Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

*Available online at*www.jcbsc.org

Section A: Food Biotechnology

CODEN (USA): JCBPAT Research Article

Antioxidant Capacity of Extractable and Non-extractable Polyphenols of Pigmented Maize

Gustavo A. Camelo-Méndez[1], Luis A. Bello-Pérez[1*]

[1] Instituto Politécnico Nacional, CEPROBI. Km. 6.6 Carr. Yautepec-Jojutla Col. San Isidro, 62731, Yautepec, Morelos, México. Phone: +52 735 3942020, Fax: +52 735 3941896

Abstract. Most research studies in food science have been focused in the field of dietary polyphenols or phenolic compounds, but there is an important part of polyphenols not extracted with organic solvents and the reports are few of those compounds. Pigmented maize (mainly blue and red) have presented significant anthocyanins content and antioxidant capacity; however, the studies about phytochemical content of pigmented maize in Mexico and their biological effects are limited. The aim of this study was to evaluate the antioxidant capacity of extractable and non-extractable polyphenols of blue and red maize. Total polyphenols was determined, as the sum of the polyphenols present in methanol: acetone: water extracts (extractable polyphenols), condensed tannins and hydrolysable polyphenols (non-extractable polyphenols) in the corresponding residues of maize samples. The non-extractable polyphenols content was higher than extractable polyphenols. It was estimated that high percentage of phenolics could be more bio-accessible in the large intestine. In general, blue and red maize presented an antioxidant capacity (FRAP and ABTS assays). These results are important for the use of maize-based foods, industrial and pharmaceutical products.

Key words. Antioxidant capacity, pigmented maize, polyphenols, tannins.

INTRODUCTION

Maize is one of the most diverse food crops found in nature and one of the most widely cultivated cereals in the world. Also, maize and milled maize including meals, flours, and bran have been integral parts of the diet of all socioeconomic classes worldwide[1]. Native white and pigmented maize have been cultivated in South

America, mainly in Peru, Bolivia and Mexico[2, 3], and used for the preparation of traditional drinks, desserts, pasta and bakery products. However, multicolored, red, purple, blue, and black maize kernels are currently produced only in small amounts for making specialty foods, regional foods or for use in ornamentation due to their colorful appearance[1].

Currently, pigmented maize has received increased attention from a nutraceutical perspective owing to its potential health benefits. Phytochemicals such as phenolics, anthocyanins, amongst others have been previously reported in several genotypes[4]. Also, these compounds are considered of interest because their antioxidant and bioactive properties as free radical inhibitors that protect the cells against oxidative damage[20].

In recent years, the use of pigmented maize (mainly blue) had a significant increase in the development of functional foods in nature, such as tortillas[5-9] and cookies[10] due to their anthocyanin content and indigestible carbohydrates.

Polyphenols content in foods and its implications on health have been widely studied[11-12], but only extractable polyphenols (EPP), which are extracted using aqueous organic solvents, have been considered in the current research[11, 13]. On the other hand, it has been reported that an appreciable amount of polyphenols, named non-extractable polyphenols (NEPP), can remain in the neglected residues of aqueous organic extraction[13] due to the ability to bind with macromolecules by covalent bonds, hydrogen bonding, and hydrophobic and hydrophilic interactions.

The aim of this study was to evaluate the antioxidant capacity of extractable and non-extractable polyphenols of blue and red maize.

METHODS

Plant material: Pigmented maize were obtained from the experimental field of INIFAP Texcoco, Edo. México, México during 2012-2013 season. Maize was named according to their pigmentation characteristics. Samples were pulverized in a cyclone mill (A10 analytical mill, Tekmar Co., Cincinnati, OH, USA), it was stored in airtight and opaque containers at 4°C.

Extraction procedure of polyphenols: Extraction procedure was performance by the method used by Ovando-Martínez *et al.*[14] with some modifications. Samples were extracted by shaking at room temperature with methanol: water acidified with HCl (70:30 v/v) and acetone: water (70:30 v/v). After centrifugation (10 min, 25 °C, 3000g) supernatants were combined and used to determine extractable polyphenols content and antioxidant capacity.

Determination of total anthocyanins and polyphenols content: Total anthocyanins content (TAC) were evaluated using the methanol: water extraction, they were determined spectrophotometrically by differential pH method[15], results were expressed as mg of cyanidin 3-glucoside/100 g dry matter. Extractable polyphenols were determined with Folin–Ciocalteau reagent[12] with some modifications[16]. Determination was performed at a wavelength of 765 nm in a spectrophotometer, total polyphenol content was expressed as mg of gallic acid (GA)/g dry matter, using a calibration curve.

Condensed tannins: Residues from the methanol/acetone/water extraction were treated with 10 ml/l HCl in butanol for 3 h at 100°C[17] for condensed tannins determination. Condensed tannins were calculated from the absorbance at 550 nm of the anthocyanidin solutions.

Hydrolysable polyphenols: Hydrolysable polyphenols were determined by a methanol/H_2SO_4 9:1 (v/v) hydrolysis at 85 °C for 20 h on the residues of the methanol/acetone/water extraction[18]. After centrifugation 10 min, 25 °C, 3000g) supernatants were combined and used to determinate the hydrolysable polyphenols by Folin–Ciocalteu method[19].

Antioxidant capacity. ABTS method: Inhibition of ABTS free radical (2, 2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) was performed by the methodology proposed by Re *et al.* [20], it was carried out measuring the absorbance at 0 and 4 min at 593 nm. Results were expressed in mg equivalent of Trolox/g of dry weight, using a calibration curve of trolox.

FRAP method: Ferric reducing antioxidant power (FRAP) method was performed by the methodology proposed by Benzie and Strain[21] with some modifications; it was carried out measuring the absorbance at 0 and 6 min at 593 nm. Results were expressed in mg equivalent of Trolox/g of dry weight, using a calibration curve of trolox.

Statistical analysis: Results were expressed as means ± standard deviation (SD). All experiments were randomized, analysis of variance (ANOVA) was performance using Statgraphics Plus version 5.1® (Manugistics, Inc., Rockville, MA, USA).

RESULTS

Extractable and non-extractable polyphenols: Food polyphenol data usually correspond to polyphenols analyzed in aqueous organic extracts of foods (extractable polyphenols), while significant amounts of potentially bioactive polyphenols that remain in the residues (non-extractable polyphenols) are ignored. The presence of important amounts of non-extractable polyphenols has been reported in specific foods and vegetables[22, 23]. Non-extractable polyphenols are high molecular weight proanthocyanidins and phenolics associated with dietary fibre and indigestible compounds that are not taken into account in chemical and biological studies[11, 13, 22-24]. Due to this, the methodology proposed by Saura-Calixto[11] was used, where extractable and non-extractable polyphenols and antioxidant capacity of blue and red corn are quantified (Figure 1).

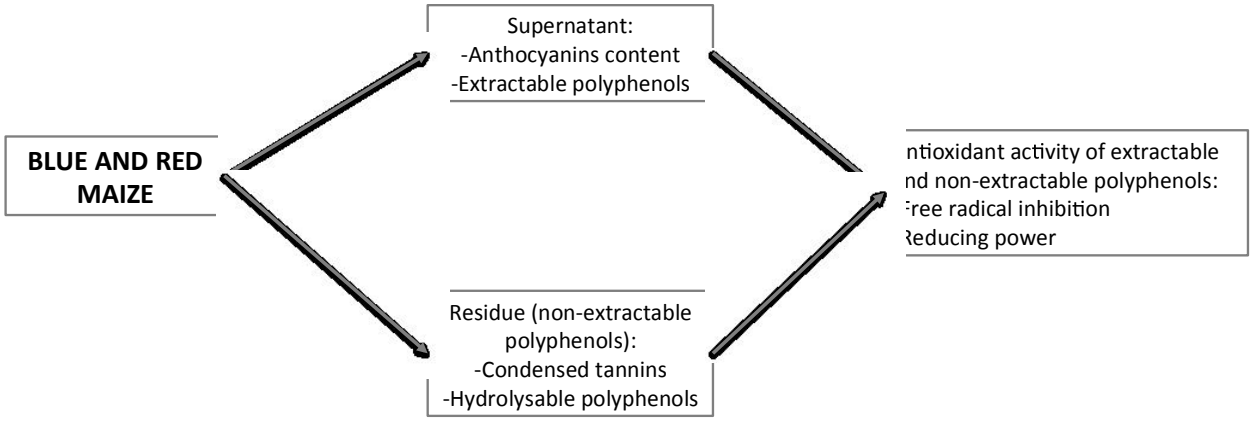

Figure 1: Schematic of the methodology used to evaluate the antioxidant capacity of red and blue maize.

Extractable and non-extractable polyphenols of samples were evaluated (Table 1). Blue and red maize did not present significant differences ($p<0.05$) in polyphenols content. Samples presented a high content of

condensed tannins, those are not bioaccesible in the small intestine, and they could travel through the gastrointestinal tract as insoluble substrates reaching the colon, where they release single polyphenols and different bioavailable metabolites by the action of bacterial microbiota[13].

Table 1: Extractable and non-extractable polyphenols content (mg/g)

Maize	TA	EP	HP	CT
Blue	0.20±0.09[a]	7.65±2.21[a]	13.55±4.04[a]	209.54±23.81[a]
Red	0.17±0.05[a]	7.15±0.47[a]	11.65±3.00[a]	207.85±13.16[a]

*** Mean ± SD (n=4), dry matter. TA: total anthocyanins, EP: extractable polyphenols, HP: hydrolysable polyphenols, CT: condensed tannins. Same letters indicate not significant differences (*p<0.05*), LSD test.**

Antioxidant capacity of pigmented maize: The antioxidant activity of extractable and hydrolysable polyphenols and condensed tannins of pigmented maize was measured by spectrophotometric methods: free radical inhibition (ABTS) and reducing power (FRAP), results are presented in Figure 2. It is important to note that this biological activity cannot only be attributed to the antioxidant characteristic of each molecule *per se*. Antioxidant activity of samples is remarkable, since they are rich in polyphenolic compounds, which play an important role in the kidnapping of free radicals[25] . Very few data on bioavailability of hydrolysable polyphenols are available in the literature. It is known that condensed tannins have low bioaccessibility in the small intestine, but polyphenols become available in the large intestine[11, 13].

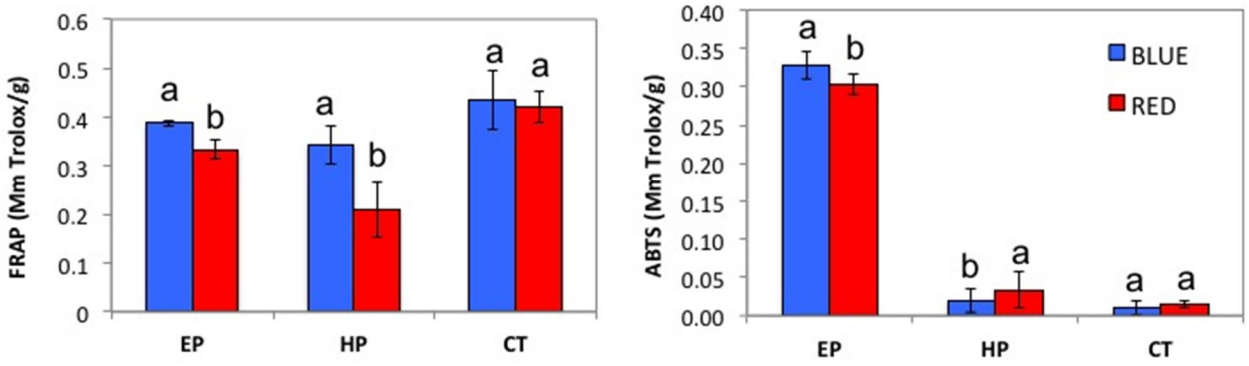

Figure 2: Reducing power (FRAP assay) and free radical inhibition (mM Trolox/g) of extractable and non-extractable polyphenols of blue and red maize. Mean ± SD (n=4), dry matter. EP: extractable polyphenols, HP: hydrolysable polyphenols, CT: condensed tannins. Same letters indicate not significant differences (*p<0.05*), LSD test.

Methodologies used in this study have been widely used for antioxidant activity in food science. However, it is important to mention that they cannot be compared because each one measures the inhibition of different radicals and reducing power of Fe^{3+}. Kondo *et al.*[26] reported that the antioxidant activity in foods are remarkable, since they are rich in polyphenolic compounds and vitamins, which play an important role in the

kidnapping of free radicals. Very few data on bioavailability of hydrolysable polyphenols are available in the literature. In order to compare with the methodologies used here, the results were presented as percentages of inhibition of free radicals and reducing capacity (Figure 3). FRAP assay presented higher antioxidant capacity (%) than ABTS assay, this could be associated with the molecules present in the extracts. FRAP assay demonstrated that red maize presented major reducing power than blue maize while in ABTS assay results were similar in percentage of inhibition.

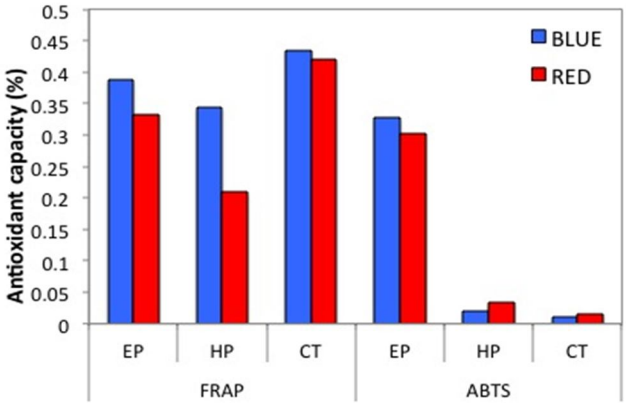

Figure 3: Antioxidant capacity (%) of extractable and non-extractable polyphenols of blue and red maize. EP: extractable polyphenols, HP: hydrolysable polyphenols, CT: condensed tannins.

Therefore, correlations between the polyphenol and tannin contents with each antioxidant methodology were evaluated. Pearson coefficients indicate significant correlations ($p<0.05$) between extractable and non-extractable polyphenols and antioxidant activity are showed in Table 2, suggesting a direct relationship between polyphenol content and antioxidant activity. Differences in Pearson coefficients among the antioxidant methodologies used in this study are due to the polarity of compounds and sensitivity characteristics of each technique.

Table 2: Correlation coefficients between assay [a]

	AT	EP	HP	CT	F-EP	F-HP	F-CT	A-EP	A-HP	A-CT
AT	1	***0.77***	***0.36***	-0.40	0.02	0.12	0.77	-0.01	-0.19	0.31
EP	-	1	0.67	-0.31	0.12	-0.10	0.38	-0.37	-0.59	0.39
HT	-	-	1	-0.32	0.33	-0.11	0.38	-0.38	***-0.82***	-0.07
CT	-	-	-	1	***-0.84***	***-0.85***	-0.31	-0.63	0.33	0.38
F-EP	-	-	-	-	1	0.70	-0.02	0.65	-0.46	-0.37
F-HP	-	-	-	-	-	1	0.16	***0.80***	0.15	-0.62
F-CT	-	-	-	-	-	-	1	0.03	0.04	-0.15
A-EP	-	-	-	-	-	-	-	1	0.22	-0.37
A-HP	-	-	-	-	-	-	-	-	1	-0.25
A-CT	-	-	-	-	-	-	-	-	-	1

[a] F-EP: FRAP assay of extractable polyphenols, F-HP: FRAP assay of hydrolysable polyphenols,F-CT: FRAP assay of condensed tannins,A-EP: ABTS assay of extractable polyphenols, A-HP: ABTS assay of hydrolysable polyphenols,A-CT: ABTS assay of condensed tannins.Significant correlations ($p<0.05$) are denoted by the use of italics and bold face.

Also, it is important to note that this biological activity cannot only be attributed to the antioxidant characteristic of each molecule *per se* (double conjugate bonds, number and position of methyl and hydroxyl groups). Further, this activity can be attributed to the complex effects of the molecules (synergistic, additive or antagonist) present in each extract and the affinity of each molecule to inhibit free radicals or reduce metals. The methods used to determine antioxidant capacity are complementary, and their sensitivity depends on the different macromolecules and bioactive compounds present in the food[27].

CONCLUSIONS

The present study revealed that extracts of blue and red maize demonstrated high phenolic content and potent antioxidant activity, achieved by free radical scavenging and reducing power assays. The results obtained shows that the phytochemical compositions do not varies greatly between the different maize samples analyzed. These differences may be explained by genetic make-up, since all varieties were grown under the same environmental conditions. The large variations observed in this study may be important for the optimum utilization of these materials for food and industrial uses.

ACKNOWLEDGEMENTS

The authors thank the support from CONACYT-Mexico, SIP-IPN, COFAA-IPN and EDI-IPN. One of the authors (GACM) also acknowledges the scholarship from CONACYT-Mexico.

REFERENCES

1. 1. J.P.Vielle-Calzada,J. Padilla. The Mexican landraces: Description classification and diversity. In: *Handbook of Maize: Its Biology.* Bennetzen JL, Hake SC. Springer. USA, 2009, 453-561.
2. E.Cuevas Montilla,S. Hillebrand,A. Antezana,P. WinterhalterSoluble and bound phenolic compounds in different Bolivian purple corn (*Zea mays* L.) cultivars. *J Agric Food Chem, 2011, 59*(13):7068-7074.
3. J.de la Rosa-Millán,E. Agama-Acevedo,A.R. Jimenez-Aparicio,L.A. Bello-Pérez Starch characterization of different blue maize varieties. *Starch/Stärke*, (2010),62: 549-557
4. L.X.López-Martínez,R.M. Oliart-Ros,G. Valerio-Alfaro,C.H. Lee,K.L. Parkin,H.S. Garcia. Antioxidant activity, phenolic compounds and anthocyanins content of eighteen strains of Mexican maize. *J Food Sci Tech*, 2009, 42:1187-1192.
5. J.Aguayo-Rojas, S. Mora-Rochín, E.O. Cuevas-Rodríguez, S.O. Serna-Saldivar, J.A. Gutierrez-Uribe, C. Reyes-Moreno, J. Milán-Carrillo. Phytochemicals and Antioxidant Capacity of Tortillas Obtained after Lime-Cooking Extrusion Process of Whole Pigmented Mexican Maize. Plant Foods Hum Nutr., 2012, 67:178–185.
6. C. De la Parra,S.O. Serna-Saldivar,R.H. Liu. Effect of Processing on the Phytochemical Profiles and Antioxidant Activity of Corn for Production of Masa, Tortillas, and Tortilla Chips. *J Agric Food Chem,2007* 55: 4177−4183
7. D.Del Pozo-Insfran,C.H. Brenes,S.O. Serna-Saldivar,S.T. Talcott Polyphenolic and antioxidant content of white and blue corn (*Zea mays* L.) products, 2006, *Food Res Int* 39: 696-703

8. 12. D.Del Pozo-Insfran,S.O. Serna Saldivar,C.H. Brenes,S.T. Talcott. Polyphenolics and Antioxidant Capacity of White and Blue Corns Processed into Tortillas and Chips. *Cereal Chem, 2*007, 84(2):162-168
9. S.Mora-Rochin,J.A. Gutiérrez-Uribe,S.O. Serna-Saldivar,P. Sánchez-Peña,C. Reyes-Moreno,J. Milán-Carrillo. Phenolic content and antioxidant activity of tortillas produced from pigmented maize processed by conventional nixtamalization or extrusion cooking. *J Cereal Sci,* 2010, 52: 502-508.
10. R.G.Utrilla-Coello,E. Agama-Acevedo,P. Osorio-Díaz,R. Reynoso-Camacho,L.A. Bello-Pérez Glycemic response in healthy rats fed with composite cereal bars. *Starch/Stärke*, 2013, 65: 354-359.
11. F.Saura-Calixto,J. Serrano,I. Goñi. Intake and bioaccessibility of total polyphenols in a whole diet. *Food Chem,* 2007, 101: 492-501.
12. V.Singleton, andJ. Rossi. Colorimetry of total phenolics with phosphomolybdic- phosphotungstenic acid reagents. *Am J Enol Viticult,* 1965, 16 (3): 144-158.
13. F.Saura-Calixto. Dietary Fiber as a Carrier of Dietary Antioxidants: An Essential Physiological Function. *J Agric Food Chem,* 2011, 59: 43–49.
14. M.Ovando-Martinez,S. Sayago-Ayerdi,E. Agama-Acevedo,I. Goñi,L.A. Bello-Perez. Unripe banana flour as an ingredient to increase the undigestible carbohydrates of pasta. *Food Chem,* 2009, 113:121-126.
15. G.A.Camelo-Méndez,J.A. Ragazzo-Sánchez,A.R. Jiménez-Aparicio,P.E. Vanegas-Espinoza,O. Paredes-López,A.A. Del Villar-Martínez, Comparative study of anthocyanin and volatile compounds content of four varieties of Mexican roselle (*Hibiscus sabdariffa* L.) by multivariable analysis. *Plant Food Hum Nutr, 2013,* 68(3): 229-234.
16. V.Ivanova,M. Stefova,B. Vojnoski,A. Dörnyei,L. Márk,V. Dimovska,T. Stafilov, andF. Kilár. Identification of polyphenolic compounds in red and white grape varieties grown in R. Macedonia and changes of their content during ripening. *Food Res Int,* 2011, 44 (9): 2851-2860.
17. J.Reed,R.E. McDowell,P.J. Van Soest,P.J. Horvarth. Condensed tannins: A factor limiting the use of cassava forage. *J Sci Food Agric,* 1982, 33: 213-220.
18. P.W. Hartzfeld,R. Forkner,D.M. Hunter,A.E. Hagerman Determination of hydrolyzable tannins (gallotannins and ellagitanins) after reaction with potassium iodate. *J Agric Food Chem,* 2002, 50: 1785-1790.
19. F.R.Montreau. Sur le dosage des composés phénoliques totaux dans les vins par la méthode Folin-Ciocalteau. *Connaiss Vigne Vin*, 197224: 397-404.
20. R.Re,N. Pellegrini,A. Proteggente,A. Pannala,M. Yang,C. Rice-Evans. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Bio Med.* 26: 1231-1237.1999
21. I.F.F.Benzie,J.J. Strain.. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal Biochem*, 1996, 239(1): 70-76.
22. L.Bravo,R. Abia,F. Saura-Calixto. Polyphenols as dietary fiber associated compounds: comparative study on *in vivo* and *in vitro* properties. *J Agric Food Chem.* 42: 1481–1487. (1994).
23. L.Bravo,E. Mañas,F. Saura-Calixto Dietary non-extractable condensed tannins as indigestible compounds: effects on faecal weight, and protein and fat excretion. *J Sci Food Agric, 1993,* 63: 63–68.
24. A.E.Quirós-Sauceda,H. Palafox-Carlos,S.G. Sáyago-Ayerdi,J.F. Ayala-Zavala,L.A. Bello-Perez,E. Alvarez-Parrilla,L.A. de la Rosa,A.F. González-Córdova andG.A. González-Aguilar Dietary fiber and phenolic compounds as functional ingredients: interaction and possible effect after ingestion. *Food Funct*, 2014, 5(6):1063-1072.

25. K.F. Adom,R.H. LiuAntioxidant activity of grains. *J Agr Food Chem., 2002,* 50:6182-6187.
26. S.Kondo,M. Kittikorn,S. Kanlayanarat. Preharvest antioxidant activities of tropical fruit and the effect of low temperature storage on antioxidants and jasmonates. *Postharvest Biol Technol,* 2005, 36: 309–318.
27. N.Bordenave,B.R. Hamaker,M.G. Ferruzzi, Nature and consequences of non-covalent interactions between flavonoids and macronutrients in foods. *Food Funct* ,2014,5: 18–34

*** Corresponding author: Luis A. Bello Pérez**
Instituto Politécnico Nacional, CEPROBI. Km. 6.6 Carr. Yautepec-Jojutla Col. San Isidro, 62731, Yautepec, Morelos, México. Phone: +52 735 3942020, Fax: +52 735 3941896
* e-mail corresponding author, (labellop@ipn.mx)