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Valorization of the whole grains *of Triticum aestivum* L. and *Triticum vulgare* L. through the investigation of their biochemical composition and *in vitro* antioxidant, anti-inflammatory, anticancer and anticalpain activities

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ABSTRACT

Two locally grown wheat species named *Triticum aestivum* L. and *Triticum vulgare* L. were studied for their phytochemical contents and their biological activities. *T. vulgare* presented the highest amounts of total phenolic compounds and ascorbic acids while *T. aestivum* was found to be rich in flavonoids, flavonols, proanthocynidins and *ortho*-diphenols. Eleven carotenoids were identified in *T. vulgare* where the most dominant compounds belongs to α-carotene and its derivatives while *T. aestivum* presented seven carotenoids. This later presented the highest DPPH radical scavenging activity and exhibited a strong reducing power in FRAP, phosphomolybdenum, hydrogen peroxide and reducing power assays. *T. vulgare* extract was found to be effective in metal chelating power and in scavenging nitric oxide radical. No significant differences in scavenging ABTS and hydroxyl radicals were noted between the two wheat species. *T. aestivum* inhibited xanthine oxidase and ROS production and showed the best cytotoxic effect while *T. vulgare* extract exhibited anti-calpain activity.

1. Introduction

Since a long time, wheat is considered as a major crop and is ranked among the most consumed grain worldwide. *Triticum aestivum* L. and *Triticum vulgare* L. are cereals belonging to the *Gramineae* (*Poaceae*) family, their height can reach 60–150 cm, but may be as short as 30 cm. Leaves are long and narrow having glabrous or hairy on one or both surface while stems are hollow and tufted. Wheat grain is oval and has a color ranging from white to red. Botanically, the grain of wheat is not a seed, but a particular fruit, a caryopsis. Its length varies from 7 à 9 mm, its width varies from 3 to 4 mm, and it has a thickness of 4–5 mm. Generally, its weight can range from 30 to 50 mg and includes three essential parts: envelopes (13–16% of the total grain weight), floury almond (82–84% of total grain weight) and the germ (2–3% of the total grain weight) (Patel and Patel, 2013).

Depending on the variety and composition, wheat is used for the elaboration of several food products such as bread and fine cakes obtained from hard wheat flour which is rich in gluten and pasta products such as macaroni and spaghetti obtained from durum wheat flour (Adom et al., 2003).

Grains represent a very interesting source of antioxidants in our

Abbreviations: EMEM, Eagle's Minimal Essential Medium.; FBS, Fetal Bovine Serum.; MTT, Methylthiazolyldiphenyl-tetrazolium bromide.; RRLC, Rapid Resolution Liquid Chromatography.; CIE, International Commission on Illumination.; DPPH, 1,1-diphenyl-2-picrilhydrazyl; $ABTS^{+}$, 2,2-azinobis(3-ethylbenzothiazoline-6-sufonic) acid.; FRAP, Ferric-Reducing Antioxidant Power; XO, Xanthine Oxidase.; ROS, Reactive Oxygen Species; RFU, Relative Fluorescence Units; HBSS, Hank's medium Balanced Salt Solution.

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daily diet. By importance, antioxidants may be listed as follows: phytoestrogens, phenolic compounds where ferulic acid represent the major component, and other substances such as lignans, phytic acid, and sterols. These metabolites were concentrated mostly in bran, nuclear envelope, and germ and have been well recognized for their potential beneficial effects in disease prevention and health promotion. Several epidemiological studies have demonstrated that there is a close relationship between consumption wheat seed and wheat based products and reducing chronic disease rates such as cardiovascular disease, diabetes, and cancer (Aydos et al., 2011; Moore et al., 2005).

Scientific reports on wheat species have essentially focused on functional characteristics in food products, preventing disease and improving yield and in this work, we are interested in the valuation of two wheat species known for their great consumption in Algeria: *T. aestivum* and *T. vulgare*. Certainly, the literature does not lack work on these two interesting cereals, but several aspects of their biological activities remain unexploited.

In this context, we studied the biochemical composition and specially phenolic compounds present in wheat and we quantified and identified by Ultra Performance Liquid Chromatography (UPLC) the major carotenoids. Antioxidant activities were determined using ten different tests depending on their operating principle and mode of action of antioxidants. Also the anti-inflammatory, anti-calpain and cytotoxic effects on cancer cells were determined.

2. Material and methods

2.1. Chemicals

Eagle's minimal essential medium (EMEM), trypsin-ethylene diamine tetra-acetic acid (EDTA), sodium pyruvate, fetal bovine serum (FBS), and L-glutamine were purchased from Gibco-BRL (Invitrogen, Scotland-UK). Methylthiazolyldiphenyl-tetrazolium bromide (MTT) and lucigenin were purchased from Sigma-Aldrich (St Quentin Fallavier, France). Antibiotics: streptomycin and penicillin were obtained from GIBCO (Cergy- Pontoise, France). The chromatographic solvents were methanol, acetonitrile, ethyl acetate (HPLC grade, procured from Merck, Darmstadt, Germany). Water was purified in a NANOpure®DIamond™system (Barnsted Inc., Dubuque, IO). Standards β-carotene, pheophytins a and b, luetin, β-cryptoxanthin, Chlorophylls *a* and *b*, antheraxanthin and zeinoxanthin were obtained from Sigma-Aldrich (Germany). All other chemical reagents used were obtained from Sigma-Aldrich (Oakville,ON) or Alfa Aesar Co. (Ward Hill, MA).

2.2. Sample treatments

T. vulgare and *T. aestivum* were harvested in August 2012 in Timezrit located at East of Bejaia City, Algeria. These cereals were identified and voucher specimens were deposited at the Herbarium of Natural History Museum of Aix-en-Provence, France (*Triticum vulgare*: D-PH-2013-37-11 and *Triticum aestivum*: D-PH-2013-37-12). Healthy and uninfected grains were selected and ground to a fine powder (diameter < 300 μm).

Cereal powders (1 g) were exhaustively extracted by maceration with 50 ml ethanol, at room temperature for 24 h. In all cases the extracts were centrifuged (6800×g/20 min) and the extraction was repeated twice. Obtained extracts were pooled and stored at −20 °C in darkness.

2.3. Antioxidant contents

2.3.1. Phenolic compounds contents

Total phenolic compounds content was determined using Maksimović et al. (2005) method and the results were expressed as mg of gallic acid equivalents per 100 g of dry weight (mg GAE/100g DW) through the calibration curve with gallic acid ($y = 0.0015 + 0.1483x$; $r^2 = 0.999$).

Flavonoids content was achieved using the method described by Amessis-Ouchemoukh et al. (2014a). Amounts of flavonoids were deduced from a standard curve ($y = -0.0008 + 0.1162x$; $r^2 = 0.994$) and calculated in mg quercetin equivalents (QE)/100g dry weight (DW).

Total flavonols in *Triticum* extracts were assayed by the method of Adedapo et al. (2008) and the amounts were expressed as mg of quercetin equivalents per 100g of dry weight (mg QE/100g DW). Quercetin was used to establish the calibration curve ($y = -0.0003 + 2.2549x$; $r^2 = 0.999$).

Proanthocyanidins content was determined by the method described by Maksimović et al. (2005) using butanol-HCl and the amounts were expressed as mg (+)-catechin equivalent (CE)/100g DW ($y = -0.1488 + 2.078x$; $r^2 = 0.999$).

Ortho-diphenols contents were determined according to the method described by Tovar et al. (2002) and were expressed as mg of gallic acid equivalents (GAE)/100g DW ($y = -0.0087 + 0.0848x$; $r^2 = 0.998$).

2.3.2. Ascorbic acid content

Ascorbic acid contents were determined according to the method described by Mau et al. (2005) and were expressed as mg ascorbic acid equivalents (AAE)/100g DW ($y = 1.0142–18.333x$, $r^2 = 0.999$).

2.3.3. Carotenoids content

The analysis of carotenoids was performed according to the method of Melendez-Martinez et al. (2007). Cereal powders (20 g) were mixed with 15 ml of hexane/acetone/methanol (2/1/1, v/v/v). The mixture was placed into ultrasonic bath (Selecta, Spain) for 5 min and extraction was repeated 3 times. After centrifugation at 4500 rpm for 10 min (20 °C), supernatants were concentrated to dryness in rotary evaporator at 30 °C, added with 150 μl of hexane and centrifuged (14000×g/5 min/4 °C). The supernatant was injected in the Rapid resolution liquid chromatography (RRLC).

Carotenoid extracts were separated and quantified by RRLC (Agilent 1260 system equipped with a diode-array detector) using polymeric C18 Poroshell 120 column (2.7 μm, 5 cm × 4.6 mm) (Agilent, Palo Alto, CA). Acetonitrile (solvent A) was used as mobile phase, methanol (solvent B) and acetate ethyl (solvent C). The gradient elution profile was as follows: 0 min, 85% A + 15% B; between 5 and 7 min, 60% A + 20% B + 20% C; between 9 and 12 min, 85% A + 15% B. The flow rate was 1 ml/min, the injection volume was 20 μl and the chromatograms were monitored at 450 nm. The identification of carotenoids was made by comparison of the chromatographic and UV/VIS spectroscopic characteristics with those of standards. The carotenoid compounds were quantified based on the area under the detected peaks against calibrated standards. Total carotenoids were expressed as sum of single quantified carotenoids (Stinco et al., 2014).

2.4. Color

The Vis-NIR reflectance spectra were acquired using a spectrometer Spectro-320 (Instrument Systems, Germany) coupled with an integrating sphere ISP-150 (Instruments Systems, Germany) and using a Certified Reflectance Standard (Labsphere, North Sutton NH, USA). Each sample was put into a quartz cell and was placed in the integrating sphere with diffuse illumination and an observation angle of 8° (geometry "d/8°"). The Specwin v. 1.8.1.6 software (Instruments Systems, Germany) recorded the reflectance