Food Chemistry 118 (2010) 300–306

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

In vitro evaluation of red and green lettuce (*Lactuca sativa*) for functional food properties

Vanisree Mulabagal [a], Mathieu Ngouajio [b], Ajay Nair [b], Yanjun Zhang [c], Aditya L. Gottumukkala [a], Muraleedharan G. Nair [a,*]

[a] Bioactive Natural Products and Phytoceuticals, Department of Horticulture, 173 National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI 48824, USA
[b] Department of Horticulture, Michigan State University, East Lansing, MI, USA
[c] UCLA Center for Human Nutrition, David Geffen School of Medicine, 900 Veteran Avenue, Los Angeles, CA 90095, USA

ARTICLE INFO

Article history:
Received 25 August 2008
Received in revised form 31 March 2009
Accepted 29 April 2009

Keywords:
Phenolics
Lactuca sativa
Anthocyanins
Antioxidant
Cyclooxygenase enzyme

ABSTRACT

Lettuce (*Lactuca sativa*) is an important leafy vegetable consumed fresh or in salad mixes. We have compared the functional food properties of selected commercial red and green lettuce varieties grown under field conditions. Both lettuce cultivars were extracted with water at biological (38 °C) and room temperatures (22 °C) at pH 2. The residues from each extraction were further extracted, sequentially with methanol and ethyl acetate. The extracts were evaluated for their *in vitro* lipid peroxidation (LPO) and cyclooxygenase enzyme (COX) inhibitory activities. Amongst the extracts tested, all three extracts of red lettuce showed higher LPO and COX-1 and -2 enzyme inhibitory activities than did the green lettuce extracts. Red lettuce contained a single anthocyanin, cyanidin-3-*O*-(6″-malonyl-β-glucopyranoside) (**1**), which immediately converted to cyanidin-3-*O*-(6″-malonyl-β-glucopyranoside methyl ester) (**2**) and cyanidin-3-*O*-β-glucopyranoside (**3**) under laboratory conditions. Anthocyanins **1** and **2** inhibited LPO by 88% and 91.5%, respectively, at 0.25 μM concentration. Also, they inhibited COX-2 enzyme by 78.9% and 84.3% and COX-1 by 64% and 65.8%, respectively, at 5 μM. The chicoric acid (**4**), amongst other phenolics, such as quercetin glucoside, ferulic and caffeic acids, isolated from both green and red lettuce, showed 85.6%, 45.6% and 94% of LPO, COX-1 and -2 enzyme inhibitions at 50 μM, respectively. This is the first report of the LPO, COX-1 and -2 enzyme inhibitory activities of compounds **1**, **2** and **4**. The variation of phenolics in the red and green lettuces, and specifically the lack of anthocyanins in green lettuce, might account for the higher biological activity obtained with the red variety in our study.

1. Introduction

Numerous studies have shown a correlation between the consumption of fresh fruits and vegetables and their health benefits. Epidemiological studies have further demonstrated the relationship between dietary habits and disease risk and established that food has a direct impact on health. Lettuce, *Lactuca sativa*, is an important dietary leafy vegetable that is primarily consumed fresh or in salad mixes due to its perception as being amongst healthier foods (Dupont, Mondi, Willamson, & Price, 2000). A number of lettuce varieties have been investigated recently and reported to contain phenolic compounds with antioxidant activities (Llorach, Martinez-Sanchez, Tomas-Barberan, Gil, & Ferrers, 2008; Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995). The health benefits of lettuce have also been attributed to the presence of Vitamin C, phenolic compounds and fibre content (Nicolle & Cardinault et al., 2004; Nicolle & Carnat et al., 2004. In folk medicine, lettuce seeds are used in the treatment of asthma, cough and as an analgesic.

In 2006, over 4,338,000 metric tons of lettuce plants were harvested from over 306,600 acres with a total value of over $2 billion. Iceberg, romaine and leaf lettuces represented 61%, 18% and 21% of the total national production (by weight), respectively. Despite a relatively small proportion of the total production (21%), leaf lettuce, including red and green types, continues to have the highest value. In 2006, leaf lettuce was valued at $8,451/acre compared to $5,596/acre and $7,016/acre for iceberg and romaine types, respectively. Iceberg lettuce is so far the most common lettuce used (especially in fast food restaurants). The antioxidant phenolics in lettuce vary amongst varieties due to growing practices, processing and storage conditions (Baur, Klaiber, Koblo, & Carle, 2004). Currently, red lettuce is popular in salad mixes due to its anthocyanin content that contributes to the higher value it fetches compared to the green lettuce (Gazula, Kleinhenz, Scheerens, & Ling, 2007). The increased demand of fresh vegetables associated with health benefits has led to an increase in the quality, quantity and variety of produce available to the consumer. Various approaches, involving

* Corresponding author. Fax: +1 (517) 432 2310.
E-mail address: nairm@msu.edu (M.G. Nair).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.04.119

environmental, cultural and management practices, have been used to enhance the quality of lettuce, specifically in the areas of phytochemical contents and health-promoting attributes (Kleinhenz, French, Gazula, & Scheerens, 2003).

Anthocyanins are water-soluble phenolic glycosides that colour fruits, flowers, vegetables and cereals. Apart from imparting colour to plants, anthocyanins exhibit an array of health-promoting benefits. We have reported that anthocyanins isolated from various plants inhibited lipid peroxidation (LPO) and cyclooxygenase (COX) enzymes (Mulabagal, Van Nocker, DeWitt, & Nair, 2007; Seeram, Cichewez, Chandra, & Nair, 2003; Tall, Seeram, Zhao, Nair, & Meyer, 2004; Wang, Nair, & Strasburg, 1999). The ability of anthocyanidins to inhibit LPO and COX enzymes has also been reported (Seeram & Nair, 2002). Both anthocyanins and anthocyanidins stimulated insulin release by rodent pancreatic β-cells (INS-1 832/13) *in vitro* (Jayaprakasam, Vareed, Olson, & Nair, 2005). Also, a purified anthocyanin mixture from *Cornus mas* fruits has demonstrated an ability to ameliorate obesity and insulin resistance in C57BL/6 mice fed a high-fat diet (Jayaprakasam, Olson, Schutzki, Tai, & Nair, 2006). In another *in vivo* study, anthocyanins up-regulated the adipocyte-specific gene and genes involved in lipid metabolism (Tsuda, Ueno, Kojo, Yoshikawa, & Osawa, 2005; Tsuda et al., 2004).

Although red lettuce costs more than green lettuce, it is becoming very popular amongst consumers. This is probably due to its red colour and its association with better health as in the case of red fruits and berries. Therefore, in this study, we have compared both green and red lettuce, using *in vitro* bioassays and chemical composition studies and evaluated their functional food advantages.

2. Materials and methods

2.1. Materials

Seeds of green (Var. North Star) and red lettuce (Var. Cherokee) were purchased from Siegers Seeds Company (Holland, MI). The COX-1 enzyme was prepared from ram seminal vesicles purchased from Oxford Biomedical research, Inc. (Oxford, MI). The COX-2 enzyme was prepared from prostaglandin endoperoxide-H synthase-2 (PGHS-2)-cloned insect cell lysate. Solvents used for isolation and purification were of ACS grade and purchased from Sigma–Aldrich Chemical Co., Inc. (St. Luis, MO). Positive controls, t-butyl hydroquinone (TBHQ), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), used in the anti-oxidant assay, were purchased from Sigma Chemical Company.

2.2. Equipment

Samples were homogenised using a Kinematica CH-6010 (Roxdale, ON, Canada) homogenizer and centrifuged (model RC5C, Sorvall Instruments, Hoffman Estates, IL) at 10,000*g* for 20 min at 4 °C. The NMR (^{1}H & ^{13}C) experiments were recorded on Varian INOVA (300 MHz) and VRX (500 MHz) instruments. The chemical shifts were measured in DMSO-*d6* and CD_3OD/DCl solutions and are expressed in δ (ppm). Fractionation of anthocyanin was carried out on a XAD-2 column (500 g, Amberlite resin, mesh size 20–50; Sigma Chemical Co., St. Louis, MO) and purified on a C-18 MPLC column (350 × 40 mm). Anthocyanin detection was carried out with a Waters 2010 HPLC system (Waters Corp.) equipped with Empower Software, Shodex Degasser, Auto sampler (Waters 717) and a Photodiode Array Detector (Waters 996). HPLC analysis was carried out by using a Capcell Pak column (DyChrom, Santa Clara, CA) C-18 column (150 × 4.6 mm i.d.; 5 μm particle size). Preparative HPLC purification of anthocyanin was carried out by using a Capcell Pak (DyChrom, Santa Clara, CA) C-18 column (250 × 4.6 mm i.d.; 5 μm particle size).

2.3. Plant material

Lettuce transplants were produced in the greenhouse using 72-cell flats filled with commercial greenhouse potting mix. At the two-leaf stage, the seedlings were transplanted to the field. Field experiments were conducted at Michigan State University Horticulture Teaching and Research Center. The lettuce seedlings were transplanted on raised beds covered with black plastic mulch and drip-irrigated using two staggered rows per bed. Spacing was 30 cm between the rows and 30 cm between plants inside each row. The plants were grown in the absence of any pesticide.

2.4. LC/MS analysis

Samples were analysed on a Surveyor HPLC system equipped with a diode array absorbance detector (DAD), measuring at 520 nm, and an autosampler cooled to 4 °C (Thermo Finnigan, San Jose, USA). An Agilent Zorbax SB C-18 column, 150 × 2.1 mm, i.d.; 5 μm particle size (Agilent, USA), was used and solvent elution consisted of a gradient system over 50 min of methanol (1% acetic acid) and H_2O (1% acetic acid) at a flow rate of 0.19 ml/min. The linear gradient system started from 5% methanol (1% acetic acid) and 95% of H_2O (1% acetic acid) to 95% methanol (1% acetic acid) and 5% of H_2O (1%) at 50 min. The column was maintained at 25 °C. After passing through the flow cell of the DAD, eluate was directed to a LCQ Advantage ion trap mass spectrometer fitted with an Eelectrospray Interface (ESI). Analyses utilised the positive ion mode (*m/z* $M + H^+$) for detection of anthocyanins. Preliminary analyses were carried out using full scan, data-dependent MS/MS scanning from *m/z* 250 to 1000. The capillary temperature was set at 275 °C and the sheath and auxiliary gas at 45 and 0 units/min, respectively. The source voltage was 4 kV. MS/MS and fragmentation were carried out with 50% energy.

2.5. Extraction of lettuce

Fresh leaves of red lettuce (1.2 kg) were blended and extracted at 22 °C with acidic water (0.1% HCl, 1 l 3×) and centrifuged. Supernatants were lyophilised to get water extract (19.1 g). The residue was further extracted with methanol (500 ml 3×), followed by ethyl acetate (500 ml 3×) and the organic extracts evaporated to dryness under reduced pressure. The yields of methanol and ethyl acetate extracts were 7 and 0.2 g, respectively. Similarly, green lettuce (300 g) leaves were blended and extracted with acidic water (200 ml 3×) and the water-soluble portions were centrifuged and lyophilised (3.4 g). Residue was extracted further with methanol (100 ml 3×), followed by ethyl acetate (100 ml 3×) and the resulting extracts evaporated under vacuum to afford 0.3 and 0.4 g of dried extracts, respectively.

To mimic *in vivo* conditions, another extraction was carried out at 38 °C and pH = 2. Red lettuce leaves (350 g) were blended with acid water (0.1% HCl, 300 ml 3×) and allowed to stand at 38 °C and pH = 2 for 4 h. The mixture was then centrifuged and the supernatant lyophilised to yield a powder (14.7 g). The residue was then extracted sequentially with methanol (200 ml 3×) and ethyl acetate (200 ml 3×). The yields of methanol and ethyl acetate extracts from this procedure were 4.9 and 0.8 g, respectively. The green lettuce leaves (200 g) were also extracted in a similar manner (75 ml 3×), followed by methanol (75 ml 3×) and ethyl acetate (75 ml 3×) to afford 3.4, 0.3 and 0.4 g of dried extracts, respectively.