Arabian Peninsula (Ali-Mohamed and Khamis, 2004) and to extract oil for pharmaceutical and cosmetic objects (Devshony et al., 1992). Some reports were appeared in literature about fatty acid compositions and lipid classes of date seed (*Phoenix dactylifera* L.) oil cultivated in Tunisia (Besbes et al., 2004a,b; Hossain et al., 2014; Saafi et al., 2008) and in other countries as Saudi Arabia, Oman, Egypt, Iraq, Iran (Basuny and Al-Marzooq, 2011; Hossain et al., 2014). Seed oil were mainly oleic acid (47.66%) and lauric acid (17.39%), followed by linoleic acid (10.54%), palmitic acid (10.20%) and myristic acid (10.06%) (Saafi et al., 2008). Lipids and oils were extracted with conventional soxhlet using petroleum solvents such as hexane and petroleum ether (Besbes et al., 2004b; Nehdi et al., 2010; Biglar et al., 2012; Kazemi and Dadkhah, 2012). Conventional solid-liquid extraction (SLE) techniques as maceration and soxhlet extraction methods consume time and use large amounts of solvents (Wang and Weller, 2006). Nowadays the development of the concept of green extraction, the environment friendly procedures are becoming more interesting. The application of new technologies, as microwave and ultrasound offer several advantages like reducing time, cost, solvent consumption and power to not affect the stability of extracted compounds, simplifing process and improving extraction quantitatively and qualitatively of extract (Arslan and Ozcan 2010; Chemat et al., 2008; Maskan, 2000; Tiwari, 2015). In this research, we applied the green extraction of oils and lipids in date seeds in which MeTHF was chosen as a good suitable solvent extraction due to its chemical and physical properties which are similar to hexane.

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This agro-solvent is produced from waste of corn cobs, oat hulls and sugar cane bagasse (Pace et al., 2012; Sicaire et al., 2015) and it has nocarcinogenic, mutagenic, and reprotoxic (CMR) properties (Antonucci et al., 2011). Several studies have been reported about the good yields of lipids compounds extraction using alternative methods and solvents (Sicaire et al., 2015; Virot et al., 2008; Dejoye Tanzi et al., 2013; Breil et al., 2016). This study deals for the first time with oils extraction from Tunisian date palm seeds using green extraction procedures as well as the determination of oils chemical compositions.

2. Material and methods

2.1. Plant material

In this study, about 100 g seeds of each date cultivar of Deglet Nour, Allig and Bellah were collected at "Tamr" stage (full ripeness), on November, January and September 2015 respectively, having all the same origin (Tozeur, Tunisia) with GPS position 33°55′6.722N/08°07′22.558E and pluviometry 8.9 mm. The seeds were immersed in water, washed to get rid of any adhering date flesh, air-dried within one week in shadow at ambient temperature and after dried in oven for 12 h at about 50 °C. Then seeds of each variety were grinded using the hammer mill with 1 mm Sieve and preserved at −20 °C until extractions.

2.2. Extraction procedures

Extractions of lipids from date palm seeds were carried out in triplicate with different procedures (Soxhlet, ultrasound, microwave and maceration) using hexane and MeTHF as solvents. Yield of the extracted oil was calculated according to the following expression:

$$\text{Oil Yield} = (W_{oil}/W_{sample}) \times 100$$

Where (W_{oil}): weight of oil obtained after extraction and (W_{sample}): weight of dry sample.

Conventional Soxhlet method (ISO 659–1988) was used for the extraction of oils from seeds of 3 different date palm cultivars. 15 g of powdered seeds were extracted using a 200 mL capacity Soxhlet apparatus in a 33 mm × 80 mm cellulose cartridge (Macherey-Nagel). The top of this latter was covered with cotton wool to avoid transfer of particles in the distillation chamber. All samples were extracted under reflux for 8 h using 300 mL of solvent placed in a 500 mL capacity flask which was putted under the soxhlet apparatus.

On the other hand, ultrasound, microwave and maceration procedures were applied for the extraction of oil from milled date palm seeds (10 g) with 100 mL of solvents (hexane and MeTHF) for 30 min.

- Ultrasound extraction was performed in a double envelope thermostatic glass reactor of 250 mL and a transducer of 6 mm, operating at a frequency of 20 kHz and a maximum input power of 130 W. The temperature was fixed at 40 °C.
- Microwave oven (Milestone NEOS-GR multimode) operating with a maximum input power of 450 W at 20 MHz was used for the extraction of lipids from date seeds. Microwaves heat up the solvent to boiling point. The solvent vapors penetrate via the dried date palm seeds and the condensation occurs on the condenser (extraction under reflux).
- Maceration was performed with mechanic agitation in a double envelope thermostatic glass reactor of 250 mL at 40 °C.

Samples extracted with MeTHF were filtered on 0.45 μm nylon filter and evaporated under reduced pressure. The obtained dry samples were re-extracted with hexane (150 mL); centrifuged (1000 rpm, 10 min, 25 °C) and then the hexane was evaporated under reduced pressure. The obtained dry extracts were stored in a freezer (−20 °C) for subsequent analyses.

2.3. Hansen solubility parameters

Hansen solubility parameters (HSPs) software (Hansen, 2007) was used to evaluate the solubility parameters of solvents. This software provides an efficient and convenient theoretical approach to characterize solute-solvent interactions according to the classical "like dissolves like" rule. HSPs are based on the concept that the total cohesive energy density is approximated by the sum of the energy densities required to overcome atomic dispersion forces (δ_d^2), molecular polar forces arising from dipole moments (δ_p^2) and hydrogen bonds (exchange of electrons, proton donor/acceptor) between molecules (δ_h^2), as given by Eq. (1):

$$\delta_{total}^2 = \delta_d^2 + \delta_p^2 + \delta_h^2 \quad (1)$$

where δ_{total} is the Hansen total solubility parameter, which is composed of three Hansen solubility parameters for dispersion (δ_d), polar (δ_p) and hydrogen bonding (δ_h). The Yamamoto (Y-MB) is the easiest method used to calculate HSPs (Benazzouz et al., 2013). Using JChemPaint version 3.3 (GitHub Pages, San Francisco, CA, USA), the chemical structures of the solvents and solutes were transformed to their simplified molecular input line entry syntax (SMILES) notations, then they were used to calculate the solubility parameters of the alternative solvent and constituents extracted from date seeds. The relative energy difference (RED) number is a simple composite affinity parameter, it has been calculated using (Eq. (2)) by HSP software to determine whether the alternative solvent and the solute are miscible:

$$RED = R_a/R_b \quad (2)$$

Where R_b is the radius of a Hansen solubility sphere, and R_a is the distance of a solvent situated within the Hansen solubility sphere.

In accordance with the classical "like to like" rule: smaller R_a is, greater affinity between solute and solvent (Filly et al., 2014). Signifies that a suitable solvent has a RED number smaller than 1, whereas unsuitable solvent has a RED number superior than 1. These solubility parameters were further modeled often using two dimensional HSP sphere for better visualization of the solute-solvent system, owing to the negligible differences between δ_D (HSPiP Version 4.0, Hansen-Solubility, Horsholm, Denmark).

2.4. High Performance Thin Layer Chromatography (HPTLC)

Two different HPTLC developments were applied to determine the classes of lipids through the separation of polar and neutral ones. Lipids were charring and quantified by a CAMAG 3 TLC scanning densitometer (CAMAG, Muttenz, Switzerland) with identification of the classes by comparison with known polar and neutral lipid standards. The extracted oils were loaded onto 20 × 10 cm HPTLC plates (Silica gel 60 F254; Merck KGaA, Darmstadt, Germany) by means of an ATS 5 automatic TLC sampler (CAMAG, Switzerland). The HPTLC silica gel plates were then developed with two eluents in an ADC2 automatic developing chamber (CAMAG, Switzerland): The first eluent (a mixture of methyl acetate/isopropanol/chloroform/methanol/KCl in a ratio of 25:25:25:10:9) running to a height of 7 cm from the origin; The second eluent is a mixture of *n*-hexane/diethyl ether/glacial acetic acid in a ratio of (70:30:2) running to a height of 7 cm from the origin. The dried plates were dipped for 6 s in a reagent (10 mg of primuline, 160 mL of acetone, 40 mL of water) and then scanned using a TLC Scanner 3 with a WinCATs software (CAMAG). Identification and quantification of lipids classes were carried out by comparison with lipid standards. The densitometry data are expressed as percent of lipid class in total date palm seeds oils.