Chapter 18

Anthocyanin Profile of Red Maize Native from Mixteco Race and Their Antiproliferative Activity on Cell Line DU145

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Abstract

Mexico is regarded as the point of origin and biodiversity of maize, which takes the colors white, yellow, blue, or red. Red maize in particular owes its coloring to a type of polyphenolic compounds known as anthocyanins. The aim of this study was to determine the profile anthocyanin of red maize, as well as their antiproliferative activity on prostate cancer cell line DU145. Three samples of red maize were analyzed. Total polyphenols, monomeric anthocyanins, antioxidant activity by DDPH and FRAP were evaluated. The sample of red maize with the highest levels of total polyphenols and monomeric anthocyanins was selected, and its anthocyanin fraction was analyzed by HPLC-ESI-MS. Twenty compounds were detected in the anthocyanin profile, and from these, 12 anthocyanins derived of cyanidin were identified. MTT assay was used to determine the antiproliferative activity of the anthocyanin fraction from red maize at different concentrations (7–1000 μg/mL), and a significant antiproliferative activity was observed at 1000 μg/mL. Microscopy analysis showed that the anthocyanin fraction of red maize induced apoptosis in cell lines DU145. This is the first report showing that anthocyanin fraction of red maize possess antiproliferative activity in the DU145 cell line.

Keywords: red maize, phenolic compounds, anthocyanin profile, antiproliferative activity, DU145 cell line

1. Introduction

Cereals are still the most important food source worldwide. Maize, sorghum, millet, wheat, rice, barley, oats, teff, and quinoa account for a large amount of the human diet. Maize (*Zea mays* L.) is the third crop by volume cultivated in the world and has a great economic importance, since it is used for animal and human foods as well as a raw material in the production of several industrial products [1]. There are over 59 maize races in Mexico [2] with different shapes and colors, such as white, yellow, blue, and red. In recent years, there has been a growing interest in pigmented varieties form the scientific point of view, since they are sources of phenolic compounds. Approximately 60% of polyphenols are flavonoids, which are regarded as the most important group of phenolic compounds. Among these are anthocyanins, the chemical compounds responsible for the color of red maize, which are located in the pericarp or aleurone layer of the grain [3].

Anthocyanins are generally regarded as the most important pigments in nature. These water-soluble compounds belong to the flavonoid family and can be found in the colors blue, red, purple, and dark violet [4]. From the chemical standpoint, anthocyanins in nature take the form of glycosides, whose aglycone is known as anthocyanidin. Anthocyanidins are made up of a benzopyril system (A–C) and a phenolic ring (B). Particular differences between anthocyanins depend upon the number of hydroxyl groups, the nature and number of glycosides attached to the molecule, the position of the point of attachment, and to the nature and number of aromatic acids joined to the glycoside in the molecule [5]. Since each anthocyanidin may be glycosylated and acylated by different acids at different points, there is a large number of chemical combinations [6]. Furthermore, the simplest or monomeric anthocyanins may react between themselves, producing more complex structures of higher molecular weights known as polymeric anthocyanins. They may also react with other compounds, and therefore, in nature more than 600 different anthocyanins are found.

On the other hand, there is a growing scientific interest on pigmented maize races such as red, due to their content of anthocyanins. Experts recommend the consumption of these because of their strong free-radical scavenging activity and chelation of divalent cations, which is given by their phenolic structure, double bonds of their rings, and hydroxyl groups. Anthocyanins may also modulate enzymes related to oxidative stress, and their preventive action in the development of disease has been reported [7].

Several epidemiological studies also reveal that the consumption of flavonoid-rich foods is associated with a lower risk of neurodegenerative diseases such as cancer [8–10]. Cancer is one of the main causes of death worldwide, causing 7.6 million deaths only in 2008. For 2030, this number of cancer victims is expected to increase to 11 million. Lung, breast, colorectal, stomach, and prostate cancers cause the majority of cancer deaths [11]. The treatment of this disease depends on the type of cancer as well as on the subtype or stage of the patient and is currently based on hormones, chemotherapy, radiotherapy, pharmaceuticals, and nanotherapy. However, these treatments are aggressive, highly expensive, and unattainable for many

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people [12]. Unfortunately, some cancer tumors are extremely resistant to current therapeutic agents; therefore, the development of new strategies in prevention, treatment, and control of cancer is urgently needed [13].

The scientific community is looking for alternatives, such as the use of natural compounds as chemopreventives or adjuvants. In this regard, red maize form Mixteco race may be an important source of flavonoids; however, the number of studies investigating the antiproliferative activity of this grain is scarce. Given the above, the aim of the present work was to evaluate the antiproliferative activity of purified anthocyanins from red maize in the prostate cancer cell line DU145.

2. Research methods

2.1. Plant material

Three samples of red maize from the Mixteco race (**Figure 1**), cultivated in the Mixteca region of the State of Oaxaca, Mexico, were used in the present study.

2.2. Preparation of red maize extract

To prepare the extracts, 200 g of red maize was ground using a coffee grinder (Krups model GX410011V) in order to reduce particle size and increase contact surface area. Then 500 mL of EtOH/ $C_6H_8O_7$ (85:15 1 M) were added, and the mix was thermosonicated in an ultrasonic homogenizer (Cole-Palmer, modelo VCX-750) for 30 min at 40% amplitude, pulse 05:05). Afterward, the extracts were left to stand for 24 h at 4°C and then centrifuged at 4000 rpm for 20 min at 5°C. Extracts were concentrated in a rotavapor (Büchi Rotavapor R-205, Büchi Vacuum Controller V-800, Büchi Heating Bath B-490) at 28°C under vacuum, and finally samples were lyophilized and stored at −20°C. This process was carried out in the dark [14].

Figure 1. Samples of red maize from Mixteco race.

2.3. Analysis of total polyphenols

Total polyphenols were analyzed by the method described by Folin and Ciocalteu and modified by Singleton and Rossi [15], which is based on an oxidation-reduction reaction and phenolic compounds react with the Folin-Ciocalteu reagent under alkaline conditions, resulting in a blue coloration that is evaluated by spectrophotometry. Previous to the analysis, a calibration curve with gallic acid was made (12 mg EAG/100 mL) and read at 730 nm. Results were expressed as mg equivalent of gallic acid/L.

2.4. Determination of monomeric anthocyanins

Quantification of anthocyanins was carried out by the pH differential method described by Giusti and Wrolstad [16]. The amount of anthocyanins was expressed as mg of cyanidin 3-glucoside/L of sample, and each sample was analyzed by triplicate.

2.5. Antioxidant activity by DPPH

The DPPH (1,1-diphenyl-2-picrylhydrazyl) method, as reported by Brand-Williams et al. [17], was used to determine antioxidant activity. A standard calibration curve (100–800 μmol) of trolox was used as reference. A measure of 2.9 mL of DPPH were vigorously mixed with 0.1 mL of each extract and then kept in the dark for 30 min at 25°C. Absorbance was read at 517 nm. Results were expressed as μmol equivalent of trolox/g of simple. All determinations were made by triplicate.

2.6. Antioxidant activity by FRAP

Analysis of antioxidant activity by FRAP (Ferric reducing antioxidant power) was carried out according to Benzie and Strain [18]. A standard trolox calibration curve (100–800 μmol) was used as reference. About 3 mL of FRAP reagent were mixed with 0.1 mL of each extract, then incubated for 5 min at 37°C, and the absorbance was read at 593 nm. Results were expressed in μmol equivalent of trolox/g of simple. All determinations were made by triplicate.

2.7. Isolation of anthocyanins

Purification of anthocyanins was made in the dark, using a chromatographic column packed with amberlite XAD-7.1 g of lyophilized red maize extract that was placed per column, and two solutions were added as mobile phases: solution A: H_2O/acetic acid (95:5 v:v) and solution B: EtOH/acetic acid (95:5 v:v). Fractions of anthocyanins were collected in ambar glass bottles and concentrated in a rotavapor (Büchi Rotavapor R-205, Büchi Vacuum Controller V-800, Büchi Heating Bath B-490) at 28°C under vacuum. Samples were lyophilized and stored at −20°C [14].

2.8. Analysis of anthocyanins by high-performance liquid chromatography with electrospray ionization mass spectrometry (HPLC-ESI-MS)

Anthocyanin analysis was carried out in a high-performance liquid chromatographer (Agilent model 1200) coupled with a mass spectrometer (Bruker model Esquire 6000), equipped with electrospray and ion trap with a nitrogen nebulizer 15 psi, nitrogen as drying gas at a rate

of 7 L/min, drying temperature of 300°C, 500 m/z target, and 50–1000 m/z scan. A Zorbax Eclipse plus C-18 column (2.1 mm × 100 mm × 3.5 μm) was used. The mobile phase consisted of acetonitrile as solvent A and 2% aqueous acetic acid solution as solvent B. The gradient was 7:93 (A:B) at 0 min, 35:65 (A:B) at 80 min, and 100% A in 35 min. Flow rate was 0.2 mL/min. Test time was of 40 min [14].

2.9. Evaluation of cell proliferation in vitro

DU-145 cells were seeded into 96-well plates at 5000 cells per well and incubated with different extract concentrations. The MTT ((3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay was performed after treatment time (24–48 h) [19]. Roswell Park Memorial Institute (RPMI) 1640 culture medium was replaced with 50 μL of MTT (5 mg/mL), and the cells were incubated for 4 h at 37°C in darkness. Formazan crystals were dissolved in 200 μL of DMSO (dimethyl sulfoxide). Absorbance was read at 570 nm on a micro plate reader (Awareness Technology, Stat Fax 4200 model).

2.10. Statistical analysis

Three independent repetitions were made for the cell culture experiments. In order to determine significant differences among data, ANOVA (analysis of variance) tests were performed followed by Tukey ($p < 0.05$) multiple range tests.

3. Results and discussion

3.1. Contents of total polyphenols, monomeric anthocyanins, and antioxidant activity

In the first part of the present work, the contents of total polyphenols, monomeric anthocyanins, and antioxidant activity by DPPH and FRAP were evaluated. Sample R-14 showed the highest values for total polyphenols (**Table 1**). Values of monomeric anthocyanins for red maize were higher than those reported for Chalco and Red Chihuahua maize [20] and lower than those reported for samples of red maize from Mexico City and Puebla, Mexico [21]. The values for antioxidant activity as evaluated by the DPPH method were between 10.5 and 12.7 μmol ET/g, whereas antioxidant activity by FRAP recorded 2.77 and 2.79 μmol ET/g. This suggests that red maize samples are a potential source of phenolic compounds with antioxidant properties. On the other hand, sample R-14 was selected to evaluate antioxidant profile and antiproliferative activity on prostate cancer cell line DU145, since this particular sample showed the highest values for total polyphenols and monomeric anthocyanins.

Samples	Total polyphenols (mg EAG/100 g)	Monomeric anthocyanins (mg C3G/100 g)	DPPH (μmol ET/g)	FRAP (μmol ET/g)
R-03	327	50.1	10.5	2.77
R-07	368	68.1	12.3	2.69
R-14	373	67.4	12.7	2.79

Table 1. Total polyphenols, monomeric anthocyanins, and antioxidant activity of red maize from Mixteco race.

3.2. Anthocyanin profile of red maize from Mixteco race

It is widely known that biological properties of anthocyanins depend upon their chemical structure, substitutions, conjugations, and polymerization. Therefore, in the present study, the anthocyanin profile of red maize was analyzed by HPLC-ESI-MS. **Figure 2** shows the total ion chromatogram (TIC) of red maize anthocyanins from Mixteco race. A total of 20 different compounds were detected, their retention times (tr) and main ions (m/z) are shown in **Table 2**. A total of 17 compounds were derived from cyanidin (287 m/z). On the other hand, 15 different compounds were identified in the anthocyanin profile of red maize from Mixteco race (**Table 2**), which includes monoglycosilated, acylated anthocyanins, and proanthocyanidins. Regarding the role of anthocyanins in the prevention of prostate cancer, a recent study shows that cyanidin-3-glucoside produces cancer cell apoptosis in line DU145 [22]. After the anthocyanin profile was completed on the selected sample, its antiproliferative activity was tested on cell line DU145.

3.3. Antiproliferative activity

Figure 3 shows the percentage of cell viability after 24 h of treatment with different concentrations of purified anthocyanins from red maize. As seen in the graph, no significant differences were detected between the different concentrations and the control ($p \leq 0.05$). This shows that red maize extract had no significant effect on prostate cancer cell line DU145 after 24 h.

Figure 4 shows the percentage of cell viability after 48 h of application of different concentrations of purified anthocyanins of red maize, where a significant reduction percentage on cell growth was observed at 1000 μg/mL as compared to the control, corresponding to 35% of cell viability; the same effect was observed in the micrograph (**Figure 5**), where a cytoplasmic vacuolization is present and the growth of live cells is haltered. Even though there are no reports on the effect of anthocyanins of red maize on cancer cell lines, recent studies show that cyanidin-3-glucoside produces an antiproliferative effect through the activation of caspase-3 on prostate cell lines LnCap and DU145 [22]. It has also been reported that flavonoids from blueberry inhibit the activity of metalloproteinase in DU145 [23]. These data suggest a potential for red maize flavonoids in the chemoprevention of prostate cancer, which is nowadays the first cause of death by cancer.

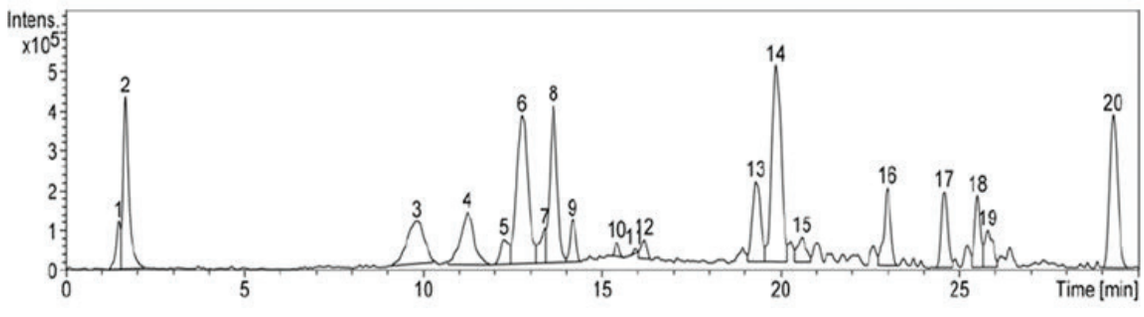

Figure 2. Anthocyanin profile of red maize from Mixteco race.

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Peak	T_R (min)	Fragment ions (m/z)	Tentative identification
1	1.5	287,509	Nonidentified
2	1.7	287,449	Cyanidin-3-glucoside
3	9.8	287,491	Cyanidin-3-O-(6′-acetyl-glucoside)
4	11.2	287,493	Nonidentified
5	12.2	287,530	Cyanidin-3-O-sambubioside-5-rhamnoside
6	12.8	287,460	Nonidentified
7	13.4	287,599	Nonidentified
8	13.6	287,754	Cyanidin-3-O-(6′-acetyl-arabinoside)
9	14.2	287,491	Cyanidin 3-(6″-malonyl) glucoside
10	15.4	287,549	Cyanidin-3-succinylglucoside
11	15.9	287,501	Cyanidin-3-O-soforoside
12	16.2	287,451	Nonidentified
13	19.3	287,433	Cyanidin-3-O-(6″-p-coumaroil-glucoside)-5-O-(6″-malonyl-glucoside)
14	19.9	287,731	Cyanidin 3-p-hydroxy-benzoyl sophoroside-5-glucoside
15	20.6	287,773	Cyanidin 3-sophoroside-5-glucoside
16	23.0	287,901	Procyanidin dimer
17	24.6	339,843	Nonidentified
18	25.5	287,901	Procyanidin dimer
19	25.8	353,901	Procyanidin dimer
20	29.3	343,885	Procyanidin type B

Table 2. Identity of anthocyanins detected in red maize from Mixteco race.

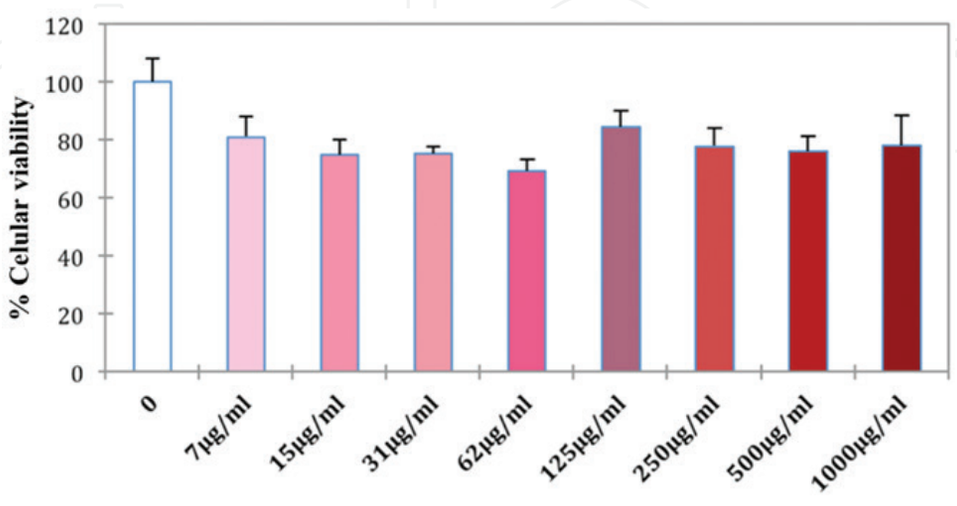

Figure 3. Percentage of cell viability after 24 h of treatment with red maize anthocyanins. Columns show the mean value and standard deviation of three independent experiments.

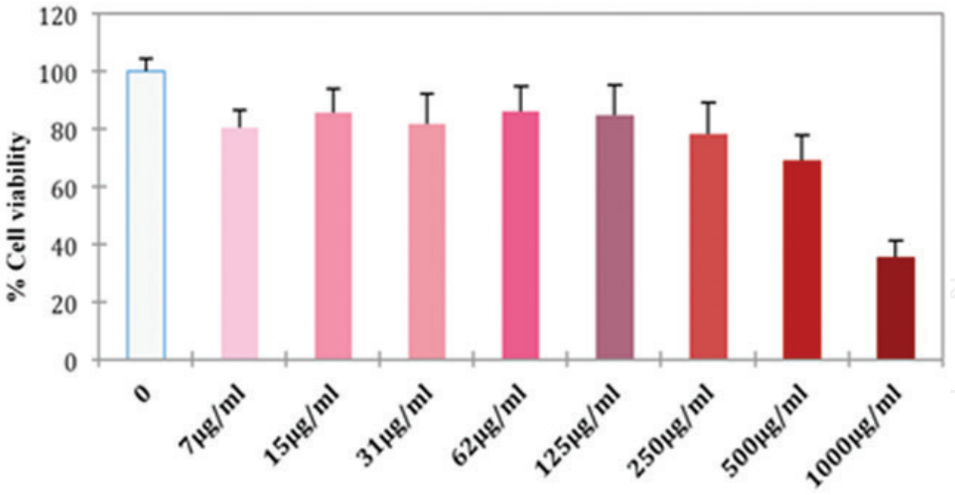

Figure 4. Percentage of cell viability after 48 h of treatment with red maize anthocyanins. Columns show the mean value and standard deviation of three independent experiments.

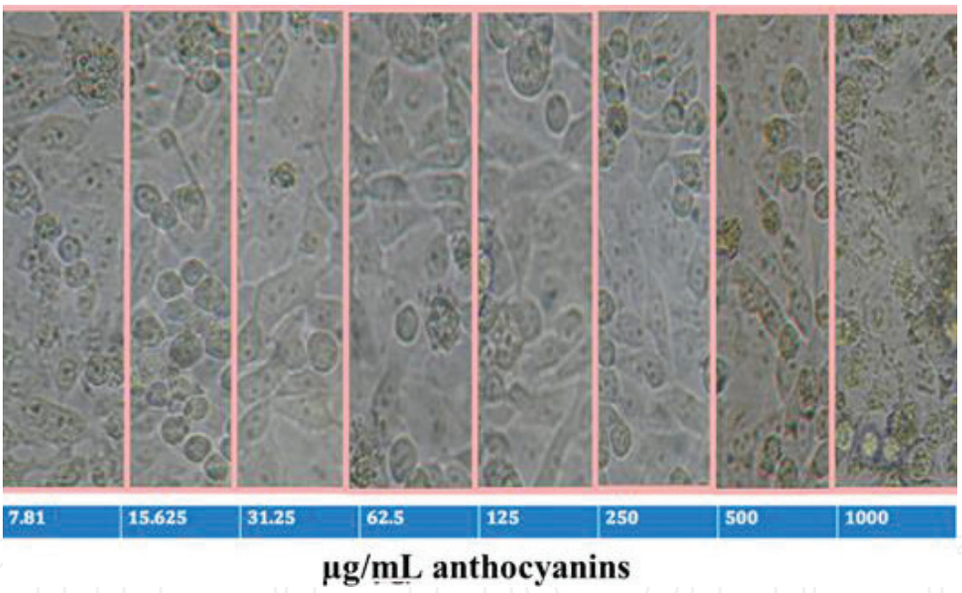

Figure 5. Microscopy of cancer cells DU145 after 48 h application of different concentrations of purified anthocyanin extract from red maize.

Figure 6 shows an image obtained by optical microscopy: a DU145 cell culture treated with the maximum concentration of red maize anthocyanins (1000 µg/mL) along with a culture in the absence of anthocyanins; after 48 h of incubation, cell vacuolization is observed in the experimental sample.

In summary, red maize from Mixteco race is a rich source of flavonoids such as anthocyanins, and their profile is mainly constituted by cyanidin-derived anthocyanins. These compounds have a potential application in the prevention of prostate cancer, showing antiproliferative activity on cell line DU145. Future research is needed.

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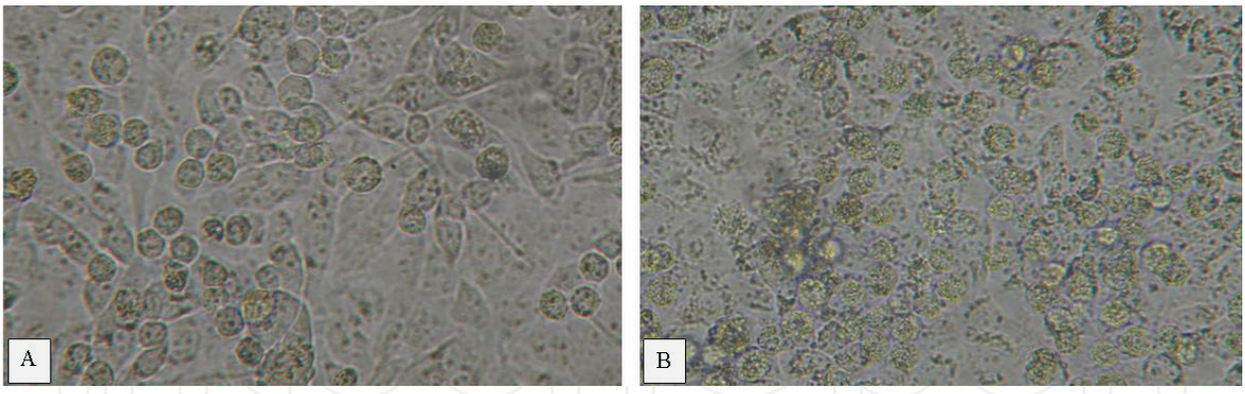

Figure 6. Microscopy of DU145 cells incubated with 1000 μg/ml of red maize anthocyanins for 48 h. (A) Control culture; (B) culture treated with anthocyanins (1000 μg/mL).

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References

[1] Paliwal RL, Granados G, Lafitte HR, Violic AD, editors. El maíz en los trópicos: Mejoramiento y producción 2001. 1st ed. Rome; Italia: FAO: 392 p.

[2] Ruiz CJ, Durán PN, Sánchez GJ, Ron PR, González ED, Medina GG, Holland BJ. Climatic adaptation and ecological descriptors of 42 maize races. Crop Sci Soc Am 2008;**48**:1502-1512. DOI: 10.2135/cropsci2007.09.0518.

[3] Trujillo EE, Mendoza CMC, Castillo GF, Ortiz CJ, Delgado DA, Carrillo SA. Acumulación de antocianinas en pericarpio y aleurona del grano y sus efectos genéticos en poblaciones criollas de maíz pigmentado. Rev Fitotec Mex 2009;**32**(4):303-309.

[4] Delgado-Vargas F, Jiménez AR, Paredes-López O. Natural pigments: Carotenoids, anthocyanins, and betalains—Characteristics, biosynthesis, processing, and stability 2003. 1st ed. Boca Raton, Florida: CRC press: 313 p. DOI: 10.1080/10408690091189257.

[5] Kong JM, Chiam SL, Goh NK, Chia TF, Brouillar C. Analysis and biological activities of anthocyanins. Phytochemistry 2003;**64**(5):923-933. DOI: 10.1016/S0031-9422(03)00438-2.

[6] Clifford MN. Anthocyanins: Nature, occurrence and dietary burden. J Sci Food Agric 2000;**80**(7):1063-1072. DOI: 10.1002/(SICI)1097-0010(20000515)80:7<1063::AID-JSFA605>3.0.CO;2-Q.

[7] Wang LS, Stoner GD. Anthocyanins and their role in cancer prevention. Cancer Lett 2008;**269**(281-290). DOI: 10.1016/j.canlet.2008.05.020.

[8] Kotesha R, Takami A, Espiniza JL. Dietary phytochemicals and cancer chemoprevention: a review. Oncotarget 2016;**7**:57517-52529. DOI: 10.18632/oncotarget.9593.

[9] Mehta M, Shike M. Diet and physical activity in the prevention of colorectal cancer. J Natl Compr Canc Netw 2014;**12**:1721-1726.

[10] Howes MJ, Simmonds MS. The role of phytochemicals as micronutrients in health and disease. Curr Opin Clin Nutr 2014;**17**:558-566. DOI: 10.1097/MCO.0000000000000115.

[11] WHO. http://www.who.int/gho/ncd/mortality_morbidity/cancer_text/en/ [Internet]. [Accessed: January 7].

[12] Wang CJ, Ying J, Kapur P, Wohlfeld B, Roehrborn C, Kim DW. Solitary recurrence of castration-resistant prostate cancer with low or undetectable levels of prostate specific antigen salvaged with local ablative radiation therapy: A case report. Oncol Lett 2016;**11**(1):713-716. DOI: 10.3892/ol.2015.3940.

[13] Priyadarsini RV, Nagini S. Cancer chemoprevention by dietary phytochemicals: promises and pitfalls. Curr Pharm Biotechnol 2012;**13**:125-136. DOI: 10.2174/138920112798868610.

[14] Alarcón AE. Polifenoles, antocianinas, actividad antioxidante y actividad biológica de tortilla y grano de maíz azul de la raza Mixteco. [Thesis] 2013. Xalapa, Veracruz. México: Universidad Veracruzana: 200 p.

[15] Singleton VL, Rossi, JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 1965;**16**:144-150.

[16] Giusti M, Wrolstad RE. Characterization and measurement of anthocyanins by UV-visible spectroscopy. Giusti M, Wrolstad RE. Characterization and measurement of anthocyanins by UV-visible spectroscopy. In: Current Protocols in Food Analytical Chemistry 2001. New York, N.Y.: John Wiley and Sons, Inc. p. F:F1:F1.2. DOI: 10.1002/0471142913.

[17] Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT—Food Sci Technol 1995;**28**:25-30. DOI: 10.1016/S0023-6438(95)80008-5.

[18] Benzie IF, Strain FJ. Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Anal Biochem 1996;**239**(1):70-76. DOI: 10.1006/abio.1996.0292.

[19] Denizot F, Lang R. Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J Immunol Methods 1986;**89**(2):271-277.

[20] Salinas MY, Martínez BF, Soto HM, Ortega PR, Arellano VJ. Efecto de a nixtamalización sobre las antocianinas del grano de maíces pigmentados. Agrociencia 2003;**37**(6):615-628.

[21] López-Martinez LX, Parkin KL, García HS. Phase II-Inducing, polyphenols content and antioxidant capacity of corn (*Zea mays* L.) from phenotypes of white, blue, red, and purple colors processed into masa and tortillas. Plant Food Hum Nutr 2011;**66**(1):41-47. DOI: 10.1007/s11130-011-0210-z.

[22] Sorrenti V, Vanella L, Acquaviva R, Cardile V, Giofre S, Giacomo DC. Cyanidin induces apoptosis and differentiation in prostate cancer cells. Int J Oncol 2015;**47**(4):1303-1310. DOI: 10.3892/ijo.2015.3130.

[23] Matchett MD, MacKinnon SL, Sweeney M, Gottschall-Pass KT, Hurta RA. Blueberry flavonoids inhibit matrix metalloproteinase activity in DU145 human prostate cancer cells. Biochem Cell Biol 2005;**83**(5):637-643.

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