Food Chemistry 201 (2016) 298–306

Contents lists available at ScienceDirect

Food Chemistry

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

Antioxidative free and bound phenolic constituents in botanical fractions of Indian specialty maize (*Zea mays* L.) genotypes

Amit K. Das [a], Vasudeva Singh [a,b,*]

[a] Dept. of Grain Science and Technology, CSIR-Central Food Technological Research Institute, Mysore 570020, Karnataka, India
[b] Dept. of Studies in Food Science and Nutrition, Manasagangotri, University of Mysore, Mysore 570006, Karnataka, India

ARTICLE INFO

Article history:
Received 5 July 2015
Received in revised form 21 January 2016
Accepted 21 January 2016
Available online 22 January 2016

Chemical compounds studied in this article:
Vanillic acid (PubChem CID: 8468)
Syringic acid (PubChem CID: 10742)
p-Hydroxybenzoic acid (PubChem CID: 135)
Caffeic acid (PubChem CID: 689043)
p-Coumaric acid (PubChem CID: 637542)
Ferulic acid (PubChem CID: 445858)
Isoferulic acid (PubChem CID: 736186)
Cyanidin 3-O-glucoside (PubChem CID: 176457)
Kaempferol (PubChem CID: 5280863)
Quercetin (PubChem CID: 5280343)

Keywords:
QPM
Popcorn
Baby corn
Sweet corn
Phenolic acids
Flavonoids
Vitamin E
Antioxidant

ABSTRACT

Specialty maize genotypes *viz.* QPM (quality protein maize), Baby corn, Popcorn and Sweet corn, which are usually consumed in whole forms can be good supplements of phenolic antioxidants. Botanical fractions of these maize genotypes were analyzed to explore the distribution of free and bound phenolics. HPLC and ESI-MS/MS results indicated the presence of vanillic, syringic, *p*-hydroxybenzoic, caffeic, *p*-coumaric, ferulic and isoferulic acids along with cyanidin-3-O-glucoside, kaempferol and quercetin. Germs of maize samples contained significantly higher free phenolics than pericarps, whereas, pericarps contained 74–83% of bound ones. QPM and Popcorn contained only 3% free phenolics whereas, Baby corn and Sweet corn had 14–17%. Unlike in peroxide scavenging and reducing capacity, anti-radical capacity of free phenolics of germs was significantly higher than that of pericarps. Free phenolics contributed 0.2–1.65%, 2–5% and 42–49% in anti-radical, peroxide scavenging and reducing capacity, respectively. Among lipophilic tocochromanols γ-tocopherol was the most abundant isomer in the samples among which Sweet corn contained the most (84.2 μg/g). Data showed that specialty maize genotypes are rich sources of hydrophilic and lipophilic bioactives and are natural antioxidants.

1. Introduction

In the last decade, maize has registered the highest growth rate among all food grains including wheat and rice because of newly emerging food habits as well as enhanced industrial requirements. The annual global production of maize in the year 2013 was 1000 million metric tons (MMT) of which India produced more than 23 MMT (FAOSTAT, 2013). Other than high-yielding field corn (dent and flint genotypes), India produces various specialty maize among which QPM (quality protein maize), Baby corn, Popcorn and Sweet corn are being popularized and cultivated by a large number of farmers. The growing popularity of maize consumption is primarily due to its suitability of replacing rice and wheat from the diet to a considerable extent. It was also reported that maize possesses higher content of phenolics than some of the other important cereals (Adom & Liu, 2002; Ndolo & Beta, 2014). Phytochemicals especially the phenolics, are mainly concentrated in the outermost layers of the grains and exhibit significant antioxidant activity with a number of other bioactivities such as cell differentiation, deactivation of pro-carcinogens, DNA repair, inhibiting N-nitrosamine formation, and change of estrogen metabolism, etc. (Shahidi, 2004). Unlike in fruits and vegetables,

* Corresponding author at: Dept. of Studies in Food Science and Nutrition, Manasagangotri, University of Mysore, Mysore 570006, India.
E-mail address: singhva2003@yahoo.co.in (V. Singh).

http://dx.doi.org/10.1016/j.foodchem.2016.01.099
0308-8146/

phenolics in cereals are present in soluble-free, soluble-ester and insoluble-bound forms (Bunzel, Ralph, Marita, Hatfield, & Steinhart, 2001; Lloyd, Siebenmorgan, & Beers, 2000). When ingested, the free phenolics are rapidly absorbed by the small intestine and subsequently conjugate, leading to less accumulation of aglycones in the blood (Scalbert & Williamson, 2000). On the other hand, bound phenolics, which are associated with cell wall polysaccharides, survive gastrointestinal digestion but are released through colonic fermentation to exert their unique benefits in the colon (Adom & Liu, 2002; Andreasen, Kroon, Williamson, & Garcia-Conesa, 2001). Therefore, emphasis on the distribution of both free and bound phenolic constituents and their antioxidant activity is key to understand the potential health benefit of whole grain consumption.

Unlike in maize, the distribution of phenolic compounds had been investigated by various authors for wheat (Adom, Sorrells, & Liu, 2005; Beta, Nam, Dexter, & Sapirstein, 2005; Siebenhandl et al., 2007) and rice (Butsat & Siriamornpun, 2010; Shao, Xu, Sun, Bao, & Beta, 2014; Ti et al., 2014). However, a few studies on various maize varieties and landraces (De la Parra, Serna Saldivar, & Liu, 2007; Lopez-Martinez et al., 2009; Zilic, Serpen, Akıllıoğlu, Gökmen, & Vančetović, 2012) depicted no data on distribution of phenolics in the kernel. Distribution of phenolic acids in botanical fractions of yellow maize (Ndolo & Beta, 2014) and bound phenolic acids in bran and endosperm of white maize (Chiremba, Taylor, Rooney, & Beta, 2012) have been studied, but these studies did not report data on of free phenolics in the kernel. Flavonoid compounds were characterized in purple and waxy maize (Abdel-Aal, Young, & Rabalski, 2006; Harakotr, Suriharn, Tangwongchai, Scott, & Lertrat, 2014) but was not observed in yellow maize (Zilic et al., 2012). In addition to the phenolic constituents, whole grain cereals are also known for their content of vitamins, minerals, trace elements and carotenoids. Among these phytochemicals vitamin E is a direct free radical scavenger and alleviates oxidative stress. Fardet, Rock, and Rémésy (2008) reported maize as the richest source of vitamin E among various other cereals including wheat. This led us to quantify the homologs of vitamin E (tocochromanols) from the Indian yellow maize of specialty genotypes. Moreover, data on detailed phytochemical profile of specialty maize is scarce more so, from this subcontinent.

It was found that the data on independent distribution of free and bound phenolics in all the botanical fractions of maize, especially specialty genotypes, are scanty in literature. Hence, the objective of this study was to explore the distribution of free and bound phenolics in the botanical fractions of maize kernels, their antioxidant capacities and the profile of tocochromanols of the whole kernel of some popular Indian specialty maize genotypes.

2. Materials and methods

2.1. Materials

All phenolic acids, flavonoids and tocopherol standards, trolox and DPPH were purchased from Sigma–Aldrich (St. Louis, MO, USA). Follin Ciocalteu's reagent was purchased from SRL Pvt. Ltd. (Mumbai, India). Hydrogen peroxide was purchased from Merck Specialities (Mumbai, India). Sep-pak C_{18} cartridges were purchased from Waters Corporation (Milford, MA, USA). Solvents used for HPLC analyses were of HPLC grade. Potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride, sodium nitrite, aluminum chloride, solvents, acids and all other general chemicals used were of analytical grade.

2.2. Sample preparation

HQPM-7, HM-4, VL Amber and Madhuri from the *Kharif* crop (sown in June and harvested in October) of 2010 are the simple random samples selected from the hybrid/composite varieties of QPM, Baby corn, Popcorn and Sweet corn, respectively, released in India during 1961–2010 by Directorate of Maize Research (DMR), New Delhi, India (Kaul et al., 2010). Each representative sample was collected from a large are of adaptation and in different agro-climatic conditions of the country. The samples were kindly provided by DMR and were stored at 5–7 °C before use. Prior to analyses, the samples were milled to separate germ and pericarp (along with aleurone layer) from the endosperm using Satake Grain Mill (Satake Co., Tokyo, Japan). The pericarp and the germ were then isolated manually. Approximately 80% of endosperm obtained through above process was again milled through McGill polisher (H.T. McGill Houston, Texas, USA) for 3 min with a pressure of 13.34 N on the lever-arm of the polisher followed by sieving through 1400 μm screen mesh. The polishing was repeated until there was no permeate from the mesh, so as to ensure complete separation of aleurone layer from the endosperm. The pericarp, germ and endosperm portions were ground individually using a laboratory milling machine (Model No. 2900000, IKA, Germany) and passed through 250 μm screen mesh.

In order to calculate the relative percentages of botanical fractions, 50 g of maize kernels were soaked in 100 ml of water for 20 min. After draining the water, the wet kernels were transversally dissected with a scalper to separate bran, germ and endosperm followed by drying in an oven at 50–60 °C and weighed (Das & Singh, 2015).

2.3. Extraction of free and bound phenolic constituents

Free phenolics were extracted using coupled method of solid–liquid extraction (SLE) and solid–phase extraction (SPE) previously reported by us (Das, Sreerama, & Singh, 2014). Briefly, five grams, on dry basis (db), of each powdered botanical fraction was extracted thrice with 80% methanol for 20 min, centrifuged at 2500×*g* for 15 min, and the supernatant was decanted. The pooled supernatant was vacuum evaporated at 50–52 °C and passed through a pre-activated Sep-Pak C_{18} cartridge. Finally, the phenolics were eluted with a known volume of methanol.

Bound phenolics were extracted according to the method of Arranz and Calixto (2010) with modification (Das et al., 2014). Briefly, one gram (db) of each powdered botanical fraction was extracted twice with 80% methanol, centrifuged and the supernatant was discarded. The residue was then refluxed in methanol/hydrochloric acid 90:10 (v/v) at 85 °C for 20 h, vacuum evaporated and adjusted to pH 2.0 before passing through a pre-activated Sep-Pak C_{18} cartridge. Finally, the phenolics were eluted with a known volume of methanol.

2.4. Determination of total phenolic and total flavonoids content

The total phenolic content was determined using the method described by Singleton, Orthofer, and Lamuela-Raventos (1999). Briefly, appropriate dilution of the extracts was oxidized with Folin–Ciocalteu reagent and the reaction was neutralized with sodium carbonate (20%). The resulting blue color was read after 30 min at 750 nm in Shimadzu UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). The results were expressed as micromoles of ferulic acid equivalents (FAE) per gram of sample (db). Total flavonoids were determined using the aluminum chloride method with modifications (Das et al., 2014). Briefly, extracts were mixed with 75 μL sodium nitrite (5%) and after every 6 min 15 μL aluminum chloride (10%) and 500 μL sodium hydroxide (1 M). After