

Carotenoids, Phenolic Compounds and Antioxidant Capacity of Five Local Italian Corn (*Zea Mays L.*) Kernels

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Abstract

Pigmented corn contains several bioactive phytonutrients that possess many biological activities, such as antioxidant, anticancer and anti-inflammation properties. In this study, the content of major nutrients (carbohydrates, fats and protein and fiber), carotenoids and phenolics, were determined for the first time in whole kernels of 5 different pigmented corn genotypes included in the regional register of biodiversity of Marche (Italy) managed by Agency for Food Service Industry in the Marche (ASSAM). In addition, the antioxidant activity was evaluated as radical scavenging activity using ORAC assay. The carotenoid compounds were characterised. Zeaxantin, the most abundant carotenoid was found in concentrations ranging between 176-218 µg/100 g. The β-carotene and lutein contents ranged from 27-39 µg/100 g and 23-49 µg/100 g, respectively. The highest content of polyphenols was found in corn Roccacontrada Rosso. All corn hydrophilic extracts inhibited significantly low density lipoprotein (LDL) peroxidation triggered by copper ions.

These results suggest that corn varieties constitute a good source of natural antioxidant and could be considered a functional food.

Keywords: Antioxidant capacity; Bioactive phytonutrients; Biodiversity; Carotenoids; Lipid peroxidation; Low-density lipoproteins (LDL); Corn (*Zea Mays L.*); Phenolic compounds

Introduction

Corn is one of the most widely cultivated cereals in the world. There are several types of genotypes, with colours such as white, yellow, violet, red, black, and blue. Pigmented corn has received increased attention from a nutraceutical perspective because it contains several bioactive phytochemicals such as carotenoids [1-5], tocopherols [6], phytic acid [7] and phenolic compounds [1,2,8,9]. Although these compounds are considered non-nutritive, the interest in their antioxidant and bioactive properties has increased due to their potential health benefits [10]. In fact it has been demonstrated that several plant phytochemicals are bioavailable, they are absorbed from intestine and exert several physiological roles as demonstrated in animal models and in human studies [11-13]. In addition to their high antioxidant and antiradical activities, the health beneficial properties of these plant metabolites have been attributed also to many other mechanisms such as anti-inflammatory properties, inhibition of enzymes, and induction of detoxification enzymes [14-17].

The present study was conducted to investigate for the first time the levels of carotenoids and antioxidant properties of different types of corn included in the regional register of biodiversity of Marche (Italy) managed by Agency for Food Service Industry in the Marche (ASSAM), an institution involved in the implementation of programs for the protection of biodiversity for agriculture of Marche Region.

Materials and Methods

Corn varieties

A collection of five genotypes of corn (year 2011) (Roccacontrada Rosso, Roccacontrada Giallo, Ottofile Pollenza, Ottofile Treia, Dodicifile Treia) under agro-ecological management in the province of Ancona and Macerata (Marche, Italy) was used in the present study. The different types of corn are included in the regional register of biodiversity of Marche (Italy), as part of development policies,

promotion and protection of agro-ecosystems and production of quality, in relation to the Regional Law No. 12 "Protection of animal and plant genetic resources of the Marche" approved June 2003. The law protects the genetic resources that are locally grown within the region. Corns were picked by hand at complete maturity stage.

Proximate composition analysis

Each sample was used to evaluate the carbohydrate, protein and fat content. The procedure AOAC method 985.29 [18] was adapted for the total dietary fiber determinations.

Preparation of hydrophilic extracts

All corn kernels were ground to a fine powder prior to extraction. 0.5 g corn powders were extracted three times with 5 mL of methanol in a flask and shaking it for 2 h at room temperature and in the absence of light. After centrifugation, the combined supernatants were reduced to dryness and resuspended in 1 mL of methanol. The extracts of supernatant fluid were kept at -20°C in the dark until further analysis for total phenolics and antioxidant activity [1].

Determination of total phenolic content

The total phenolic content of hydrophilic extracts was determined using the Folin-Ciocalteu colorimetric method [19]. Briefly, 25 µL

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of extracts were oxidized with the Folin-Ciocalteu reagent, and the reaction was then neutralized with sodium carbonate. Then mixtures were allowed to stand at room temperature. After 60 minutes, absorbance was measured at 760 nm against a blank containing an extraction solvent instead of sample. The total phenolic content of each sample was determined by means of a calibration curve prepared with gallic acid and expressed as mean of mg of gallic acid equivalents (GAE) per 100 g of dry weight of sample.

ORAC assay

Total antioxidant capacity of corn hydrophilic extract was evaluated using oxygen radical absorbance capacity method (ORAC) [20]. This assay is based on the degree of inhibition of fluorescein oxidation by antioxidants that scavenge peroxy radicals generated from the thermal degradation of 2,2'-azobis (2-methylpropionamide) dihydrochloride (AAPH). Fluorescence emission intensity was evaluated using 485 nm as excitation wavelength and 530 nm as emission wavelength. Fluorescence loss was monitored in a Synergy microplate reader. The fluorescence was measured every 2 min for 4 h. All samples were analyzed in triplicate. The final ORAC values were calculated using the net area under the decay curves. Data were expressed as micro molar Trolox equivalents (TE) per 100 g of dry weight sample.

Carotenoid analysis

Carotenoids were quantified according to previously reports by Scott & Eldridge [4], using HPLC system with ultraviolet visible detector. The separation was performed isocratically on a Spherisorb ODS2 column (3 mm, 4.0×250 mm² with titanium frits). The mobile phase consisted of acetonitrile/dioxane-50 methanol-50 isopropanol/triethylamine (80/15/5/0.1) at a flow rate of 1.0 mL/min. The alcohol component contained 150 mm ammonium acetate. A photodiode array (PDA) detector was programmed to measure carotenoids at 450 nm. The calibrants included lutein, zeaxanthin, β -cryptoxanthin, lycopene, α -carotene, and β -carotene. Quantification and quality control were performed by external standard calibration using peak areas [4].

Isolation of low density lipoproteins (LDL) and copper-triggered oxidation

Blood samples were obtained at 8.00 a.m. after overnight fasting from healthy donors by venipuncture and were collected in heparin-containing vacutainer tubes. Plasma was prepared by centrifugation at 3000 rpm for 20 minutes and thereafter used for the preparation of lipoproteins. Low density lipoproteins (LDL) (density between 1.025 and 1.063 g/ml) were isolated by single vertical spin density gradient ultracentrifugation for 1.30 hrs at 65,000 rpm and dialysed at 4°C for 24 hrs against 5 mM phosphate buffer saline (PBS), pH 7.4 [21]. A pool of LDL from different subjects was used in the following experiments. Protein concentration of LDL was determined by the method of Bradford [22].

Ability of corn extracts to protect LDL from oxidation

Antioxidant activity of hydrophilic extracts from corn was assessed by monitoring the conjugated diene formation at 234 nm during copper-induced oxidation of LDL incubated in the absence or in the presence of corn extracts [23]. Conjugated dienes were evaluated in a 96-well microplate reader. Briefly, a volume of LDL was diluted in water to a final concentration of 100 μ g/ml per well, 0.01 ml of corn extract were added. The volume of each well was adjusted with deionized water to reach the final volume of 0.2 ml. The oxidation reaction was initiated by adding 0.1 mM Cu²⁺ (final concentration 5 μ M) to each well and

conjugated dienes were monitored at 234 nm for 4 hrs at 37°C. The lag phase was then determined graphically.

Data analysis

Mean value and standard deviation (SD) were calculated. ANOVA analysis and Duncan's multiple range test for mean separation were performed and a p<0.05 was considered statistically significant. Pearson correlation coefficients and their significance levels were calculated for linear regression analysis. (Microcal Origin 5.0 for Windows, OriginLab, Northampton, MA).

Results

Composition

Table 1 shows major nutrients of corn varieties included in our study. There were no significant differences as far as concerns carbohydrate, fat; protein and fiber content (Table 1).

Total phenol content and total antioxidant activity

Total phenolic content ranged from 115.4 \pm 9.3 mg/100 g to 175.5 \pm 6.8 mg/100 g of dry weight of corn extracts. The highest value was observed in Roccacontrada Rosso. As shown in Figure 1, the antioxidant capacity of hydrophilic corn extracts evaluated by ORAC assay ranged from 1827.5 \pm 90.5 μ mol TE/100 g to 2429.3 \pm 406.8 μ mol TE/100 g. The highest values were observed in Ottofile Treia and Roccacontrada Rosso. A significant positive correlation was established between the levels of total phenolic compounds and the ORAC values (r=0.83, n=15, p<0.002).

Carotenoids

The carotenoid content of the five corn varieties is shown in Table 2. The major carotenoids identified by HPLC were in the order zeaxanthin> α -cryptoxanthin>lutein> β -carotene> α -carotene> β -cryptoxanthin (Table 2). Total carotenoid concentration was highest in Dodicifile Treia. The highest concentration of lutein and zeaxanthin has been observed in Dodicifile Treia. The highest value of β -carotene was in Roccacontrada Rosso.

	Carbohydrate	Protein	Fat	Total fiber
RoccacontradaRosso	78 \pm 1	8.8 \pm 0.2	4.2 \pm 0.3	7.5 \pm 0.6
RoccacontradaGiallo	77 \pm 1	9.1 \pm 0.7	5.5 \pm 0.3	8.3 \pm 0.3
Ottofile Pollenza	77 \pm 2	7.8 \pm 1.1	4.9 \pm 0.5	7.2 \pm 0.3
Ottofile Treia	76 \pm 1	8.1 \pm 0.6	3.8 \pm 1.2	7.8 \pm 0.5
Dodicifile Treia	76 \pm 2	8.5 \pm 0.2	4.0 \pm 0.4	8.1 \pm 0.9

Table 1: Major nutrients of different varieties of local Italian corn (*Zea Mays L.*) kernels (g/100 g).

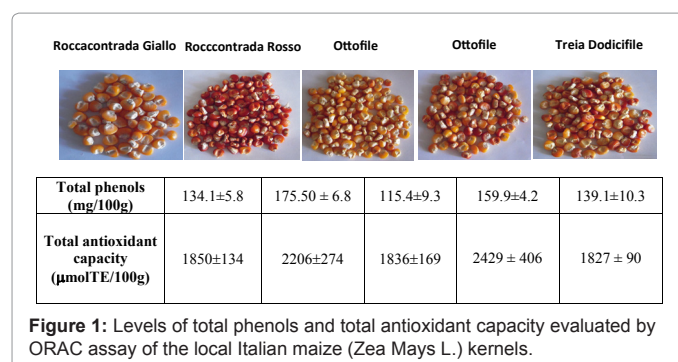


Figure 1: Levels of total phenols and total antioxidant capacity evaluated by ORAC assay of the local Italian maize (*Zea Mays L.*) kernels.

Effect of hydrophilic extracts on copper-triggered low density lipoproteins (LDL) oxidation

To investigate the ability of hydrophilic extracts of corn to inhibit copper-triggered LDL oxidation, we compared the increase of conjugated diene in the absence and in the presence of corn hydrophilic extracts.

As shown in Figure 2, the lag times observed during Cu²⁺-triggered peroxidation were significantly longer in the presence of all corn extracts with respect to the lag-time observed in LDL oxidized in the absence of extracts (ox-LDL), suggesting a protective effects against Cu²⁺-triggered lipid peroxidation of LDL. No significant differences were demonstrated between different types of corn included in our study.

Discussion and Conclusion

Previous studies have shown that corn composition is modulated by genetic factors, by stages of maturation, and processing [1,3,4,6,7,9]. The levels of phytonutrients such carotenoids and polyphenols differ significantly in different corn genotype [1,2,4].

This study investigated for the first time carotenoid content and antioxidant properties of different corn varieties harvested in Regione Marche (Italy). Zeaxanthin, α -cryptoxanthin and lutein were present at higher levels; lower levels of β -carotene, α -carotene and β -cryptoxanthin were observed, in good agreement with other authors [1,4].

We demonstrated that Roccacontrada Rosso has the highest value of polyphenols. These results are in good agreement with previous studies that reported that pigmented corns have high total phenol content greater than that of the other corns at some maturity stage [1,2].

Using ORAC assay, the higher value was observed in Ottofile Treia.

	Roccacon- trada Rosso	Roccacon- trada Giallo	Pollenza Ottofile	Ottofile Treia	Dodicifile Treia
Lutein	26.2 ± 1.2	23.1 ± 1.3	32.2 ± 1.2	31.3 ± 1.2	49.6 ± 2.3
Zeaxanthin	194.3 ± 0.5	176.2 ± 1.2	215.4 ± 3.1	183.4 ± 1.8	218.2 ± 2.8
α -Cryptoxanthin	75.2 ± 0.7	66.1 ± 0.9	71.3 ± 2.2	53.4 ± 1.3	68.3 ± 1.6
β -Cryptoxanthin	4.1 ± 0.3	4.5 ± 0.3	3.5 ± 2.1	4.2 ± 1.6	5.6 ± 1.5
α -Carotene	5.3 ± 1.3	4.9 ± 0.6	3.8 ± 1.6	5.6 ± 1.2	4.9 ± 1.7
β -Carotene	39.3 ± 0.5	30.3 ± 0.6	27.3 ± 1.5	30.3 ± 1.3	35.7 ± 1.3

Table 2: Content of carotenoids in different varieties of the local Italian corn (*Zea Mays L.*) kernels ($\mu\text{g}/100\text{ g}$).

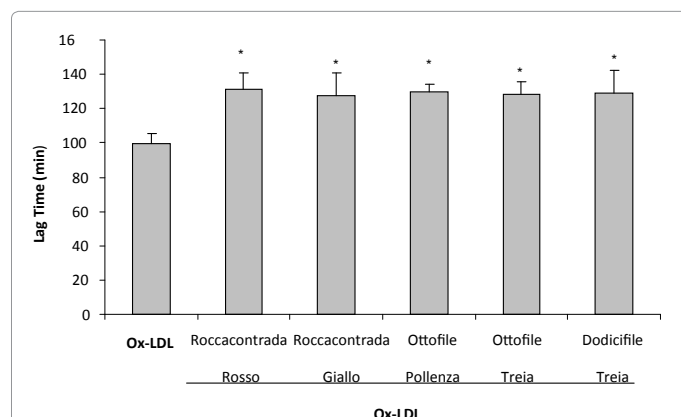


Figure 2: Antioxidant effect of the local Italian maize (*Zea Mays L.*) kernels expressed as lag time relative to control (ox-LDL). * $p < 0.001$ vs control Cu²⁺-oxidized LDL.

ORAC, as well as other *in vitro* methods used to measure antioxidant activity, does not provide information about the bioavailability or metabolism of these compounds in biological systems; however these methods are useful to screen and compare antioxidant activity levels among a wide variety of samples [24].

The hydrophilic antioxidant activity represents approximately 98% of the total antioxidant activity in corn [5]. Previous studies have demonstrated that polyphenols play a key role against oxidative damage and are the most important contributors to the antioxidant capacity in plant food [25,26]. These data were confirmed by the significant positive correlation between total phenol contents and ORAC values, established in our experimental conditions.

Other authors have demonstrated that a purple corn extract contains various bioactive phenolic compounds that exhibit considerable *in vitro* antioxidant activity in the isolated mouse organs as experimental model [27]. Furthermore, phenolic fractions from Andean purple corn (*Zea Mays L.*) exert anti-mutagenic properties [15].

In the present study we demonstrated that corn hydrophilic extracts exert protective effects against lipid peroxidation of LDL. In fact, a longer lag-time of conjugated dienes was observed in LDL oxidized in the presence of corn extracts with respect to LDL incubated alone. LDL oxidized *in vitro* by copper ions has been widely used as experimental model to study the protective effect exerted by isolated phytonutrients and fruit extracts [28-30]. LDL, the main carriers of cholesterol in human plasma, is highly susceptible to free radicals. Structural and functional modifications, induced by lipid peroxidation, have been widely demonstrated in oxidized LDL [23] and lipid peroxidation is considered an atherogenic modification for lipoproteins [31]. The antioxidant activity of corn extracts on human LDL may depend on several factors such as hydrogen-donating ability and metal chelation, notably partitioning efficiency of the antioxidant molecules as well as protein binding ability of phenol compounds [32].

As far as concerns the physiological relevance of our results, previous studies have shown that corn polyphenols are slightly bioavailable in rats [11], however human studies have not been carried out. Our results confirm that Italian corn varieties included in our study could present a good source of phenol and carotenoid compounds. Information about the health-promoting components of local corn varieties could lead to a better understanding and an increased consumption of these, including their use as functional foods.

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