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Immature mulberry fruits richness of promising constituents in contrast with mature ones: A comparative study among three Tunisian species

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ABSTRACT

Mulberry is one of the most consumed fruit for its special taste, and its nutritional and medicinal properties. The present study aims to track for the first time the chemical changes occurring in three Tunisian Mulberry species: *Morus alba* L., *Morus nigra* L. and *Morus rubra* L. fruits according to four classified maturity stages. Nutritional composition showed that mulberries content varied from species to the other and from a stage to another. Protein increases during maturation while Ash decrease with surprisingly the highest amounts in *Morus rubra* L. Fatty acid composition proved richness of polyunsaturated fatty acids, with a light decrease from immature stages to mature ones. Mulberries volatile components showed a heterogeneous composition such as mineral composition with a decrease during maturation of calcium, iron, magnesium and manganese amounts). Also, polyphenols and flavonoids content became lower, reducing antioxidant potential with maturation while anthocyanins content became higher. Results proved that mulberry powdered fruits, specially *Morus rubra* L. have high nutritional potential and suggest immature stages valorization for human beings.

1. Introduction

The discovery of new drugs from medicinal plants is a preoccupation for human health due to synthetic antioxidants and drugs potential danger (Fukushima and Tsuda, 1985). Plants can be used as natural antioxidants due to their ability to reduce oxidative stress. Many nutrients, such as mulberry fruit are able to treat disorders. Traditionally, mulberry fruit is useful to treat tired, anemic, or constipated patients and to repair kidney or urinary incontinence problems (Nadkarni, 1976). Mature mulberry fruits can be consumed fresh for only a short period because of their fragility and delicateness. The mulberry fruit can also be consumed in processed forms, such as juices, syrups, or dried fruits, due to its nutritive value (Ercisli and Orhan, 2007; Gundogdu et al., 2011). An earlier study reported that mulberry fruits powder was able to inhibit normal cells transformation into cancerous cells due to their antimutagenic activity (Hou, 2003). More recently, Singhal et al. in 2010 indicated that mulberry powder is able to prevent diseases associated with chronic inflammation with an anti-aging effect on cells because it combats free radical damage. It was proved also that fruit powder regulates lipid homeostasis in the human body and controls carbohydrate digestion (Liu et al., 2009). The most commonly known species in the *Morus* genus are white mulberry (*Morus alba* L.), black mulberry (*Morus nigra* L.) and red mulberry (*Morus rubra* L.) (Gundogdu et al., 2011). The increasing interest in the nutritional potential of mulberry fruit, and the high popularity of these fruits, motivated investigation on the chemical content and antioxidant power of these fruits during maturation in order to find new promising sources of natural antioxidants. Fruit ripening is usually accompanied by a change in the skin color due to a change in the pigments concentration in superficial tissues (Agati et al., 2005). Recently, the evaluation of the nutritional changes of fruit during ripening has become important for natural bioactive compounds extraction. To the best of authors knowledge, there is no

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study showing the evolution of mineral, nutritional, and volatiles composition, as well as of the fatty acids profile and anthocyanin progress during mulberry maturity. Furthermore, the present work highlights the antioxidant potential, phytochemical composition and bioactive compounds of *Morus* fruits at four maturity stages for the three most consumed species, *Morus alba* L. (MA), *M. rubra* L. (MR) and *M. nigra* L. (MN).

2. Materials and methods

2.1. Standards and reagents

2,2-Diphenyl-1-picrylhydrazyl(DPPH), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), gallic acid and Folin–Ciocalteu's reagent were purchased from Sigma–Aldrich Co. (St. Louis, France).

2.2. Samples

Mulberry fruits of three species: *Morus alba* (MA), *M. nigra* (MN) and *M. rubra* (MR) grown under the same climatic conditions were collected at four different developmental stages (S1, S2, S3, S4). The samples were collected from Monastir during 2015 Mulberry season (between May and June). Only healthy fruits without any type of infection or physical damage were processed. In the laboratory, all the fruits were washed twice with distilled water, cleaned, and dried at 40 °C until constant weight. A small quantity was kept fresh to be used in the corresponding analyses, while the major quantity was reduced to fine powder and stored until use.

2.3. Morphologic parameters and proximate composition

For each stage of maturity, fruit length and width dimensions were measured using a digital caliper (accuracy 0.01 mm) while fruit weight was measured by using a digital balance with a sensitivity of 0.001 g. Also, moisture, protein, fat, carbohydrates and ash were determined following the AOAC procedures (AOAC, 1995). The moisture content was evaluated by the weight difference before and after drying; crude protein content of the samples was estimated by the Lowry method; the crude fat was determined by extracting a known weight of powdered sample with *n*-hexane in a Soxhlet apparatus; the ash content was determined by incineration at 500 ± 15 °C using a Chamber furnace (Lenton Thermal Designs Ltd). Total carbohydrates were calculated by difference.

2.4. Mineral composition

The method described in the AOAC procedures was used with some modifications (Horwitz and Latimer, 2005). A sample of 2 g was incinerated for 5 h at 550 °C. The residue of incineration was extracted with 1 mL of 1% nitric acid and boiled for 5 min. After cooling, the volume was adjusted to 50 mL with nitric acid (1%) and then decanted and filtered. Fe, Cu, Mn, Zn, Ca, Mg, and K were directly measured by inductively coupled plasma-optical emission analytical spectrometry (Perkin ELmer model OPTIMA 2100 DV). When needed, an additional 1/10 (*v*/*v*) dilution of the sample extracts and standards was performed to avoid interferences between different elements (ISO, 2007). Sodium was assayed by flame spectrophotometry, using the spectrophotometer flame "SHERWOOD model 410" by direct passage of the solution above obtained (ISO, 1993).

2.5. Fatty acids

For each studied sample, fatty acid composition of the lipids extracted by soxlet apparatus were determined as described by Tekaya et al., 2013 with slight changes about methylation procedure. Fatty acid contents were determined by a capillary gas chromatographic method indicated in EEC Regulation 2568/91. Fatty acid methyl esters (FAME) were obtained as follow: lipid fractions were mixted with hexane (2 mL) boron trifluoride (3%; 0.5 mL), N H_2SO_4 (0.2 mL; 1 N) and sodium chloride (10%; 1.5 mL). Heptadecanoic acid methyl ester (C17:0) was used as the internal standard. Fatty acid analyses were carried out using an HP6890 gas chromatograph equipped with a capillary column (50 m × 0.25 mm i.d., 0.2 μm film thickness). The oven temperature was 165 °C. The carrier gas was helium at a flow rate of 1.2 mL min-1. The injector and flame ionisation detector (FID) temperatures were 190 and 250 °C respectively. The split ratio was1:50 and the injected volume was 1 μL. Identification of FAMEs was performed by comparing each sample with a standard FAME reference mixture. All fatty acid peak areas were calculated using HP Chem Station software and recorded as peak area percentages.

Fatty acid contents were determined by a capillary gas chro-matographic method described in EEC Regulation 2568/91. Fatty acid methyl esters (FAMEs) were prepared by saponification/methylation with methanolic KOH. Fatty acid analyses were carriedoutusingan HP6890 gaschromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with a CP-6173-SIL88 capillary col- umn (50 m × 0.25 mm i.d., 0.2 μm film thickness). The oven temperature was 165 °C. The carrier gas was helium at a flow rate of 1.2 mL min−1. The injector and flame ionisation detector (FID)temperatures were 190 and 250 °C respectively. The split ratio was 1:50 and the injected volume was 1 μL. Identification of FAMEs was performed by comparing each sample with a standard FAME reference mixture. All fatty acid peak areas were calculated using HP ChemStation software and recorded as peak area percentages. Fatty acid contents were determined by a capillary gas chro- matographic method described in EEC Regulation 2568/91. Fatty acid methyl esters (FAMEs) were prepared by saponification/methylation with methanolic KOH. Fatty acid analyses were carriedoutusingan HP6890 gaschromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with a CP-6173-SIL88 capillary col-umn (50 m × 0.25 mm i.d., 0.2 μm film thickness). The oven temperature was 165 °C. The carrier gas was helium at a flow rate of 1.2 mL min−1. The injector and flame ionisation detector (FID) temperatures were 190 and 250 °C respectively. The split ratio was 1:50 and the injected volume was 1 μL. Identification of FAMEs was performed by comparing each sample with a standard FAME reference mixture. All fatty acid peak areas were calculated using HP ChemStation software and recorded as peak area percentages.

2.6. Volatile compounds analysis

Solid Phase Micro-extraction (SPME) analyses were performed as described by El Arem et al. (2012) with slight modifications. Briefly, powdered dry fruit were inserted into a 5 mL vial and allowed to equilibrate for 30 min. A special fibre was exposed to the headspace for 25 min. Then, the fibre was transferred to the injection port of the GC-EIMS system. The resulting relative peaks areas were quantitatively compared. Analytical conditions were as follows: injector and transfer line temperatures were 250 and 240 °C, respectively; oven temperature was programmed from 60 to 240 °C at 3 °C/min; carrier gas was helium at 1 mL/min; split less injection. Retention times served for identification of constituents comparing their linear retention indices (LRI) on computer matching against library mass spectra, and MS literature data (Adams, 1995; Stenhagen et al., 1974; Swigar and Silvestein, 1981).

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