INSTITUTO TECNOLÓGICO Y DE ESTUDIOS SUPERIORES DE MONTERREY

CAMPUS MONTERREY
DIVISIÓN DE BIOTECNOLOGIA Y ALIMENTOS
PROGRAMA DE GRADUADOS EN BIOTECNOLOGIA

PHYTOCHEMICAL AND NUTRACEUTICAL PROFILES OF BLUE MAIZE (*ZEA MAYS)* HYBRIDS EVALUATED IN TWO LOCATIONS

TESIS

PRESENTADA COMO REQUISITO PARCIAL PARA OBTENER EL GRADO ACADEMICO DE:

MAESTRO EN CIENCIAS CON ESPECIALIDAD EN BIOTECNOLOGIA

POR:

MARCOS URIAS PERALDI

MONTERREY, N.L. **DICIEMBRE DE 2010**

Recent years have seen an increased interest in blue maize (*Zea mays*) due to its proven nutraceutical properties. In recent times blue maize hybrids have been developed through a landrace starting point and by genetic improvement. These hybrids are being evaluated for their environmental stability and agronomic profile. With this objective, 25 promising hybrid blue maize crosses were selected from the improvement program at INIFAP-Bajío and planted in 2 contrasting environments located in the Bajío area and Morelia.

The objective of this work was to evaluate the phytochemical and nutraceutical profiles of these hybrids and to select those with the best features. An initial bulk analysis was elaborated to elucidate any significant differences between both locations. Free and bound phenolic acids were evaluated using the Folin-Ciocalteu method as were their antioxidant capacity using the Oxygen Radical Absorbance Capacity (ORAC) method. Bound ferulic acid content was determined by High Pressure Liquid Chromatography (HPLC) and total monomeric anthocyanin content was determined by the pH differential method. Soluble carbohydrates, protein methods and biophysical properties like kernel color and endosperm texture were also determined. The results indicate a significant difference among the 25 genotypes evaluated. With a number of genotypes having free and bound phenolic acids content higher than those previously reported; their respective antioxidant capacities also showed high levels. Bound phenolic acid showed a significant difference between environments whereas anthocyanins were not affected by the environment. Soluble carbohydrate and soluble protein determination confirmed that white maize conversion retains key phytochemical properties. Results show a surprising correlation between chroma and anthocyanin content as well as for other kernel color measurements. Overall, the 25 hybrid blue maize genotypes evaluated were little influenced by the environment and retained high levels of health promoting phytochemicals and nutraceutical activity. A number of genotypes exhibited high phytochemical and nutraceutical profiles that can be used as a basis for crop improvement in further studies involving breeding blue maize. Especially genotype 22 which showed stability among regions and across most phytochemical traits, having high anthocyanin content, ferulic acid and antioxidant capacity.

A mi mama Laura, por su amor y apoyo incondicional que me brinda la fuerza para querer seguir adelante y superarme a pesar de la adversidad. Así como mantener una vida con ideales firmes. A mi papa Javier, quien me ha enseñado a superarme día a día, alcanzar nuevas metas y nunca dejar de sorprenderme. A ambos les ofrezco esta tesis como símbolo del esfuerzo que han hecho para verme superar.

A mis hermanos Javier y Mónica quienes en todo momento me apoyaron y ofrecieron su ayuda en todas las facetas de mi vida como estudiante de maestría.

A mis abuelos Alfredo y Graciela quienes se mostraron siempre dispuestos a escuchar los quehaceres de un estudio sobre maices azules

Por su confianza y apoyo en todo momento quiero que sepan que este logro es de todos ustedes.

Quiero agradecer a todas aquellas personas que de alguna manera u otra me ayudaron a realizar esta tesis. En el periodo de dos años, un sin número de personas comentaron, analizaron y contribuyeron al escrito de este proyecto que sin ellos las cosas no hubieran resultado, así que gracias a todos ustedes. Agradezco a Dios y sus planes divinos que me han llevado por el camino de la ciencia y el aprendizaje.

Enérgicamente agradezco al Dr. Silverio García Lara, mi co-asesor quien despertó en mi la curiosidad y pasión por la ciencia y el cuestionamiento. Su visión me mantuvo en el camino de la superación y por ello le agradezco. Admiro su capacidad de asombro y su vocación que sin saberlo me ha contagiado.

Extiendo un agradecimiento al Dr. Sergio Serna Saldívar, mi co-asesor quien se mantuvo siempre en la mayor disposición para el desarrollo de mi tesis. Fungió papeles de asesor y de maestro, y agradezco haber sido alumno de tan prestigiado investigador.

Agradezco a la Dra. Janet Gutiérrez Uribe quien se empeño en verme superar los obstáculos a los que me enfrente. Gracias por sus contribuciones diarias a mis experimentos, estudios y salud mental.

Agradezco a la maestra en ciencias Perla Ramos Parra por su ayuda diaria durante el periodo de la maestría. En especial le agradezco por responder mis preguntas y por ayudarme a cuestionar mis protocolos y métodos de experimentación.

Agradezco la valiosa ayuda que me brindaron Carlos, Alejandro, Melissa y Mario, quienes dejan su huella en este documento.

Agradezco al Dr. Ricardo Ernesto Preciado y al INIFAP por la contribución de los materiales con los que se trabajo. Agradezco al personal de INIFAP y CIMMYT quienes brindaron información indispensable para el desarrollo de la tesis.

Les agradezco la comprensión, ayuda y buenos momentos que me hicieron pasar mis amigos, a quienes debo las gracias por ayudarme a mantener la cordura. Así mismo, agradezco a todo el personal del Centro de Biotecnología FEMSA, alumnos, maestros, administrativos y personal de limpieza por su cooperación durante el desarrollo del presente proyecto.

Table of contents

List of tables

List of figures

Chapter I: Introduction

Maize (*Zea mays*) has become the primary staple food in many nations, including Mexico. Worldwide, maize production has never been higher and demand has increased alarmingly through the last decade. Now, more than ever, corn/maize has become the primary food source for millions of cattle, households and countless food service industries. Such interest in maize has prompted an increase in germplasm diversity available for commercialization and research. Genotypes include common Latin American varieties as well as modified varieties which exhibit nutritional properties well above other non-modified genotypes. Pigmented corn varieties have been ever present, but have endured low recognition due to the overwhelming attention placed on white and yellow corns. Pigmented varieties studies have increased through the last decade at a rate of 10% annually.

Extensive selective breeding to improve nutrition, agronomics and yield has led to a characterization of key nutritional and antinutritional components (Harrigan *et al*., 2007). Contributing factors to this variation include (i) differences between ecotypes adapted for growth and productivity in different geographical regions and environments; (ii) differences arising from changes in climatic conditions and from different levels of abiotic stress; (iii) breeding for different endpoints such as protein composition, yield or morphology (Harrigan *et al*., 2007).

The importance of maize in Mexico and other Latin American countries has increased over the last decade. This grain has become of vital importance to industrial applications and human consumption. As reported in Food and Agricultural Organizations 2008 annual report, 7,353,940 Ha of maize were harvested in Mexico with a yield of 24,320,100 tons of production. In 2007 FAO reported Mexico had a consumption of 12,993,719 tons of maize corresponding to a 125 kg annual consumption per capita (FAO, 2008).

FAO also described that 1,064 calories per day are derived from maize product consumption including mainly tortillas (FAO, 2008). This represents Mexico's primary food source and is intrinsically embedded within the culture. The maize tortilla is one of the basic food sources in Mexico and Central America. The Mexican population receives most

of their calcium (Serna-Saldívar *et al.*, 1990; Gonzalez *et al.*, 2004) from nixtamalized products. Tortilla, either prepared in the traditional method or from commercial corn flour, represents up to 67% of the total energy intake of the Mexican population, with the highest intake in rural areas (Rosado *et al.*, 2005).

The nutritional value of blue corns with floury endosperm is higher than yellow or white dent kernels. In general, the protein and mineral content of blue corn are higher than that of most dent corns (Dickerson, 1990); the blue corn contains higher levels of flavonoids especially anthocyanins, which are currently thought to be excellent sources of antioxidants for functional foods (Betran *et al.,* 2001). Interest in the phytochemical constituents of fruits, vegetables and grains has increased in recent years due to consumer awareness of their various health and nutraceutical benefits (Del Pozo-insfran *et al*., 2006). Numerous studies have investigated the polyphenolic composition of various cereal grains, not only for their roles on cell wall structure but also for their antioxidant and bioactive properties (Del Pozo-insfran *et al*., 2006). At present, blue and purple corn kernels are used for making blue or pink tortillas, their anthocyanin content holds promise as functional foods or functional food colorants (Abdel-Aal *et al*., 2006). The study of blue maize represents a unique opportunity to enhance its use and take advantage of its nutritional and industrial properties.

Chapter II: Background

2.1 Blue maize

Maize is a monocot that belongs to the grass family *Poaceae,* the *Zea* genus and *mays* species (**Table 2.1**). It is a cross pollinating angiosperm that is believed to have evolved from teosinte (derived from "*teocintli*" in nahuatl language: "*teotl*"= sacred and "*cintli*"= dried ear of corn) species some 10,000 years ago (Vavilov *et al.,* 1931). Domestication resulted in a group of ancestral landraces that subsequently spread throughout Mexico and Central America and eventually to the rest of the world. Morphologically, the maize plant contains the female (ear) and male (tassel) flowers in separate places on the plant. Kernel development occurs in the ears, or cobs; each ear has about 300 to 1 000 kernels, weighing between 190 and 300 g per 1,000 kernels, in a variable number of rows (12 to 16) (Andrade *et al*., 2000).

Table 2.1 Taxonomic classification of *Zea mays*

Kingdom	*Plantae*
Subkingdom	*Tracheobionta*
Division	*Magnoliophyta*
Class	*Liliopsida*
Subclass	*Commelinidae*
Order	*Cyperales*
Family	*Poaceae*
Genus	*Zea*
Species	*mays*

The kernel is mainly composed of 3 anatomical parts: the pericarp (structure providing protection and rigidness), the germ (first reserve tissue) and the endosperm (second reserve tissue). The pericarp is mainly composed of hemicellulose and cellulose fibers, ferulic acid monomers and dimers, p-coumaric acid monomers and lignin. The endosperm consists of a single layer of aleurone cells and corneous, floury, and peripheral endosperm (**Figure 2.1**). The aleurone layer of a mature maize kernel consists of a single file of cuboidal, densely cytoplasmic cells (Scanlon *et al*., 1997). The proportion of floury to corneous endosperm varies from all corneous to nearly all floury endosperm in certain varieties. The floury endosperm consists of a discontinuous protein matrix with large

spherical starch granules and empty spaces between starch granules, protein bodies, and matrix. These spaces refract light rays and appear light to reflected light or opaque to transmitted light. In maize grown in southwest United States and Mexico, the aleurone layer contains anthocyanin pigments that give the characteristic blue color (Betran *et al*., 2001). In corn varieties, pigmentation can occur in the pericarp, aleurone, and starchy endosperm (Espinoza-Trujillo *et al*., 2009); in Mexican blue corn this pigment is specifically located in the aleurone layer. The vast majority of anthocyanins present in plant cell tissues are specifically located inside the vacuole in a plant cell. These flavonoid pigments are transported in sub cellular structures called anthocyanoplasts from their production point inside the endoplasmic reticulum to the vacuole (Kitamura, 2006). In some cases, the pigmentation is so intense that kernels appear to be black (Betran *et al*., 2001).

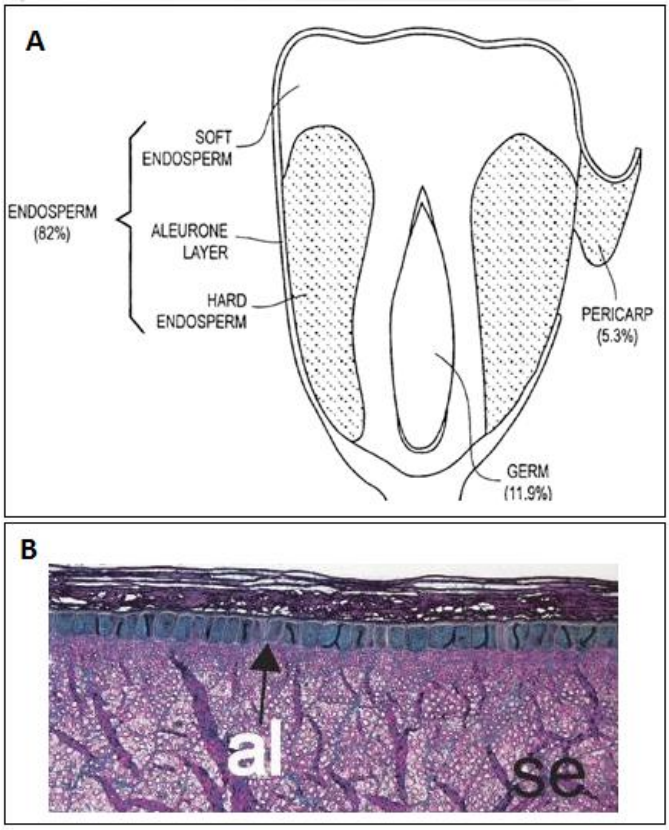


Figure 2.1 Corn kernel diagram (A) Pericarp, endosperm, germ and aleurone diagram. (B) Kernel cross-section stained with ABB/Sudan black and PAS reagent, al (aleurone), se (starchy endosperm) (Modified from May, 1987).

Recently pigmented materials have received increased interest due to the anthocyanin contents (Ultrilla-Coello *et al.,* 2009). These include blue, purple and red-pigmented kernels with well-established antioxidant and bioactive properties (Del Pozo-Insfran *et al.,* 2006). These pigments are associated with the aleurone layer of the endosperm and affect the visible color of the grain (**Figure 2.1**). Generally kernels of small to medium size produce more intense blue coloration since they posses a higher proportion of aleurone layer and are less diluted by the starchy endosperm (Del Pozo-Insfran *et al*., 2006).

Figure 2.2 Landrace blue corn images showing texture, corncob and kernel (adapted from Preciado, 2010).

Blue corn varieties have poor stalk strength and often lodge prior to harvest. High winds break off the stalks and cause significant losses of grain and lower quality of the kernels. Some blue corn hybrids that have improved yields, plant height, and maturity have been developed (Betran *et al.*, 2001). These hybrids have a harder endosperm and can be more easily processed with the standard equipment presently being used in the industry (Betran *et al.*, 2001).

2.1.1 Blue maize in México

Mexico is considered as the place of origin and diversity of maize. This diversity has been classified as 59 races based on morphological, biochemical, genetic and molecular characteristics (Warburton *et al*., 2008). Corn varieties have been classified on basis of grain pigment. Color is determined by the frequency of pigments like carotenoids in yellow corn (Egesel *et al*., 2003) and anthocyanins in blue corn (Irani *et al*., 2003). These pigmented corn varieties are present in 41 races and show distinct kernel color or stalk and foliage color (Ortega *et al*., 1991).

An estimated national corn production of 25.1 million tons was reported in 2008, which represents a 43.1% increase with respect to data from the year 2000.The average yield per hectare is 3.3 tons. This output is an estimated 34% higher than that reported in 2000 (SAGARPA 2010). During 2008, the Northern state of Sinaloa occupied the first place in national production with a total of 5.27 million tons, which represents 22% of national production. Average yield per hectare in the state was 9.32 tons, being also the highest in Mexico. The state of Jalisco occupied the second place in maize production with a total of 3.08 million tons harvested with an average yield of 5.36 tons. The central state of Estado de Mexico is the third most important maize producer in the country with 1.8 million tons harvested with an average yield of 3.23 tons.

The central highlands of Mexico (also known as "Valles altos") are an extensive area comprising several states located at 2200 to 2600 meters above sea level (**Figure 2.3**). This area harvests a comprised total of up to 1.5 million hectares, representing 20% of the national surface. Approximately a third of producers plant less than a third of their seasonal corn crops with blue or pigmented corn, this sums up to 159,000 hectares with a yield of 300,000 tons (Antonio-Miguel *et al.,* 2004; Arellano-Vazquez *et al*., 2003). The main areas for harvesting pigmented corn in Mexico are the states of Puebla, Tlaxcala, Mexico and Hidalgo, where the average regional yield is less than 4 tons per hectare.

Figure 2.3 Country diagram depicting the central and northern highlands area "Valles altos" where most of the domestic blue corn is produced in Mexico (Adapted from Vielle-Calzada *et al*., 2009)

Blue corn varieties represent a niche market due to its low commercial exploitation and higher price. Pigmented maize varieties (**Figure 2.2**) have tonalities varying in blue, purple and red. Some of the most common races include: elotes cónicos originally from the states of Puebla, Tlaxcala, México, Querétaro and Guanajuato; chalqueño from the state of México; cónico norteño from Zacatecas; gordo from Chihuahua and bolita from the southern state of Oaxaca (Sanchez *et al*., 2000). Other varieties are Chalco race, arrocillo and purépecha that are most commonly found in central or southern Mexico. (Antonio-Miguel *et al.,* 2004)

2.1.2 Blue maize products

Blue corn has been utilized in Mexico for human consumption for centuries (Antonio-Miguel *et al*., 2004). In Mexico, traditional uses for blue corn include its use in tortillas, tortilla chips, tamales, atole (beverage) and tejuino (alcoholic beverage obtained from corn kernel fermentation).

Although blue corn products have been around for thousands of years (**Table 2.2**), it was until recently that blue corn has claimed spotlight in the international market as a functional food (Serna-Saldívar *et al*., 2001). Consumption of blue corn tortillas and chips has greatly increased in United States mainly stemming from the southwestern states of New Mexico, Arizona and Utah (Dickerson *et al*., 1990). Recognition of health promoting benefits in blue corn consumption has widened product diversity and now they are present in health food supermarkets. Products vary from pancakes, corn flakes, chips, muffins and various extruded snacks (Betran *et al*., 2001).

Recently, flexible biofilms have been developed for their use in industrial packaging (Rojas *et al*., 2010). Blue corn polymers are being employed for biodegradable and edible containers due to their excellent antioxidant properties and the added value of oxidation protection for the food they contain. Other common industrial uses include anthocyanin extraction for pigments used in textile coloration (Jing *et al*., 2007a). Pure anthocyanins in the food market can cost approximately $1,000USD per kilogram (Cisneros-Zevallos 2003; Espinoza-Trujillo *et al*., 2010).

Table 2.2 Traditional blue corn uses and industrial products

Component	Industrial use	Reference
Anthocyanins	Natural pigment for textile and food industries	Zhao *et al*., 2008; Jing *et al.,* 2007b; Cevallos-Casals *et al*., 2004
Cellulose, lignin and hemicellulose	Bioactive containers with antioxidant capacity	Rojas *et al*., 2010
Foliage	Artesanal uses, tamal wraps	Salinas-Moreno *et al*., 2008
Kernel	Non-fermented beverage (chicha morada)	Antonio-Miguel *et al*., 2004; Jones, 2005
Kernel	Tortillas, tortilla chips, masa, tlacoyos, atole	Betran *et al*., 2001; De la Parra *et al*., 2007; Dickerson, 2003

2.2 Phytochemical and nutraceutical composition in cereal grains

Chemical components known as phytochemicals are bioactive secondary metabolites present in plants (Wildman, 2000). It has been shown that a large number of phytochemicals present in cereal grains posses additive synergistic effects that have

protective health benefits (Adom *et al*., 2002). Plants utilize such compounds as protection mechanisms, and in turn, their consumption has been attributed to several health benefits.
A large number of nutraceuticals components have been shown to be present in a wide variety of grains (**Figure 2.4**). A nutraceutical is a bioactive phytochemical which provides desirable health benefits beyond basic nutrition (Liu, 2007; Slavin, 2001). More than 5000 different phytochemicals have been found in plants (Adom *et al*., 2002).

Whole grains are rich in soluble fiber, insoluble fiber and bioactive compounds. Barley, rye, wheat, corn, sorghum, oats and rice are a major source of compounds like lignans (Martinez-Tome *et al*., 2004), phenolics (gallic acid, *p*-hydroxybenzoic acid, ferulic acid, cinnamic acid) (Rooney *et al*., 2007), flavonoids (anthocyanins, flavones, flavanones) (Adom *et al*., 2002), isoprenoids (Sakakibara *et al*., 2003), carbohydrates and lipids (Liu, 2007) .

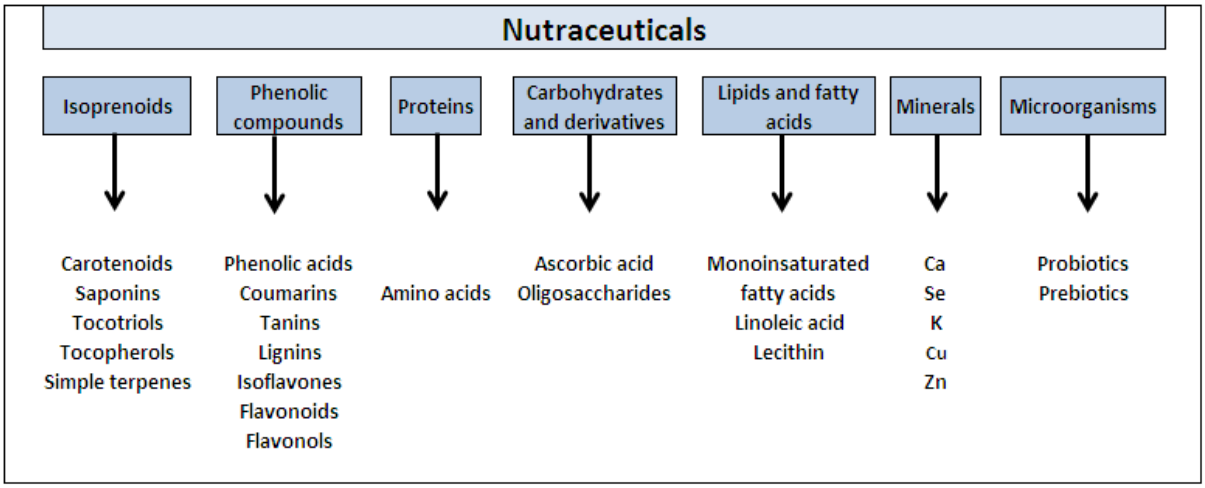


Figure 2.4 Nutraceutical classification of the main components associated to cereals (Adapted from Wildman, 2000)

Maize varieties of diverse genotypes vary significantly in nutraceutical substances like flavonoids, tocopherols, polyphenols, carotenes and xantophylls relevant to human health (Della Penna, 1999). Corn has a higher antioxidant capacity when compared to wheat, oat, and rice (De la Parra *et al.*, 2007). Blue, purple and red-pigmented corn kernels are rich in anthocyanins with well documented antioxidant and bioactive properties (Adom *et al*., 2002; Del Pozo-infran *et al*., 2006). Epidemiological studies suggest that

phytochemical content fights oxidative stress, which raises the occurrence of degenerative diseases like cancer and cardiovascular diseases (Temple, 2000).

2.2.1 Phytochemical composition in blue maize

The chemical composition of blue corn has been noted as superior in certain phytochemicals and minerals when compared to yellow and white corn genotypes (Dickerson, 2003; Ultrilla-Coello *et al*., 2009). In protein content (**Table 2.3**) present in the endosperm, blue corn genotypes averages 8.3% whereas the contrasting white genotype approximately 7.5%. Apparent amylose content present ranges from 23.1% to 26.3 % for blue corn and white corn, respectively. Starch present in the endosperm consisted of 84.1% for the blue corn genotype and 78.7% for the white corn (Ultrilla-Coello *et al*., 2009).

Table 2.3 Chemical composition in blue and white corn genotypes

Content (%)*	Blue corn	White corn
Moisture	6.3	7.4
Protein	8.3	7.5
Lipids	0.2	0.5
Ash	0.3	0.6
Apparent amylose	23.1	26.3
Total starch	84.1	78.7
Damaged starch	4.5	4.2

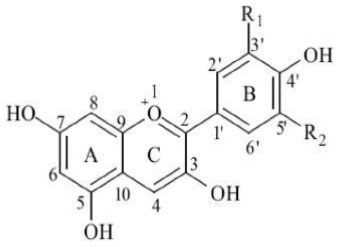
*Adapted from Hernandez-Uribe *et al*., 2007

Hernández-Uribe and colleagues (2007) showed correlation between the amount of starch present in blue corn and the amount of protein and lipids when compared to white corn genotypes. It was found that both components interact with amylose and large chains of amylopectin in order to attenuate starch retrogradation. Results are intimately related to a lower glycemic index when compared to white corn genotypes.

2.2.1.1 Anthocyanins

Anthocyanins are a type of flavonoid (**Figure 2.5**) commonly found in grains, fruits, flowers and vegetables. They are especially attractive because of their intense coloring, exhibiting hues that appear blue, red and orange (Espinoza-Trujillo *et al.,* 2009; Salinas-Moreno *et al.,* 2003). They are naturally occurring pigments in the form of glucosides having glucose, galactose, rhamnose, xylose and arabinose adhered to an aglycone nucleus (Wang *et al.,* 2008). Differing from other flavonoids, anthocyanins contain a net positive charge when in acidic solution. They are water soluble and depending on the pH and presence of chelating ions, they have an intense blue or purple color when in solution (Giusti *et al.,* 2001). The aglycone or de-glycosilated form of an anthocyanin is known as an anthocyanidin.

These pigments play an important role as antioxidants and in protecting DNA and the photosynthetic apparatus from high radiation changes (Irani *et al*., 2005). Other suggested functions of anthocyanins are protection against cold stress or providing drought resistance (Christie *et al*., 1994).



Anthocyanin name	R1	R2
Delphinidin	OH	OH
Petunidin	OCH_3	H
Cyanidin	OH	H
Pelargonidin	H	H
Peonidin	OCH_3	H
Malvidin	OCH_3	OCH_3

Figure 2.5 Anthocyanidin skeleton showing corresponding functional groups in rings A, B and C (Adapted from Moreno *et al*., 2005; Welch *et al*., 2008).

The most studied anthocyanidins are cyanidin, delphinidin, pelargonidin, malvidin, petunidin and peonidin (Moreno *et al.,* 2005). The sugar component of the anthocyanin is found conjugated to the anthocyanidin skeleton through a C3 hydroxyl on ring C. More than 500 different types of anthocyanins are known, in part due to the position of the glycoside on the anthocyanidin skeleton (Abdel-Aal *et al*., 2006). Additional complexities include anthocyanin acylation which along with glycosidic patterns create complex molecules that exhibit vivid colors.

De la Parra and colleagues (2007) determined that blue corn contained 36.87 mg of cyanidin-3-glucoside/100g of sample. This amount is about 10-fold greater compared to a high carotenoid corn which contains 4.63mg of cyanidin-3-glucoside/100g of sample (**Table 2.4**) (De la Parra *et al.,* 2007).

Table 2.4 Anthocyanin content in corn genotypes

Genotype	Anthocyanin content (mg of cyanidin-3-glucoside/100g)
White corn	1.33 ± .02
Yellow corn	0.57 ± .01
Red corn	9.75 ± .44
Blue corn	36.87 ± 0.71
High carotenoid corn	4.63 ± 0.06

*Adapted from De la Parra *et al*., 2007

Several studies have determined individual anthocyanin content in several blue corn genotypes. It has been stated that the main anthocyanin present in blue corn is cyanidin-3-glucoside (**Table 2.5**) (Abdel-Aal *et al.,* 1999; Jing *et al*., 2007). As observed, cianidin-3-glucoside is the main anthocyanin present in blue corn; other anthocyanins like pelargonidin and peonidin have been shown to have equal concentrations among them (Welch *et al*., 2008). Values reported for total anthocyanin content ranged from 321 mg/kg for a Mexican blue genotype to 196.7 mg/kg for a New Mexico harvested blue corn named "cutie blue" (Abdel-Aal *et al*., 2006).

Table 2.5 Total and individual anthocyanin content in blue corn genotypes

Component	Concentration (mg/kg)	Genotype
Total anthocyanin	321.00 *	Mexican blue 1
	307.00 *	American blue 1
	342.20 *	Mexican blue 2
	260.91 *	American blue 2
	322.72 ¥	Shaman blue
	196.76 ¥	Cutie blue
Cyanidin-3-glucoside	110.20 ¥	NA
Cyanidin-3-glucoside	15.43 §	NA
Pelargonidin-3-glucoside	2.33 §	NA
Peonidin-3-glucoside	4.44 §	NA
Acylated cyanidin-3-glucoside	10.37 §	NA
Acylated pelargonidin-3-glucoside	2.83 §	NA
Acylated peonidin-3-glucoside	4.85 §	NA

* Del Pozo-Insfran *et al*., 2006

¥ Abdel-Aal *et al*., 2006

§ Pedreschi *et al*., 2007

2.2.1.1.1 Anthocyanin biosynthesis

Anthocyanin synthesis has been widely studied, and most of the enzymes and genes involved have been characterized (Cone, 2007; Coe *et al*., 1998). The first step in the anthocyanin synthesis (**Figure 2. 6**) pathway involves the condensation of 3 malonyl-CoA molecules with p-coumaroyl-CoA to produce a chalcone, this step is catalyzed by the enzyme chalcone synthase (CHS) (Pascual-Teresa *et al*., 2008). The isomerization of the chalcone to a flavanone occurs with the enzyme chalcone-flavanone isomerase (CHI). Flavanones (naringenin, eriodyctiol) are converted to dihydroflavonols by hydroxylation of the C3 carbon in the C ring by flavanone-3-hydroxylase (F3H).Flavanones and dihydroflavonols can also be hydroxylated on the 3' position of the B ring by flavonoid 3'-hydroxylase (F3'H) and in the 3' and 5' position by flavonoid-3',5'-hydroxylase (F3'5'H). Dihydroflavonols are reduced to leucoanthocyanidins by dihydroflavonol 4-reductase (DFR), the colorless leucoanthocyanidins are converted to colored anthocyanidins by

anthocyanidin synthase (ANS). Anthocyanidins (Cyanidin, Pelargonidin and Delphinidin) are the glycosylated by UDP flavonoid 3-0-glucosyl transferase (FGT) and transported to the vacuole by action of glutathione-S-transferase (GST) and a multidrug resistance-like transporter (MRP) for sequestering anthocyanins in the vacuole.

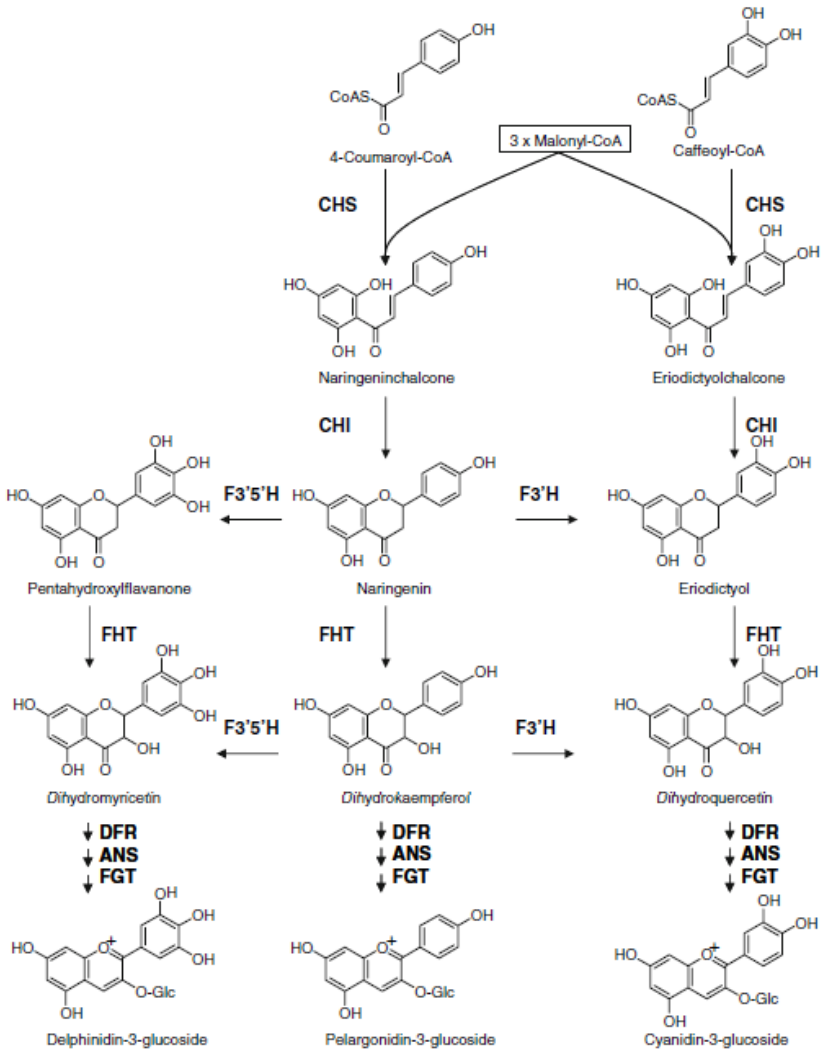


Figure 2.6 Anthocyanin biosynthesis pathway (CHS:Chalcone synthase; CHI:Chalcone isomerase; F3'5'H: Flavonoid 3'5' hydroxylase; F3'H: Flavonoid 3'hydroxylase; FHT: Flavanone 3' hydroxylase DFR; Dihydroflavonol reductase; ANS: Anthocyanidin synthase; FGT: UDP Flavonoid-3-*O*-glucosyl transferase (Adapted from Pascual-Teresa *et al*., 2008)

2.2.1.1.2 Anthocyanin antioxidant capacity

Antioxidant capacity showed by anthocyanins has been attributed to their phenolic structure (Wang *et al*., 2008; Temple, 2000). The anthocyanin phenol structure has the ability to scavenge free radicals like superoxide (O-), oxygen (O_2), peroxide (ROO-), hydrogen peroxide (H_2O_2) and hydroxide radicals (OH). The antioxidant action is attributed specifically to the presence of hydroxyl groups to position 3' of ring C and positions 3', 4' and 5' of ring B of the anthocyanidins skeleton (Cone, 2007). In general, the scavenging capacity of anthocyanidins (aglycone molecule) is superior to that of glycosilated anthocyanin molecules. This capacity is lowered if the amount of sugars attached to the skeleton increases.

Table 2.6 Nutraceutical properties of blue corn anthocyanin extracts

Antioxidant capacity	Cortes *et al*., 2006; Wang *et al*., 2008; Cevallos- Casals *et al*., 2003a; Abdel-Aal., 2006
Anticarcinogenesis activity	Pedreschi *et al*., 2007; Wang *et al*., 2008; Shih *et al*., 2005; Hagiwara *et al*., 2001; Garcia-Alonso *et al.*, 2004
Prevents obesity	Tsuda et al., 2008
Prevents hyperglycemia	Tsuda et al., 2003; De Munter *et al*., 2007
Antimutagenic action	Pedreschi *et al*., 2006; Shih *et al*., 2005; Liu *et al*., 2002
Anti-inflammatory	Wang *et al*., 2008; Tsuda *et al.*, 2002
Antimicrobial	Cevallos-Casals *et al*., 2003b; Puupponen-Pimia *et al*., 2001
Prevents cardiovascular diseases	Garcia-Alonso *et al.*, 2004
Protection against hepatic damage	Wang *et al*., 2000

Antioxidant effects have been demonstrated in several cell cultures including: colon, endothelial, liver, leukocytes and keratinocytes. Such studies (**Table 2.6**) have proven anthocyanin anti-toxic and anti-carcinogenic effects. Such include, free radical scavenging, increases in cellular free radical absorption capacity, phase II enzyme stimulation, reduction of DNA adducts caused by free radicals, reduction in lipid peroxidation, and

mutagenesis by carcinogenics and toxins, as well as reduction in cell proliferation by signaling pathways (Wang *et al*., 2008).

2.2.1.2 Polyphenols

Glycosilation and arrangement possibilities have led to the supposition that there exist more than a million different polyphenols (Sakakibara *et al.,* 2003). Phenolics are compounds that posses one or more rings with one or more hydroxyl groups attached (Liu, 2007). Phenolics posses antioxidant activity due mainly by their redox properties, which allow then to act as reducing agents, hydrogen donators, and singlet oxygen quenchers (Kahkonen *et al*., 1999). Their bioactivity (**Figure 2.5**) is attributed to the aglycone structure and not to the sugar residues; the antioxidative potency is due to the orthodiol (catechol) structure of the aglycone. Polyphenols can be classified as simple polyphenols like benzoic and cinnamic acid, flavonoids, catechins, chalcones, anthocyanins and anthraquinones (Sakakibara *et al*., 2003). Phenolic acids posses functions that are essential for plant growth, reproduction, color and defense against pathogens, parasites and plant predators. Phenols present in distinct areas of the corn kernel have relevant properties such as the tolerance to *Fusarium spp*. in the germ (Bakan *et al*., 2003). Endosperm phenols facilitate color development in dough and tortilla (Salinas-Moreno *et al*., 2007) and those present in the pericarp have been found to be related to tolerance to storage pests (Arnason *et al*., 1992). These compounds are implicated in two defense concepts, the phenolic fortification of cell walls and the deterrent effect of fiber content (Scanlon *et al*., 1997; Garcia-Lara *et al*., 2004).

Phenolic acids are commonly found ligated to the plant cell wall by esterification to proteins and ligated to cellulose and lignin (Cabrera-Soto *et al.,* 2009). De la Parra and colleagues (2007) observed high phenolic content in blue corn when compared to other genotypes (**Table 2.7**).

Table 2.7 Total phenolic content in white, yellow, red, blue and high carotenoid corn

Corn genotype	Free	Bound	Total
	(mg GAE/100g)		
White	34.7 ± 0.4	226.0 ± 6.3	260.7 ± 6.1
Yellow	43.6 ±1.8	242.2 ± 13.1	285.8 ± 14.0
Red	38.2 ± 0.4	205.6 ± 4.5	243.8 ± 4.6
Blue	45.5 ± 0.5	220.7 ± 0.5	266.2 ± 0.7
High carotenoid	50.0 ± 2.5	270.1 ± 9.4	320.1 ± 7.6

*Adapted from De la Parra *et al*., 2007

The majority of phenolics are found attached to the plant cell wall (Adom *et al*., 2002). Extraction methods that involve hydrolysis in order to liberate bound phenolics report (>80%) of total phenolics to be bound to the cell wall and (<20%) for free phenolics Adom *et al*., 2005).

2.2.1.2.1 Ferulic acid

Ferulic is the most abundant hydroxycinnamic acid present in the *Gramineae* family (Adom *et al*., 2002; De la Parra *et al*., 2007). It is found covalently bound by an ester to the cell wall, with 75% occurring in the aleurone and pericarp layers of the corn kernel and 10% in the endosperm (Sakakibara *et al*., 2003).

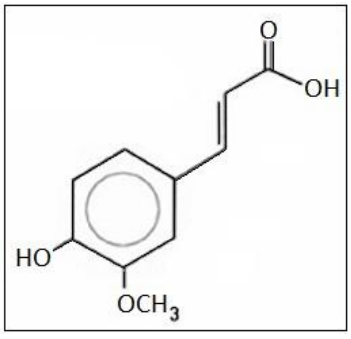


Figure 2.7 Ferulic acid chemical structure (adapted from Sakakibara *et al*., 2003)

The main components that strengthen the cell wall as mechanical barriers are ferulic and *p*-coumaric acid which are attached to hemicelluloses through pentose sugars. Studies

have shown the presence of ferulic acid and *p*-coumaric acid along with ferulic acid derivatives like 5-5', 8-O-4, 8-5' and 8-8' dihydroferulic acid (Garcia-Lara *et al*., 2004; Del Pozo-Insfran *et al.,* 2006). Previous studies have shown that formation of ferulic acid dimmers may increase the mechanical strength of the cell wall by the cross-linking of hemicelluloses and hence reduce degradability (Bergvinson *et al.,* 1995). Ferulic acid (**Figure 2.9**) is composed of an aromatic ring and shows one or more hydroxyl groups attached. The difference in total polyphenolic compounds between blue and white corn genotypes is attributed to the amount of free and sterified ferulic acid, responsible for white corn kernel endosperm hardness compared to the more floury blue corn endosperm. De la Parra and colleagues (2007) revealed a higher ferulic acid content in blue corn genotypes when compared (**Table 2.8)** to white and yellow corn.

Table 2.8 Polyphenolic and ferulic acid content in blue corn genotypes

Catechin	13.9 -21.4 mg/kg*
Free ferulic acid	202-927 mg/Kg*
Bound ferulic acid	127.8 mg/100g†
Total phenolic acids	451 mg/kg*
	1310 mg/kg*
Protochatecuic acid	14.61 mg/g§
Free phenolic acids	45.5 mg GAE/100g†
Bound phenolic acids	220.7 mg GAE/100g†

* Del Pozo-Insfran *et al*., 2006
† De la Parra *et al*., 2007
§ Pedreschi *et al*., 2007

When compared to other cereal grains, blue corn has up to 3 times higher ferulic acid content than wheat (Adom *et al.,* 2002). In such studies, blue corn genotypes contain 906. 13 (µmol ferulic acid/100gr of dry sample) of total ferulic acid, wheat contains 333.44, oats 184.66 and rice 153.39 (µmol ferulic acid/100gr of dry sample).

2.3 Hybrid blue corn

Hybrid blue corn is developed by crossing white inbred lines with elite donor blue color corns. This procedure is evaluated in order to combine blue color with properties

expected from the white corn donor. The methodology (**Figure 2.8**) commonly used for white corn conversion into blue follows that set by Betran and colleagues (2001). A donor for blue color maize line is selected and crossed with a white inbred line. The F_1 progeny is selected for deep blue kernel color and selfed to stabilize the cross and later, deep blue F_2 generation progeny kernels are selected. F_2 plants are crossed with the white inbred line; the result is blue color-1 progeny (BC1). Blue color-1plants are selfed and F_2 kernels selected, BC1 F_2 plants are crossed once more with the white inbred line. Progeny blue color-2 is selfed to give F_2 kernels, the experimental converted line.

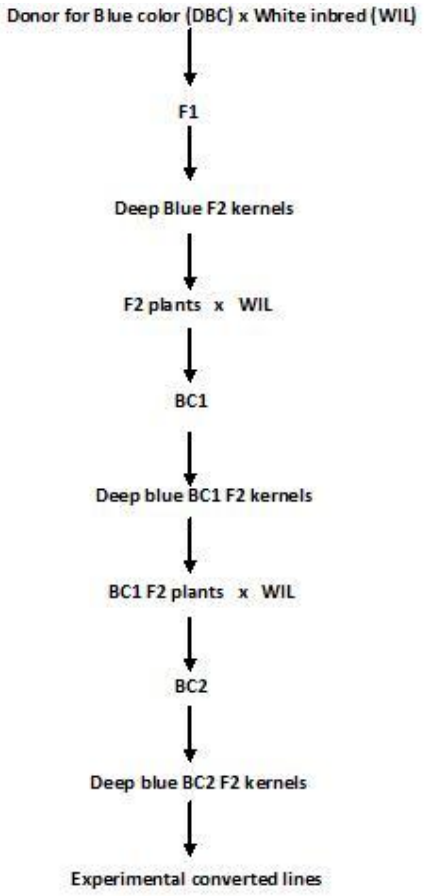


Figure 2.8 Blue corn conversion diagram involving white inbred lines and blue color donor corn (adapted from Betran *et al*., 2001).

2.3.1Genotype and environment interactions

Many researchers have emphasized the importance of genotype-environment (G x E) interaction in both breeding and variety testing (Westcott, 1986). Corn cultivars are tested in differing environments to determine the genetic stability of the tested genotypes. It has been stated that environment plays a critical role in stalk, fruit (corncob), foliage and root maturity. The key factors for corn stalk development including humidity, altitude, sun exposure, salinity and soil nutrients are key to corn stalk development (Blum, 1988). The main objective in corn improvement is the yield stability and the stable response to optimal and suboptimal conditions (Cordova, 1991).

The importance that factors responsible for yield stability have across contrasting environments and the identification of such factors is relevant for the elucidation of yield stability as well as for paving the way for other selection factors (Blum, 1988). Selection for yield, protein content, phytochemical composition and carbohydrate availability now play critical roles in commercial cultivar development.

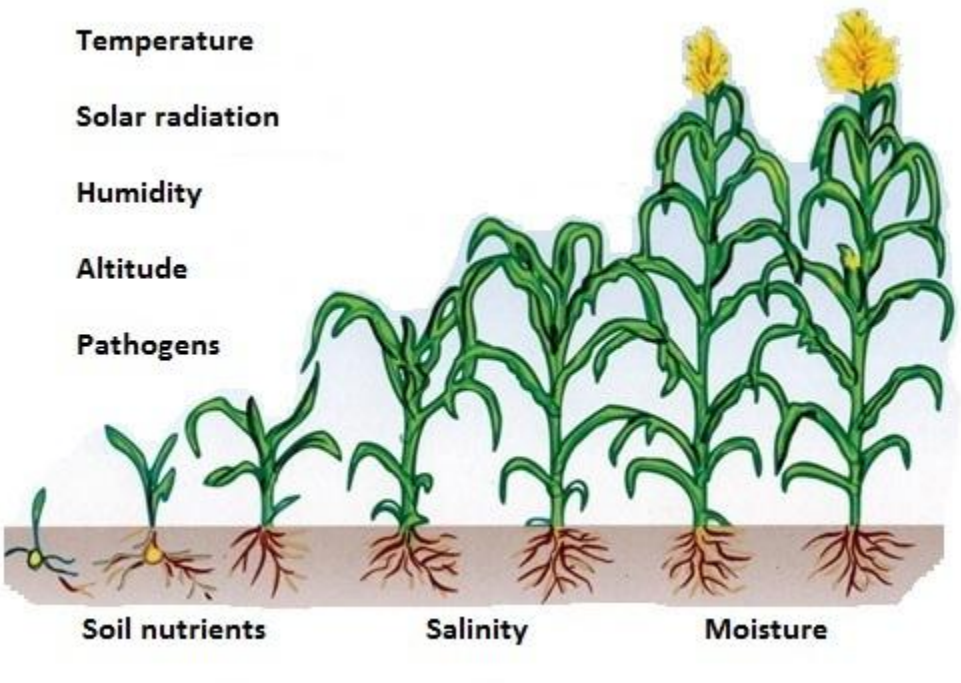


Figure 2.9 Corn plant maturation diagram showing factors affecting plant development (Adapted from Ribaut *et al*., 2006; Tollenaar *et al*., 2000)

The phenotype (P) is assumed to represent the genotypic (G) and environmental (E) effects, plus the interaction between them (G x E), or more formally P = G + E + G x E. It has been stated that the estimation of G, and to some extent G x E, has been more emphasized to date than E (Ribaut *et al*., 2006). The interaction may be due to heterogeneity of the genotypic variance across environments or also from the imperfect correlation of genotypic performance across differing environments (Kang *et al.,* 2004). This interaction is the reason for multi-environment trials (MET), the development of advanced materials that must be evaluated in multiple locations for a minimum of two years.

2.4 Objectives

Based on the background shown, the objectives of the present study involved the extraction and quantification of a series of phytochemical compounds. In a broad sense, the objective was to screen the phytochemical content of a number of hybrid blue corn genotypes and identify which phytochemical or nutraceutical traits significantly varied within each environment and between both differing environments.

Specific actions detailed in the present work include the following:

- Free and bound phenolic acid extraction and quantification.
- Determination of antioxidant capacity of free and bound phenolic acids.
- Ferulic acid quantification by HPLC.
- Determination of anthocyanin content.
- Soluble carbohydrate determination.
- Soluble protein determination.
- Phenotypic color determination.

Chapter III: Materials and methods

3.1 Maize Germplasm and Hybrid Production

Hybrid blue maize kernels utilized in this investigation were provided by Dr. Ernesto Preciado at Instituto Nacional de Investigaciones Forestales Agricolas y Pecuarias (INIFAP). Hybrid blue maize (*Zea mays*) genotypes provided consist of: 100 gram corn samples from 25 different elite crosses (**Table 3.1**) harvested in 2 contrasting subtropical regions. One harvested from the Bajío region (N 20°31′44″ W 100°48′54″, altitude 1,750 masl, Celaya Guanajuato, southeastern Mexico) consists of 3 repetitions (R1, R2 and R3) the other was harvested in the Morelia region (N 19°42′10″NW 101°11′32″, altitude 1,921 masl, Michoacán, west-central Mexico) and consists of 2 repetitions (R4 and R5).

Table 3.1 Hybrid blue maize germplasm obtained from 25 crosses harvested in 2 contrasting regions.

		BAJIO			MORELIA	
Genotype	**Cross**	**R 1**	**R 2**	**R 3**	**R 4**	**R 5**
1	23 x 53	1	107	160	1	104
2	33 x 58	3	98	149	3	74
3	63 x 26	4	92	124	4	81
4	87 x 160	9	66	167	8	96
5	121 x 84	11	85	150	10	84
6	124 x 144	12	69	163	11	105
7	136 x 197	15	111	152	13	103
8	144 x 124	16	101	134	14	71
9	160 x 87	20	99	173	17	65
10	171 x 131	22	97	180	19	79
11	193 x 210	24	93	178	21	108
12	207 x 192	26	71	175	23	67
13	222 x 323	28	75	154	25	92
14	225 x 242	29	83	169	26	75
15	242 x 225	30	72	147	27	69
16	249 x 302	31	81	139	28	63
17	254 x 315	32	82	148	29	98
18	285 x 67	38	67	157	34	106
19	292 x 160	39	61	161	35	56
20	301 x 156	40	79	126	36	66
21	293 x 227	41	90	162	37	93
22	324 x 190	44	88	121	40	107
23	352 x 38	45	87	138	41	89
24	356 x 152	47	106	145	42	80
25	364 x 224	50	104	144	45	78

Corn harvested from 3 repetitions in the bajío region (R1, R2 and R3) were planted in three different blocks in Celaya during 2008 and given an identification number. The experimental plot was elaborated with rows of 5.16 meters long and a separation between them of 0.76 meters. 250 kg per hectare of fertilizer 18-24-12 plus micro elements was applied. With this amount of fertilizer, 45 units of nitrogen (N), 60 of phosphorus (P) and 30 of potassium (K) were applied. The second fertilization event was applied when stalks showed the tenth leave, and consisted of the addition of 430 kg of urea per hectare, to cover the formula 244-60-30. Watering occurred in the form of natural precipitation, with one minor watering event.

Pre-emergent weed control was applied employing 4 L/hectare of Syngenta PRIMAGRAM GOLD herbicide with atrazine 33.7% and metolachlor 26% as active ingredients. For control during crop development, 1L/hectare of Syngenta Gramoxone herbicide was utilized, with Paraquat 25% as the active ingredient. For pest control, 20kg of Furadan 5G with carbofuran as the active ingredient were utilized to protect the crops. As a control during the vegetative step, 1.5 L/hectare of Lorsban was used for *Spodoptera frugiperda* worm.

Corn harvested from the Morelia region (R4 and R5) was planted in two different blocks during 2008 and given identification numbers. The experimental plot was elaborated with rows of 5.16 meters long and with a separation of 0.76 meters. Fertilization consisted of the physical mix of 250-30-00; applying 62.21 kg of diammonium phosphate and 246.23 kg of urea to cover half of nitrogen (N) and all the phosphorus (P) content. The second fertilization event was applied using 271.73 kg of urea to cover the rest of nitrogen (N). Watering occurred as natural precipitation, with one minor watering event.

Pre-emergent weed control was applied employing 4 L/hectare of Syngenta PRIMAGRAM GOLD herbicide with atrazine 33.7% and metolachlor 26% as active ingredients. For control during crop development, localized applications of 2L/hectare of Faena and Marel 480, as for one application of 1L/hectare of Gramoccil before flowering. For pest control, 20kg of Furadan 5G from FMC corporation with carbofuran as the active ingredient were utilized to protect crops from soil pests. Foliage pests were controlled by addition of 250 mL/hectare of cypermethrin.

Corn harvested was dried and shelled for seed shipment. Each sample of 100g was labeled and sent to the Biotechnology center in Monterrey. All corn samples were ground utilizing a (Krups GX 4100-11, Medford Massachusetts) grinder equipped with a 0.5HP motor. Ground corn samples were placed in 20 mL scintillation vials and stored at -20°C for use the following day.

For bulk analysis, a blue maize landrace from the State of Mexico (2200 masl) was included to compare the experimental content of the traits evaluated in the present study.

3.2 Chemicals and reagents

Folin-Ciocalteu reagent, Bradford reagent, Gallic acid, (±)-6-Hydroxy-2,5,7,7-tetramethylchromane-2-carboxylic acid (Trolox), 3,5-dinitrosalicylic acid (DNS) and 2,2'-Azobis (2-methylpropionio-amidine) were obtained from Sigma Aldrich (St. Louis, MO). Sodium hydroxide, sodium bicarbonate, sodium monobasic phosphate, sodium dibasic phosphate, potassium chloride, n-Hexane, ethyl acetate, hydrochloric acid, methanol and ethanol were obtained from Desarrollo de Especialidades Químicas S.A. De C.V (San Nicolas de los Garza, Nuevo León). Acetonitrile and HPLC-water were obtained from VWR (West Chester, PA), trifluoroacetic acid and glucose from Merck (Darmsladt, Germany) and sodium acetate from Baker (Phillipsburg, N.J.). Fluorescein from Riedel-de Haen (Seelze, Germany) and bovine serum albumin (BSA) from Thermo scientific (Waltham, MA).

3.3 Biophysical properties determination

Corn genotypes obtained were subjected to a variety of procedures in order to assess their biophysical properties. Procedures include corn kernel color by instrumental color measurements, 1000 kernel weight, percent moisture, flotation index and endosperm texture of each corn hybrid.

3.3.1 Kernel color

Kernel color data for 25 crosses across 2 contrasting regions were measured following a previously modified protocol by Salinas-Moreno (1992). Color characteristics were measured utilizing whole kernels placed evenly in a petri dish. Measurements include (Figure 3.2) parameters L (lightness), a*(red-green), and b*(blue-yellow) from the CIE (Commission Internationale de l'Eclairage) color system, utilizing a Minolta color meter (Model CR-300 series, Minolta Co., Ltd. Osaka, Japan). Chroma (saturation), hue (tone), and E values (indication of color index) were calculated using three equations: Chroma = $(a^2 + b^2)^{½}$; Hue = $\tan^{-1}$ (b/a) ; and E= $(L^2+ a^2+b^2)^{1/2}$.

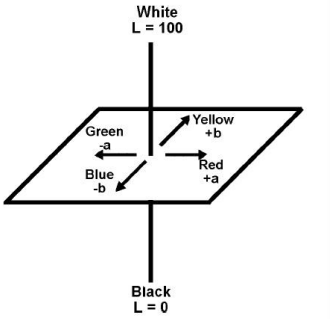


Figure 3.1 The CIE color scale utilized determining L, a* and b* values from blue corn (adapted from Lopez *et al*., 2004)

3.3.2 1000 kernel weight

Weight measurements followed a modified protocol based on Salinas-Moreno (1992). Weight was determined by randomly selecting 100 corn kernels among each of the 25 provided genotypes. Kernels were weighed in an analytical balance (Mettler Toledo AB104-S/FACT, Switzerland) and were done in triplicate.

3.3.3 Percent moisture

Method followed a protocol previously reported by Salinas-Moreno *et al.* (2006). To calculate percent moisture of each corn genotype, 3 grams previously dried and weighed were (Mettler Toledo AB104-S/FACT, Switzerland) placed in aluminum trays and

weighed. Samples were placed in a VWR oven at 90°C for 3 days. Then, samples were removed, placed in a dessicator for 20 minutes and weighed again. The change in weight was calculated and represented as a percentage. All samples were elaborated in triplicate.

3.3.4 Flotation index

Flotation index measurement followed a modified protocol based on Salinas-Moreno *et al*. (2006). Ten kernels were selected randomly by triplicate. A 55 % Sodium nitrate solution was prepared and was placed in a 300mL beaker, temperature was maintained at 35ºC. Next, 10 kernels were introduced and mixed for 30 second intervals for 5 minutes. Readings of floating kernels were taken by triplicate.

3.3.5 Endosperm texture

Endosperm texture was elaborated by a method previously described by Salinas-Moreno *et al*. (2006) and modified by our laboratory. Corn kernels were selected randomly and were dissected with a scalpel longitudinally in order to expose endosperm. Endosperm type was evaluated on a subjective scale from 1 to 5. The scale range is 1 (completely floury), 2.5 (intermediate) (floury/vitreous), and 5 (completely vitreous). The dissection was elaborated by triplicate.

3.4 Extraction and analysis methods

To facilitate germplasm characterization, a bulk analysis was necessary in order to identify if any significant differences existed between all crosses in both regions. Hence for a bulk analysis, all crosses present in region 1 were “bulked” together as were all other crosses in the second region. This mix of 25 crosses would provide the necessary information so as to dictate if any difference exists between both regions and subsequently the direction of the present study. Once a preliminary bulk analysis has been conducted, a necessary analysis of all 25 crosses was conducted to detect significant differences among all crosses.

3.4.1 Bulk analysis

The preliminary bulk analysis consisted of creating a mix of 2 corn kernels from each cross and repetitions of each region. This "mix" contained all 25 crosses and repetitions which were harvested in this particular region. Each amount of grains selected were done so in a random manner and carefully ground as described above. This procedure was repeated for both regions in triplicate.

3.4.1.1 Free phenolic acid extraction

Extractions were elaborated using the method described by De la Parra *et al*. (2006). Initially, 500 mg of ground blue maize sample were weighed. Next, 5 ml of 80% ethanol were added for free phenolic acid extraction. Samples were shaken for 10 minutes and later centrifuged (Allegra X12, Beckman Coulter Inc., Brea, CA) for 10 minutes at 2500rpm. Supernatant (≈4.5mL) was concentrated to 1mL at 35°C using a vacuum evaporator (Savant SC250 DDA Speed Vac Plus centrifugal, Holbrook, NY) and later pellet and supernatant were stored at -20°C for further analysis of free phenolics and free ferulic acid.

3.4.1.2 Bound phenolic acid extraction

Extractions were elaborated using the method described by Adom *et al*. (2002) and later modified by Gutierrez-Uribe *et al*. (2010). Initially, 500 mg pellet obtained after free phenolic acid extractions was utilized for extraction of bound phenolic acids. For hydrolysis, 10mL of 2M NaOH was added to each sample. Samples were treated for 20 seconds with nitrogen gas to displace oxygen. Samples were subjected to a hot water bath at 95°C for 30 minutes with moderate shaking every 3 minutes. Then, each sample was neutralized with 2 mL of 12M HCl and vortexed for 2 minutes. 10 mL of hexane were added; samples were shaken for 10 minutes at 70 rpm and later centrifuged at 4500 rpm (Allegra X12, Beckman Coulter Inc., Brea, CA) for 10 minutes to remove lipids. Upper phase (hexane) was discarded, then10 mL of ethyl acetate was added, shaken for 10 minutes at 70 rpm and centrifuged at 2500 g for 10 minutes. The upper phase (ethyl

acetate) was recovered and stored. Addition-recovery of ethyl acetate was repeated 5 times. Ethyl acetate accumulated ≈45mL was concentrated to dryness 35°C using a vacuum evaporator (Savant SC250 DDA Speed Vac Plus centrifugal, Holbrook, NY) and re-suspended in 2 mL of 50% methanol and stored at -20°C.

3.4.1.3. Free ferulic acid extraction

Extractions were performed as described by Gutierrez-Uribe *et al.* (2010). Free phenolic acid suspension was used for free ferulic acid analysis.

3.4.1.4. Soluble-conjugated ferulic acid extraction

Extractions were performed as described by Gutierrez-Uribe *et al.* (2010) with slight modifications. An aliquot of 0.500mL of free phenolic acid extract was hydrolysed with 0.500mL of 2M NaOH shaken for 10 minutes. Then, samples were treated with nitrogen gas until dry. For neutralization, 0.500mL of 2M HCl were added and tubes were vortexed for 30 seconds. Next, 2 mL of hexane were added, vortexed for 30 seconds and shaken for 10 minutes to remove lipids. Upper phase (hexane) was discarded, and then 2 mL of ethyl acetate were added and vortexed for 30 seconds. Upper phase (ethyl acetate) was recovered and stored. Addition of ethyl acetate was repeated 5 times. Ethyl acetate accumulated ≈9 mL was concentrated to dryness (Savant SC250 DDA Speed Vac Plus centrifugal, Holbrook, NY) and resuspended in 200µL of 50% methanol and 800 µL of HPLC grade water and later stored at -20°C.

3.4.1.5 Bound ferulic acid extraction

Extracts were obtained as described by Gutierrez-Uribe *et al*. (2010) and De la Parra *et al*. (2007) with slight modifications. Bound phenolic acid suspension was utilized for ferulic acid analysis.

3.4.1.6 Total monomeric anthocyanin content (TAC) extraction

Extractions were performed as described by De la Parra *et al.* (2007) with modifications. Initially, 100 mg of ground blue maize sample were introduced into a 1.1 mL tubes. 1 mL of 95% acidified ethanol was added for free phenolic extraction. Samples were shaken for 10 minutes and later centrifuged in a Beckman Coulter Allegra X12 centrifuge (SX4750 rotor with plate carrier) for 5 minutes at 2500RPM. Supernatant and pellet were removed and stored at -20°C for storage.

3.4.2 Compound analysis

3.4.2.1 Phenolic acid quantification

Free and bound phenolic acids were quantified by the he Folin-Ciocalteu method originally published by Singleton *et al*., (1999) and modified by Salinas-Moreno *et al*., (2006). Sample preparation consisted of 10µL of each sample and the addition of 100µL of 0.25 M Folin-Ciocalteu reagent in a 1.1mL tube. Mixture was let stand for 3 minutes and followed by addition of 100µL of 1N Sodium bicarbonate. This mixture was left to stand for 7 minutes and followed by the addition of 400µL of distilled water. The Folin reagent-Na_2CO_3-H_2O mixture was mixed thoroughly by vortexing and let to stand for 1 hour. An aliquot of 200µL of each mixture was transferred to a 96 well transparent costar plate for absorbance reading at 726nm in a microplate reader (Synergy HT multiple-detection, BioTek, Inc., Winooski, VT). Sample was compared to a previously prepared gallic acid standard curve elaborated by the same method. Phenolics were expressed as Gallic acid micrograms equivalents per 100 grams of dry sample weight.

3.4.2.2 Antioxidant capacity quantification

Antioxidant capacity was determined using the oxygen radical absorbance capacity (ORAC) method initially described by Cao *et al*. (1993) and later modified by Talcott *et al*. (2003) and Del Pozo-Insfran *et al*. (2006).

Samples were diluted in 75 mM pH 7.4 phosphate buffered saline (PBS) prior to use. A stock solution of 0.4 mM of disodic fluorescein was prepared utilizing PBS as a solvent for dissolution and as substrate for the fluorescence reaction. Next, a 153 mM radical generator is prepared utilizing 2,2'-Azobis (2-amidinopropane dihydrocloride)(AAPH) and dissolved in PBS buffer. Each well of a 96 well costar black microplate was added with 25µL of each sample and appropriate amount of previously prepared Trolox standard curve. Excitation/Fluorescence is measured at 485/538 nm at 37°C in a microplate reader (Synergy HT multiple-detection, BioTek, Inc., Winooski, VT). Data was expressed as µmole Trolox equivalents/L of sample and computed to µmole Trolox equivalents/g of sample.

3.4.2.3 Total monomeric anthocyanin analysis

Anthocyanin analysis was performed by the pH differential spectrophotometric method previously described by Wrolstad (1976). Each individual sample was diluted in pH 4.5 0.025 M sodium acetate buffer and pH 1 0.025 M KCl buffer. Absorbance was measured at wavelengths 515 for absorbance of the pigment and 700 nm for turbidity correction.

Total monomeric anthocyanins present in the original sample were calculated as follows:

Mg/L = (Absorbance*Molecular weight* Dilution factor*1000)(ε*1)

Where:

Absorbance: $A = (A_{515nm} - A_{700nm})_{pH\ 1.0} - (A_{515nm} - A_{700nm})_{pH\ 4.5}$

Cyanidin-3-glucoside molecular weight: 449.2 g/mol

Extinction coefficient (ε) = 29,000 (L/cm/mol)

3.4.2.4 Ferulic acid quantification

Ferulic acid analysis was developed using a modified method based on Gutierrez-Uribe *et al.* (2010). Extracts were analyzed using a HPLC-DAD equipped with a Zorbax SB-Aq, 4.6mm ID x 150 mm (3.5µ) reverse column. Isocratic elution of 20% (v/v) acetonitrile in water adjusted to pH 2 with trifluoroacetic acid at a flow rate of 0.5 mL/min.

Detection was set at 280 nm and integrated by HP-Agilent software (Chemstation for LC Copyright Agilent technologies, 1990-2003). Peak identification of ferulic acid in sample extracts were based on retention time of a ferulic acid standard. Twenty µL injections were used for all runs, and peak areas were integrated for all calculations.

3.4.3 Cross analysis

An amount of 10 blue corn kernels were randomly selected from each of the provided 25 crosses. The analysis of 25 crosses in both regions results in a total of 125 different corn germplasm available for this study, considering the repetitions of each region.

3.4.3.1 Free phenolic acids extraction

Extractions were elaborated as described in the bulk analysis section of the present study. Some modifications included initial sample weight of 100 mg and an extraction volume of 1 ml of 80%. Samples were centrifuged for 5 minutes at 1500rpm in a Beckman Coulter Allegra X12 centrifuge (SX4750 rotor with plate carrier).

3.4.3.2 Bound phenolic acids extraction

Extractions were elaborated as described in the bulk analysis section of the present study. Some modifications include initial sample weight of 100 mg and a hydrolysis volume of 400µL of 6M NaOH. Samples were neutralized with 200 µL of 12M HCl and vortexed for 10 seconds. 300µL of hexane were added and then samples were shaken for 10 minutes and later centrifuged at 1500 rpm in a Beckman Coulter Allegra X12 centrifuge (SX4750 rotor with plate carrier) for 5 minutes for lipid removal. 300µL of ethyl acetate were added to each sample, shaken for 10 minutes and centrifuged at 1500 rpm for 5 minutes. Addition-recovery of ethyl acetate was repeated 4 times. Ethyl acetate accumulated ≈1200µL was concentrated to dryness and resuspended in 400µL of 50% methanol and stored at -20°C.

3.4.3.3 Bound ferulic acid extraction

Extractions were elaborated as described in the bulk analysis section of the present study. Bound phenolic acid suspension was used for bound ferulic acid analysis.

3.4.3.4 Total monomeric anthocyanin content (TAC) extraction

Extractions were elaborated as described in the bulk analysis section of the present study. Modifications include: 100 mg sample and an extraction volume of 1mL of acidified 95% ethanol. Samples were shaken for 10 minutes and later centrifuged in a Beckman Coulter Allegra X12 centrifuge (SX4750 rotor with plate carrier) for 5 minutes at 2500rpm.

3.4.3.5 Soluble carbohydrates extraction

Soluble carbohydrates were extracted according to a method previously described by Harrigan *et al*., (2007). Initially, 100 mg of each sample are weighed using an analytical balance (Mettler Toledo AB104-S/FACT, Switzerland) and placed in a 1.1 ml Neptune tube. Samples were added with 500µL of 80% ethanol (80/20)(v/v), vortexed for 5 minutes and let stand at 5°C over night. The following day, samples were placed with 400µL of distilled H_2O and vortexed for 5 minutes. Samples were then subjected to a hot water bath at 80°C for 5 minutes. Samples were centrifuged in a Beckman Coulter Allegra X12 centrifuge (SX4750 rotor with plate carrier) for 5 minutes at 4000 RPM. The supernatant was recovered and stored at 4°C until use.

3.4.3.6 Albumin and globulins extraction (soluble protein)

Soluble protein is extracted according to a method previously described by Harrigan *et al*., (2007). Initially, 100 mg of each sample were weighed using an analytical balance (Mettler Toledo AB104-S/FACT, Switzerland) and placed in a 1.1 ml Neptune tube. Samples were added with 500µL of 75 mM PBS buffer at pH 7.4. Samples are vortexed for 5 minutes and let stand at 5°C during the night. The following day, samples were vortexed

for 5 minutes. Samples were centrifuged in a Beckman Coulter Allegra X12 centrifuge (SX4750 rotor with plate carrier) for 5 minutes at 4000 RPM. Supernatant was recovered and stored at 4°C until use.

3.4.4 Compound analysis

3.4.4.1 Phenolic acid quantification

Free and bound phenolic acids were quantified by the method described in the bulk analysis section of the present study.

3.4.4.2 Antioxidant capacity quantification

Antioxidant capacity was determined using the oxygen radical absorbance capacity (ORAC) method described in the bulk analysis section of the present study.

3.4.4.3 Total monomeric anthocyanin analysis

Method utilized for quantification of total monomeric anthocyanins is described in the bulk analysis section of the present study.

3.4.4.4 Soluble carbohydrate analysis

Soluble carbohydrates were quantified based on the method previously described by Davila (2007). A volume of 250µL of each sample was dissolved in 750µL of 3,5-dinitrosalicylic acid (DNS). An appropriate amount of each mixture was measured at 540 nm in a microplate reader (Synergy HT multiple-detection, BioTek, Inc., Winooski, VT) and compared to a glucose standard curve.

3.4.4.5 Albumin and globulins quantification (soluble protein)

Soluble protein analysis was elaborated with modification according to the method proposed by Bradford (1976). An amount of 10 µL of soluble protein (albumin) suspended in PBS buffer was mixed with 200 µL of Bradford reagent and placed in a 96 well microplate by duplicate. The solution was measured at 595 nm in a microplate spectrophotometer (Synergy HT multiple-detection, BioTek, Inc., Winooski, VT) and compared to a bovine serum albumin (BSA) standard curve.

3.4.4.6 Ferulic acid quantification

Ferulic acid analysis was elaborated using the method previously discussed in the bulk section of this study.

3.5 Statistical analysis

The data were subjected to analysis of variance to determine differences among samples followed by Tukey's test and LSD using Statistix v.7 (Analytical Software, Tallahassee, FL). ANOVA calculations and Pearson correlation values were analyzed utilizing JMP 5.0 statistical software (SAS institute inc., Cary, NC, USA). A $p < 0.05$ was considered significant. Broad-sense heritability (h^2_B) for the traits was estimated using the variance components method according to Hallauer *et al*., (1991). Broad heritability measures the proportion of phenotypic variance that is attributable to an effect for the whole genotype, comprising the sum additive, dominance, and epistatic effects. Means were calculated by 3 determinations ± standard error.

Chapter IV: Results and discussion

4.1 Bulk analysis

4.1.1 Phenolic acid quantification

Results obtained from the phenolic acid quantification in bulk analysis are shown in **Table 4.1**. The Bajío and Morelia regions together with the landraces were analyzed for free and bound phenolic acid through the proposed bulk method. The Morelia region contains an amount of free phenolic acid within 67.00 and 62.06 mg GAE/100g for the Bajío region. A maize landrace from the State of Mexico (2200 masl) was included to compare the range of the concentrations. The Landrace showed a concentration of 77.91 mg GAE/100g free phenolic acid. Free phenolic acid concentrations previously reported by De la Parra and colleagues (2007) indicated that the white (34.70 mg GAE/100g) and blue (45.50 mg GAE/100g) corn genotypes contained lower phenolic concentrations when compared with the blue genotypes analyzed in the present study.

Bound phenolic acids present in the grain account for the majority of the total amount (**Table 4.1**). The materials harvested in the Bajío region contained 206.71 mg GAE/100g for bound phenolics whereas the counterparts planted in the Morelia region showed a concentration of 177.58 mg GAE/ 100g. Interestingly, the landrace presented a concentration of 264.11 mg GAE/100g. Studies have reported phenolic composition in a variety of cereal grains (Liu *et al*., 2002; Sakakibara *et al*., 2007) especially wheat, barley and corn. These studies demonstrated that most of the phenolic content in corn is attached to the cell wall. The results obtained from the bulk analysis, suggest that >80% of total phenolics in fact occurred in the bound form.

As was proposed, bulk analyses were developed in order to distinguish if in fact there is a variation between regions. No significant difference was detected among both regions and landrace, suggesting the corn genotypes are stable.

Table 4.1 Phenolic acid content from bulk analysis of 25 elite hybrid crosses from blue maize grown in two environments and landrace control

Type of corn	Free	Bound	Total
	mg GAE/100g		
Bajío	62.06 ± 4.00 a	206.71 ± 26.50 a	268.77
Morelia	67.00 ± 6.12 a	177.58 ± 20.41 a	244.58
Landrace	77.91 ± 8.52 a	264.11 ± 39.13 a	342.08

*Values within columns with no letters in common are significantly different ($p<0.05$)

4.1.2 Antioxidant capacity quantification

Antioxidant capacity values measured for free phenolic compounds and bound phenolic compounds are shown on **Table 4.2**. Previous reports (De la Parra *et al*., 2007; Del Pozo-Insfran *et al*., 2007; Adom *et al*., 2002) conclude that bound phenolic acids are the primary contributors to antioxidant activity; the results in the present study are consistent with those observations. The bulk analysis study shows that materials planted at the Bajío region showed antioxidant capacity from free phenolic acids of 756.57 µM TE/100g whereas those of the Morelia region had 618.29 µM TE/100g. These showed lower ORAC compared to Landrace (829.25 µM TE/100g). The bulk from Morelia region showed an antioxidant capacity for bound phenolic acid of 2701.49 µM TE/100g when compared to 2687.67 µM TE/100g from the Bajío region and 2631.66 µM TE/100g from the landrace control.

Table 4.2 Phenolic acid antioxidant capacity from bulk analysis of 25 elite hybrid crosses from blue maize grown in two environments and landrace control

Type of corn	Free	Bound
	µM TE/100g	
Bajío	756.57 ± 64.21 a	2687.67 ± 92.80 a
Morelia	618.29 ± 69.67 a	2701.49 ± 145.25 a
Landrace	829.25 ± 62.68 a	2631.66 ± 133.28 a

*Values within columns with no letters in common are significantly different ($p<0.05$)

The phytochemical differences uncovered from the bulk analysis and reflected by the variation in the standard deviation, permit the next step in the analysis of the germplasm received. As observed, no variation was detected among both environment from the

phenolic acid fractions content and their respective antioxidant capacity values. Bulk analysis studies were key to elucidate differences that permitted the following analysis of the 25 genotypes in both contrasting regions in order to properly determine if environment plays a role among the genotypes.

4.2.3 Ferulic acid quantification

Results obtained from bulk analysis of ferulic acid (FA) content in blue hybrid corn are shown on **Table 4.3**. The first column shows a slight difference between free ferulic acid content between bulk analysis for the Bajío and Morelia regions compared to the landrace corn. The materials harvested from the Bajío region contained 836.83 µg of free FA/100g while those of the Morelia region 862.45 µg of ferulic acid/100g. Landrace shows a concentration of 1247.60 µg of ferulic acid/100g which indicates a significant difference when compared to Bajío and Morelia. Suggesting free ferulic acid content may be affected by altitude differences among the evaluated corns, where Landrace was harvested at 2,200 masl, whereas Bajío and Morelia at 1,750 and 1,950 masl, respectively. Also, types of corn showed variability among free, soluble conjugated and bound FA content and may be attributed to altitude differences.

The second column depicts concentrations obtained from soluble conjugated extraction of ferulic acid with the Bajío region having a concentration of 893.40 µg of FA/100g and the Morelia region with 842.22 µg of FA/100g, while landrace presented the a concentration of 794.70 µg of FA/100g. The adjacent column shows a bound ferulic acid content of 122,091.33 and 119,848.33 µg of FA/100g for the Bajío and Morelia regions, respectively. A landrace control sample exhibits content of 146,990.00 µg of FA/100g. Results indicate that ferulic acid is mainly present in bound form (Sakakibara *et al*., 2003) as previously observed.

Table 4.3 Ferulic acid content from bulk analysis of 25 elite hybrid crosses from blue maize grown in two environments and landrace control

Type of corn	Free	Soluble conjugated	Bound
	μg of ferulic acid/100g		
Bajío	836.83 ± 91.24 b	893.40 ± 195.21 a	122091.33 ± 25407.15 a
Morelia	862.45 ± 5.25 b	842.22 ± 116.28 a	119848.33 ± 8543.52 a
Landrace	1247.60 ± 43.92 a	794.70 ± 80.47 a	146990.00 ± 22143.27 a

*Values within columns with no letters in common are significantly different ($p<0.05$)

Ferulic acid concentrations obtained from the bulk analysis are similar to those recovered from De la Parra and colleagues (2006) where free ferulic acid was 683 μg of FA/100g. The same investigators showed soluble conjugated content of 1451 μg of FA/100g, and bound phenolic acids of 127,851 μg of FA/100g. Results obtained from the bulk analysis of all 25 genotypes, show no significant differences among ferulic acid content, suggesting that a full analysis of all 25 genotypes should be evaluated.

4.2.4 Total monomeric anthocyanin quantification

Bulk analysis showed that materials harvested from the Bajío region contained an average concentration of monomeric anthocyanins of 304.60 mg cyanidin-3-glucoside equivalents/kg of monomeric anthocyanins equivalent to cyanidin 3-glucoside (**Table 4.4**). The Morelia region contained 300.00 mg/kg and the landrace control previously utilized by our lab a concentration of 311.03 mg/kg. These concentrations compare with those obtained by Del Pozo-Insfran (2006) where Mexican and American corn genotypes contained 321 and 307 mg/kg, respectively. No significant differences were observed among environments and suggests that this is not a factor associated to anthocyanin content. While this may be true for the bulk analysis, evaluation of all 25 genotypes harvested from both environments must be determined. Particularly considering that the observed variability among Bajío and Morelia regions was different with the Morelia region showing close to double that of the Bajío and double that of the landrace control.

Table 4.4 Monomeric anthocyanin content from bulk analysis of 25 elite hybrid crosses from blue maize grown in two environments and landrace control

Type of corn	Monomeric anthocyanins
	mg of cyanidin-3-glucoside equivalents/ kg
Bajío	304.60 ± 8.21 a
Morelia	300.00 ± 12.19 a
Landrace	311.03 ± 6.07 a

*Values within columns with no letters in common are significantly different (P<0.05)

4.2 Biophysical properties

4.2.1 Kernel color

Measurements obtained for color a*, b* and L properties for the 25 blue corn genotypes across both regions are presented in **Tables 4.5, 4.6 and 4.7**. As can be observed there is a significant difference between genotypes and environments (**Table 4.5)** for a* values. Positive values indicate red color in the CIE spectrum (**Figure 4.1**). A higher positive value indicates a more intense red color when compared to a number closer to 0. The value does not necessarily mean that the sample is red or green, it only means that it has a red or green shading. The mean for each region was 6.89 for Bajío and 5.82 for Morelia; this suggested that the Bajío region showed a higher red a* value. **Figure 4.1** shows a color representation of approximate area in the color gamut at L=40 for mean a* values. The genotypes in the Bajío region with the highest a* values are 24 (356 x 152), 5 (124 x 84) and 19 (292 x 160) with 22.25, 12.8 and 12.41 respectively. The Morelia region showed high mean a* values for genotypes 5 (121 x 84), 24 (356 x 152) and 9 (160 x 87) with 18.43, 18.26 and 16.04, respectively. Del Pozo-Insfran and colleagues (2007) showed a* values for white and blue corn tortillas of -1.8 and 8.6, respectively. Salinas-Moreno *et al.* (2003) showed a* values of 1.68 and 3.56 for blue and red flour. The lower values obtained within each environment were genotypes 22 (324 x 190), 23 (352 x 38), 10 (171 x 131) and 8 (144 x 124) for the Bajío region with 0.46, 1.85, 1.82 and 1.81 a* values. As for the Morelia region, genotypes 22 (324 x 190), 25 (364 x 224), 3 (63 x 26) and 4 (87 x 160) showed a* values of 0.06, 1.21, 2.24 and 2.82, respectively. The results indicated that genotype 24 (356 x 152) has an intense red color, and was indeed corroborated as it showed the highest a* value indicating redness. Analysis of variance showed a* values are

significantly affected by the environment ($p<0.0093$) and thus explained the difference in mean for both environments. Suggesting red to green shading is affected by environmental factors like temperature and UV light. Analysis of variance for genotypic effects ($p<0.0001$) suggested differences among genotypes, hence the variability among all genotypes in both environments. This is also evident in the genotype-environment interaction ($p<0.0001$) where significant differences exist from the a* values exhibited and how the corns react to environmental stresses. Heritability values (0.8138) on **Table 4.5** indicate a high proportion of the phenotypic variance of this particular trait is due to genotype-environment effects as observed in **Table 4.20**. The same cannot be said for heritability of b* values (0.7429) which is lower in part due to having no significant differences among environments.

As discussed above, determination of b* values for colorimetric analysis showed there is no significant difference between environments ($p<0.3893$). In contrast, genotypic differences ($p<0.0001$) were observed among the materials harvested from both regions as were the genotype-environment interactions ($p<0.0114$), indicating that genotype plays a determining role in b* value, more than what environment alone does. As for CIE spectrum values, negative b* values indicate blue and positive b* values a yellow color. A value closer to 0 would suggest a less pure blue color while a more negative b* value shows a more defined blue color. Again, these values do not necessarily mean that a sample is yellow or blue in color. It simply means that the sample has yellow or blue shading. As lower values are associated with blue color, the lowest b* are from genotypes 7 (136 x 197), 17 (254 x 315), 3 (63 x 26) and 4 (87 x 160) from the Bajío region with 1.73, 3.03, 3.44 and 3.44 respectively (**Table 4.6**). The Morelia region showed the lowest b* value in genotypes 19 (292 x 160), 13 (222 x 323), 14 (225 x 242) and 17 (254 x 315) with 1.08, 2.71, 3.10 and 3.33 respectively. **Figure 4.1** indicates a color representation of approximate area in the color gamut at L=40 for mean b* values. Genotypes 3 (63 x 26), 4 (87 x 160), 5 (121 x 84), 7 (136 x 197) and 19 (292 x 160) showed differences among both regions and thus present different b* values in each region. Genotypes 3 (63 x 26) and 4 (87 x 160) indicate lower b* values in the Bajío region as does genotype 7 (136 x 197), which is 7x greater in the contrasting region. While genotypes 5 (121 x 84) and 19 (292 x 160) show lower b *values in the Morelia region. The lower value observed in each region must

be affected by genotype-environment interactions ($p<0.0114$) that influence a more defined blue color. Thus, suggesting that genotype-environment interactions are specific for every distinct genotype. Previous studies by Del Pozo-Insfran (2007) showed b* values of 18 for white tortillas, and -0.2 for Mexican blue tortillas and 0.9 for American blue tortillas. Salinas-Moreno and colleagues (2003) showed b* values of 1.52 from blue corn flour and 4.78 for red corn flour. Differences in b* values for genotypes 3 (63 x 26), 4 (87 x 160), 7 (136 x 197) and 19 (292 x 160) among both environments showed some of the genotypes react differently to their environment.

The lightness of a color sample is represented by the "L" value (**Table 4.7**), it is based on the percent of light reflectance. If the L value is zero, the color sample is black, if the value is 100, the sample is white. The mean L values were 39.27 for Bajío and 41.50 for Morelia. As can be observed on **Figure 4.1**, HUE color representation based on an L=40 shows an approximation of the color the samples exhibit based on the mean of a* and b* values. The range of genotype mean for both environments were genotypes 1 (23 x 53) and 24 (356 x 152) with 53.9 and 30.6 L values, respectively. Indicating that genotype 1 (23 x 53) had a brighter lightness than genotype 24 (356 x 152). As for means for each region, the Morelia region shows a larger L value, indicating brighter lightness. Studies by Del Pozo-Insfran and colleagues (2007) showed L values of 67.3 and 40 for white and blue tortillas respectively. Salinas-Moreno *et al*. (2003) showed lightness values of 64.31 and 62.08 for blue and red corn flour respectively.

Analysis of variance for L values indicated environment ($p<0.0273$) plays a critical role in determining lightness as does genotype ($p<0.0001$). This is reflected by the significant influence that the G x E interaction has on lightness values for the corns ($p<0.0001$). However, L values may be impacted by genotype and interaction, but this color parameter showed a low heritability (0.2106). Suggesting that lightness characteristics in the kernel are not carried over to future generations as readily as a* and b*.

The color parameter E (**Table 4.7**) is an indication of color index, which showed a significant difference between environment ($p<0.0487$) and genotype ($p<0.0007$). Reports showed E values of 69.7 and 40.4 for white and blue tortillas (Del Pozo-Insfran *et al*., 2007). These results are comparable to the mean across both regions of 40.83 and 42.87 for Bajío and Morelia, where a larger E value indicates a brighter tone. Results indicated G x E

interactions (p<.0002) are determinants for the index magnitude. It is however, a low heritability value (0.0770) which indicates that this particular trait may not be passed on to future generations. This phenotypic trait may be determined by genotypic and environmental factors, but is not readily passed on which may indicate that variation is mainly due to environmental factors and not as much from genotypic processes.

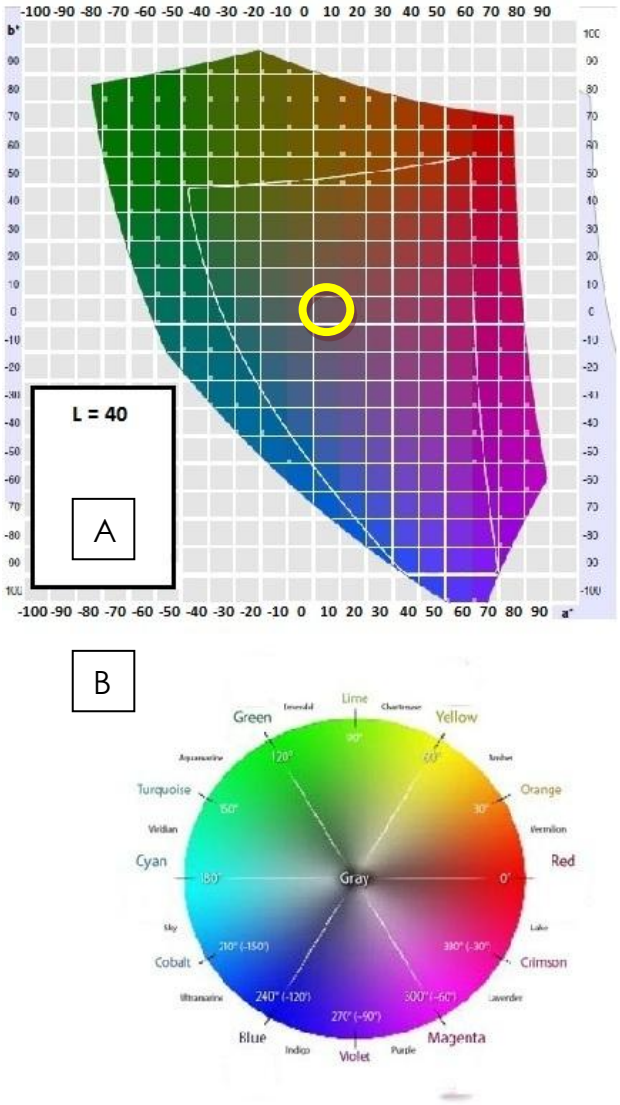


Figure 4.1 (a) Color gamut at L=40 exhibiting (yellow circle) approximate location of mean a* and b* values for 25 blue corn hybrids (b) color wheel representing HUE in degrees (Adapted from Wyszecki *et al*., 1982).

HUE is the calculation of color based on a* and b* values (**Table 4.8**). HUE is measured in degrees, exhibiting 1º as red color, 45° a yellow color, 90 º lime color, 180° cyan, 240° blue, 300° magenta and 360° red color (**Figure 4.1**). The HUE mean for all genotypes in the Bajío region was 43.3° and 47.1° for the Morelia region. The range for the Bajío region was 18.7° for genotypes 14 (225 x 242) and 85.2° for genotype 22 (324 x 190). The Morelia region showed a HUE range of 8.4° for genotype 19 (290 x 160) to 80.9 for genotype 22 (324 x 190). Based on HUE color wheel expressed in degrees, a HUE value of 70.8 ° (mean for genotype 1) would showed a color between a red and yellow shade. Del Pozo-Insfran (2007) showed similar results for American and Mexican blue tortillas with hue values of 5.9º and 357.6° respectively. Additionally, Cevallos-Casals and colleagues (2004) showed HUE value of 20 for a purple corn. Analysis of variance (**Table 4.20**) shows this trait did not exhibit a significant difference between environments ($p<0.1150$), suggesting HUE is only affected by genotypic variation ($p<0.0001$) and G x E interactions ($p<0.0017$). Stability among environments may have contributed to a low heritability (0.6571), which may be causing phenotypic effects to not be passed on to future generations as readily.

The chroma (saturation) indicates de intensity or purity of a color (**Table 4.8**). Colors with high chroma are the most brilliant, and colors with low chroma are dull. The mean for all the genotypes in the Bajío region was 10.9 which was slightly higher than 10.2 for the Morelia region. The chroma range for the Bajío region was 7.3 for genotype 8 (144 x 124) and 17.3 for genotype 23 (352 x 38). The Morelia region showed a range of 4.1 from genotype 25 (364 x 224) and 19 for genotype 21 (293 x 227). Del Pozo-Insfran (2007) showed similar results for Mexican and American blue tortillas with chroma values of 6.2-8.8. ANOVA results showed that environment effects do not cause a change in chroma values ($p<0.3162$) and thus chroma may be caused by genotypic variation as is demonstrated ($p<0.0001$). The G x E interaction ($p<0.0006$) suggests environment may not be acting directly upon chroma response, but the interaction of the environment with genotypic expression does. This parameter (chroma) is used to determine the amount of color saturation in the maize kernel and it has recently been shown that a positive correlation between chroma and anthocyanins exists (Yang *et al*., 2009). Results showed a

high heritability (0.8528) which may contribute to successfully transmitting chroma effects to future generations. A high heritability value fortifies the hypothesis that chroma values may be correlated to anthocyanin content as has been previously discussed. Such correlation may lead to selection of blue corn on the basis of colorimetric analysis by chroma values and indicating anthocyanin content.

Table 4.5 Colorimetric value a* for 25 elite hybrid crosses from blue maize grown in two environments

	a*					
Genotype	Bajío		Morelia			Genotype mean
1	1.97 ± 0.33	jkl	3.00 ± 0.43	ijkl		2.52
2	2.05 ± 0.35	jkl	2.84 ± 0.98	ijkl		2.46
3	7.57 ± 1.37	defghijk	2.24 ± 0.62	ijkl		4.96
4	8.26 ± 1.54	cdefghijk	2.82 ± 0.74	ijkl		5.55
5	12.80 ± 1.50	bcde	18.43 ± 0.89	ab		15.67
6	2.77 ± 0.68	ijkl	3.83 ± 0.60	ghijkl		3.32
7	3.93 ± 0.69	ijkl	3.44 ± 0.50	hijkl		3.70
8	1.81 ± 0.44	kl	2.87 ± 1.11	ijkl		2.31
9	7.21 ± 1.12	defghijkl	16.04 ± 0.58	abc		11.67
10	1.82 ± 0.47	kl	2.77 ± 1.09	ijkl		2.32
11	9.51 ± 1.70	cdefghi	14.66 ± 1.63	abcd		12.10
12	5.45 ± 2.10	fghijkl	4.03 ± 1.29	ghijkl		4.75
13	3.58 ± 0.77	hijkl	3.61 ± 0.96	hijkl		3.68
14	11.33 ± 1.23	bcdefgh	4.91 ± 1.97	efghijkl		8.10
15	11.94 ± 2.34	bcdefg	4.64 ± 0.97	efghijkl		8.36
16	9.17 ± 2.54	cdefghij	3.63 ± 0.27	ghijkl		6.48
17	7.51 ± 1.44	defghijk	4.19 ± 1.27	ghijkl		5.80
18	5.97 ± 1.68	efghijkl	6.33 ± 2.13	defghijkl		6.14
19	12.41 ± 1.40	bcdef	7.81 ± 0.55	cdefghijkl		10.10
20	8.90 ± 1.69	cdefghijk	7.11 ± 0.99	cdefghijkl		8.07
21	5.21 ± 2.12	fghijkl	3.23 ± 0.87	hijkl		4.20
22	0.46 ± 0.13	l	1.06 ± 0.20	jkl		0.88
23	1.85 ± 0.54	kl	2.67 ± 0.25	ijkl		2.30
24	22.25 ± 2.57	a	18.26 ± 0.65	ab		20.36
25	6.59 ± 1.66	defghijkl	1.21 ± 0.47	jkl		3.90
Mean	6.89		5.82			
LSD						1.55
Heritability						0.8138

*Values within columns with no letters in common are significantly different ($p<0.05$)

Table 4.6 Colorimetric value b* for 25 elite hybrid crosses from blue maize grown in two environments

	b*						
Genotype	Bajío			Morelia			Genotype mean
1	12.70	± 2.26	abc	14.12	± 1.86	ab	13.41
2	7.66	± 0.73	abcdef	6.91	± 1.24	abcdef	7.33
3	3.44	± 1.51	def	8.16	± 1.96	abcdef	5.88
4	3.44	± 0.43	def	7.85	± 2.17	abcdef	5.61
5	8.75	± 0.81	abcdef	4.41	± 0.27	def	6.64
6	5.35	± 1.62	def	4.88	± 2.07	def	5.17
7	1.73	± 0.15	f	7.12	± 3.05	abcdef	4.45
8	3.36	± 0.62	def	4.36	± 0.96	def	3.92
9	3.99	± 0.91	def	3.14	± 0.55	def	3.64
10	3.42	± 0.48	def	5.53	± 0.66	bcdef	4.52
11	5.82	± 0.65	cdef	7.17	± 2.02	abcdef	6.50
12	4.14	± 0.88	def	6.25	± 0.68	abcdef	5.20
13	3.60	± 1.10	def	2.71	± 1.25	ef	3.23
14	3.88	± 0.57	def	3.10	± 0.89	def	3.59
15	3.88	± 0.66	def	4.22	± 0.46	def	4.03
16	6.23	± 2.48	bcdef	9.82	± 4.75	abcde	8.04
17	3.03	± 0.96	def	3.33	± 1.35	def	3.28
18	6.12	± 1.43	cdef	5.38	± 1.29	cdef	5.85
19	7.09	± 1.36	abcdef	1.08	± 0.31	f	4.12
20	3.51	± 0.72	def	3.19	± 0.42	def	3.31
21	4.52	± 0.96	def	2.34	± 0.59	def	3.47
22	5.92	± 0.41	cdef	6.90	± 1.30	abcdef	6.41
23	5.02	± 0.73	def	5.84	± 1.62	bcdef	5.47
24	14.15	± 1.65	a	10.97	± 0.93	abcd	12.61
25	6.48	± 0.56	bcdef	7.11	± 1.34	abcdef	6.86
Mean	5.49			5.83			
LSD							1.52
Heritability							0.7429

*Values within columns with no letters in common are significantly different ($p<0.05$)

Table 4.7 Colorimetric L and E values for 25 elite hybrid crosses from blue maize grown in two environments

	L			E value		
Genotype	Bajío	Morelia	Genotype mean	Bajío	Morelia	Genotype mean
1	49.21 ± 4.77 ab	58.57 ± 2.06 a	53.96	50.94 ± 5.14 ab	60.39 ± 2.34 a	55.79
2	47.41 ± 4.59 ab	43.59 ± 6.94 ab	45.55	48.09 ± 4.61 abc	44.32 ± 6.90 abc	46.26
3	36.39 ± 4.76 b	46.46 ± 4.35 ab	41.42	37.54 ± 4.86 bc	47.31 ± 4.52 abc	42.44
4	37.55 ± 0.88 b	50.07 ± 7.00 ab	43.81	38.73 ± 1.12 cb	50.85 ± 7.15 abc	44.81
5	45.18 ± 2.99 ab	35.41 ± 1.86 b	40.34	48.01 ± 2.68 abc	40.23 ± 1.56 abc	44.12
6	37.72 ± 5.05 b	42.48 ± 3.32 ab	40.17	38.27 ± 5.24 bc	43.04 ± 3.53 abc	40.70
7	33.60 ± 1.95 b	48.15 ± 8.42 ab	40.90	33.93 ± 1.89 bc	48.94 ± 8.70 abc	41.41
8	31.95 ± 1.90 b	41.31 ± 1.30 ab	36.60	32.21 ± 1.94 c	41.72 ± 1.30 abc	37.07
9	43.86 ± 2.33 ab	36.02 ± 1.20 b	39.91	44.77 ± 2.23 abc	39.57 ± 1.36 abc	42.28
10	45.02 ± 2.58 ab	46.54 ± 1.59 ab	45.84	45.20 ± 2.61 abc	47.00 ± 1.59 abc	46.12
11	39.36 ± 3.05 ab	40.67 ± 6.11 ab	40.07	41.19 ± 2.85 abc	43.99 ± 6.25 abc	42.64
12	35.71 ± 3.31 b	47.69 ± 3.01 ab	41.72	36.69 ± 3.38 bc	48.35 ± 2.93 abc	42.57
13	39.60 ± 3.30 ab	40.14 ± 0.68 ab	39.93	40.04 ± 3.29 abc	40.48 ± 0.71 abc	40.33
14	37.83 ± 3.29 b	36.43 ± 3.07 ab	37.17	39.81 ± 3.24 abc	36.99 ± 3.39 bc	38.46
15	36.71 ± 1.50 b	40.58 ± 3.88 ab	38.69	39.09 ± 1.87 bc	41.08 ± 3.95 abc	40.18
16	32.37 ± 1.00 b	43.26 ± 4.73 ab	37.82	34.91 ± 1.99 bc	44.97 ± 5.59 abc	39.91
17	43.23 ± 4.03 ab	39.90 ± 2.80 ab	41.64	44.15 ± 4.02 abc	40.38 ± 2.81 abc	42.30
18	44.14 ± 4.78 ab	37.64 ± 3.32 ab	40.95	45.32 ± 4.60 abc	38.68 ± 3.71 abc	42.07
19	48.24 ± 3.58 ab	38.25 ± 4.77 ab	43.26	50.46 ± 3.67 abc	39.13 ± 4.59 abc	44.85
20	37.27 ± 1.54 b	35.08 ± 2.82 b	36.25	38.72 ± 1.44 bc	35.95 ± 2.95 bc	37.32
21	39.36 ± 2.12 ab	31.63 ± 2.76 b	35.55	40.35 ± 1.90 abc	31.93 ± 2.74 bc	36.13
22	36.28 ± 3.32 b	45.92 ± 3.69 ab	41.12	36.78 ± 3.31 bc	46.47 ± 3.84 abc	41.64
23	37.03 ± 2.35 b	38.64 ± 2.78 ab	37.81	37.45 ± 2.40 bc	39.24 ± 2.94 abc	38.31
24	31.87 ± 1.50 b	29.39 ± 1.63 b	30.60	41.59 ± 2.80 abc	36.32 ± 1.82 bc	39.07
25	34.92 ± 1.50 b	43.79 ± 2.40 ab	39.47	36.41 ± 1.07 bc	44.43 ± 2.50 abc	40.42
Mean	39.27	41.50		40.83	42.87	
LSD			3.88			3.99
Heritability			0.2106			0.0770

*Values within columns with no letters in common are significantly different ($p<0.05$)

Table 4.8 Hue and chroma values of 25 elite hybrid crosses from blue maize grown in two environments

Genotype	HUE°			Chroma		
	Region			Region		
	Bajío	Morelia	Genotype mean	Bajío	Morelia	Genotype mean
1	79.4 ± 2.9	77.9 ± 0.9	78.7	12.9 ± 2.2	14.4 ± 1.9	13.7
2	73.9 ± 3.8	67.8 ± 8.6	70.8	11.1 ± 1.4	12.2 ± 2.0	11.7
3	19.5 ± 6.9	70.4 ± 7.9	44.9	13.1 ± 1.5	8.7 ± 0.7	10.9
4	24.5 ± 2.5	64.1 ± 13.0	44.3	12.9 ± 1.6	7.1 ± 0.4	10.0
5	35.4 ± 5.0	13.4 ± 0.5	24.4	10.7 ± 1.6	7.2 ± 0.5	9.0
6	62.2 ± 6.7	46.3 ± 11.3	54.2	8.0 ± 0.6	7.7 ± 1.2	7.9
7	26.4 ± 3.8	52.7 ± 14.7	39.6	7.4 ± 0.3	10.2 ± 2.2	8.8
8	59.4 ± 6.9	55.5 ± 15.0	57.5	7.3 ± 0.8	8.5 ± 2.7	7.9
9	30.1 ± 7.9	11.0 ± 1.7	20.5	9.4 ± 1.5	6.5 ± 1.5	8.0
10	62.4 ± 5.6	66.2 ± 8.3	64.3	8.2 ± 1.9	7.5 ± 1.0	7.8
11	34.6 ± 6.8	25.4 ± 6.4	30.0	8.6 ± 1.8	8.7 ± 1.7	8.7
12	46.6 ± 12.4	58.1 ± 10.6	52.4	6.4 ± 0.9	10.4 ± 1.3	8.4
13	41.6 ± 12.5	35.3 ± 11.8	38.5	8.7 ± 1.1	7.2 ± 1.6	7.9
14	18.7 ± 2.0	34.4 ± 4.4	26.5	8.9 ± 1.2	8.7 ± 2.3	8.8
15	21.1 ± 4.5	43.6 ± 5.6	32.4	8.5 ± 1.4	9.4 ± 1.9	9.0
16	30.2 ± 4.8	51.4 ± 16.5	40.8	9.0 ± 1.6	8.8 ± 1.6	8.9
17	25.7 ± 8.9	38.9 ± 17.3	32.3	7.9 ± 1.6	13.7 ± 1.5	10.8
18	47.3 ± 11.2	43.0 ± 7.2	45.1	10.1 ± 1.3	16.7 ± 1.4	13.4
19	29.4 ± 5.0	8.4 ± 2.9	18.9	11.2 ± 1.8	15.7 ± 1.6	13.4
20	23.8 ± 4.9	24.6 ± 2.2	24.2	14.9 ± 1.4	16.4 ± 1.4	15.6
21	53.1 ± 12.6	38.3 ± 13.5	45.7	15.8 ± 1.1	19.0 ± 0.9	17.4
22	85.2 ± 1.6	80.9 ± 2.1	83.1	16.7 ± 1.2	14.6 ± 3.2	15.6
23	70.5 ± 5.6	60.7 ± 8.4	65.6	17.3 ± 1.2	7.9 ± 1.9	12.6
24	33.1 ± 3.0	30.9 ± 1.4	32.0	16.0 ± 2.2	4.3 ± 0.9	10.1
25	47.7 ± 5.8	78.2 ± 5.2	63.0	11.7 ± 2.7	4.1 ± 0.7	7.9
Mean	43.3	47.1		10.9	10.2	
LSD			10.70			2.30
Heritabiity			0.6571			0.8528

*Values within columns with no letters in common are significantly different ($p<0.05$)

4.2.2 1000 kernel weight

Values for 1000 kernel weigh recovered from 25 genotypes across both contrasting regions are presented (**Table 4.9**) ordered by genotype. As can be observed, genotype

1(23x53) from the Bajío region and genotype 13 (222 x 323) from Morelia region show the highest kernel weight of 434.2g and 437.1g respectively. Mean weight by genotype indicated that crosses 13 (222 x 323) and 2 (33 x 58) were the heaviest with 429.6 and 420.8 g/1000 kernels respectively. Materials harvested from the Bajío region had a total mean weight of 356.4g and Morelia region has a total mean weight of 364.7g. Antonio-Miguel and colleagues (2004) showed a range of 1000 kernel weight of chalqueño race blue corn of 385g to 463g and Del Pozo-Insfran and colleagues (2007) also obtained weights for an American blue corn harvested from New Mexico with 320g and a Mexican blue corn harvested from Toluca with 382g.

As proposed by Serna-Saldívar *et al*. (2001), a large kernel, having a larger surface compared to a smaller corn kernel, does not necessarily have a higher concentration of anthocyanins. Rather, it is proposed that the relationship is the inverse, smaller corn kernel means higher anthocyanin concentration. In accordance with these stipulations, the lightest kernels were those from genotype 23 (352 x 38), 22 (324 x 190) and 8 (144 x 124) from the Bajío region and genotypes 22 (324 x 190) and 15 (242 x 225) from the Morelia region. Total monomeric anthocyanin content (*Section: 4.3.5 Total monomeric anthocyanin quantification)* shows anthocyanin content for the above mentioned genotypes. From the Bajío region, genotypes 23 (352 x 38), 22 (324 x 190) and 8 (144 x 124) showed anthocyanin content of 504.08, 767.70 and 422.15 mg equivalents to cianidin-3-glucoside/kg, respectively. Genotypes 22 (324 x 190) and 15 (242 x 225) from the Morelia region showed anthocyanin content of 788.19 and 414.13 mg/kg, respectively. No significant correlation was found between anthocyanin content and 1000 kernel weight (0.0401), however, genotype 22 (324 x 190) was one of the lightest genotypes and contained high anthocyanin content.

4.2.3 Percent moisture

The corn kernel moistures are depicted in **Table 4.9**. There was no significant difference between the grain moisture within the genotype and both environments. Mean moisture in the Bajío and Morelia regions were 11.2 and 11.3%, respectively. As both

environments are subtropical and roughly differ in 150 meters in altitude, no difference in moisture was expected.

Table 4.9 Biophysical properties of 25 elite hybrid crosses from blue maize grown in two environments

Genotype	1000 kernel weight (g)			% Moisture		
	Region			Region		
	Bajío	Morelia	Genotype mean	Bajío	Morelia	Genotype mean
1	434.2 ± 16.1	364.7 ± 41.1	399.4	11.2 ± 0.0	11.3 ± 0.0	11.2
2	427.6 ± 15.5	414.1 ± 6.1	420.8	11.2 ± 0.0	11.3 ± 0.0	11.2
3	401.1 ± 29.2	413.3 ± 4.4	407.2	11.1 ± 0.0	11.2 ± 0.0	11.1
4	357.0 ± 15.4	318.5 ± 10.2	337.8	11.1 ± 0.0	11.2 ± 0.0	11.2
5	372.5 ± 9.2	379.5 ± 1.5	376.0	11.2 ± 0.0	11.2 ± 0.0	11.2
6	348.1 ± 19.1	359.0 ± 10.8	353.6	11.3 ± 0.1	11.2 ± 0.1	11.3
7	306.2 ± 25.8	356.7 ± 27.3	331.5	11.2 ± 0.0	11.2 ± 0.0	11.2
8	301.0 ± 31.1	343.3 ± 28.5	322.2	11.4 ± 0.0	11.2 ± 0.0	11.3
9	336.8 ± 15.8	330.8 ± 262	333.8	11.2 ± 0.0	11.2 ± 0.0	11.2
10	334.9 ± 8.1	326.5 ± 14.2	330.7	11.2 ± 0.0	11.2 ± 0.0	11.2
11	384.1 ± 18.4	360.9 ± 12.5	372.5	11.0 ± 0.0	11.2 ± 0.1	11.1
12	337.0 ± 12.5	362.4 ± 4.4	349.7	11.2 ± 0.0	11.3 ± 0.0	11.2
13	422.1 ± 61.8	437.1 ± 76.5	429.6	11.2 ± 0.0	11.3 ± 0.0	11.3
14	353.7 ± 49.1	360.4 ± 11.8	357.1	11.2 ± 0.1	11.3 ± 0.0	11.2
15	373.4 ± 196	334.1 ± 19.1	353.7	11.2 ± 0.0	11.3 ± 0.0	11.2
16	390.6 ± 25.2	371.9 ± 10.3	381.3	11.2 ± 0.0	11.3 ± 0.0	11.2
17	363.2 ± 16.1	381.1 ± 8.1	372.2	11.2 ± 0.1	11.2 ± 0.0	11.2
18	375.5 ± 9.7	389.1 ± 24.5	382.3	11.2 ± 0.0	11.3 ± 0.0	11.2
19	322.7 ± 31.8	368.2 ± 21.9	345.5	11.3 ± 0.0	11.3 ± 0.0	11.3
20	358.2 ± 26.9	391.4 ± 22.8	374.8	11.3 ± 0.0	11.3 ± 0.0	11.3
21	368.2 ± 32.1	335.8 ± 34.4	352.0	11.3 ± 0.0	11.3 ± 0.0	11.3
22	301.8 ± 39.1	301.1 ± 0.9	301.4	11.3 ± 0.0	11.3 ± 0.0	11.3
23	297.3 ± 55.2	368.3 ± 2.6	332.8	11.3 ± 0.0	11.3 ± 0.1	11.3
24	339.9 ± 10.1	359.0 ± 32.4	349.4	11.3 ± 0.0	11.3 ± 0.1	11.3
25	302.6 ± 61.2	389.1 ± 20.1	345.9	11.2 ± 0.0	11.3 ± 0.1	11.2
Mean	356.4	364.7		11.2	11.3	
LSD			32.83			0.12
Heritabiity			0.6726			0.5758

4.2.4 Flotation index

Flotation index (FI) is an indirect measure of kernel hardness (Salinas-Moreno *et al*., 1996), where flotation percentages are: 0-12% (very hard), (13-37%) hard, (38-62%) intermediate, (63-87%) soft, very soft (88-100%). Flotation percentage can indirectly determine hardness depending on the amount of kernels that float. The mean for materials planted in the Bajío region was 51.95% and that of the Morelia region was 55.7% (**Table 4.10**). These results would suggest that overall, kernels in the Morelia region posses more floury endosperm when compared to the Bajío region. The genotypes with the highest FI in the Bajío region are 13 (222 x 323), 2 (33 x 58), 5 (121 x 84) and 7 (136 x 197) with 99.3, 92.9, 86.3 and 81% respectively. The genotypes with the highest FI in the Morelia region are 1 (23 x 53), 15 (242 x 225), 21 (293 x 227) and 14 (225 x 242) with 96.6, 93.2, 90.5 and 89.5% whish would suggest they all posses very soft kernel hardness.

In contrast, the genotypes with the lowest FI in the Bajío region were 3 (63 x 26), 6 (124 x 144) and 4 (87 x 160) with 13.0, 10.0 and 20.3% respectively. The Morelia region shows genotypes 8 (144 x 124) and 3 (63 x 26) with FI of 10.0 and 16.3% respectively. Previous results showed maize flotation index of 25, 42 and 76% for Puma, Almoloya de Juarez and Ixtlahuaca corn genotypes and 82.2% for elotes chalqueños (Salinas-Moreno *et al*., 1996; Salinas-Moreno *et al*., 2003). Flotation index is said to be affected by environmental factors as seen by ANOVA ($p<0.0001$) as well as differences in genotype ($p<0.0001$). Also, G x E interaction showed it affects the materials harvested from both regions ($p<0.0001$). Heritability (0.8037) indicated FI is a trait which is carried onto future generations readily, suggesting its plays an important role in kernel content. A low correlation was found between flotation index and 1000 kernel weight (0.1666) which indicates kernel weight is associated with flotation and thus justifies a high heritability value. As observed on **Table 4.9** and **4.10**, genotypes 1(23 x 53), 2 (33 x 58) and 13 (222 x 323) contained the heaviest kernels and also showed a high flotation index.

4.1.5 Endosperm texture

Blue corn is known for its characteristical floury endosperm. The proportion of floury or chalky endosperm to corneous or vitreous endosperm is subjectively read. Endosperm type is scaled from 1 to 5 with 1 (completely floury), 2 (floury), 3

(floury/vitreous), 4 (vitreous) and 5 (completely vitreous). No significant differences were showed between environments, however there is considerable variability between genotypes ($p<0.05$). Also, G x E interaction showed it affects endosperm texture ($p<0.0001$), suggesting environmental stresses affecting each genotype determine whether a kernel contains citreous or corneous texture. As can be observed on **Table 4.11**, genotypes 13 (222 x 323), 19 (292 x 160), 20 (301 x 156) and 18 (285 x 67) showed low values, corresponding to completely or near completely floury endosperm. The Morelia region shows more genotypes with this type of endosperm with genotypes 17 (254 x 315), 15 (242 x 225), 14 (225 x 242), 13 (222 x 323) and 5 (121 x 84) with values near or below 1 (completely floury). This characteristic of blue corn is not necessarily positive due to the troubles arising from industrial processing of floury corn. Nixtamalization procedures prefer corns with (vitreous/floury) endosperm, alluding to a corn that is not completely floury and not completely corneous either. The most vitreous genotypes from the Bajío region were 3 (63 x 26), 23 (352 x 38), 24 (356 x 152) and 25 (364 x 224) with values above 3(vitreous/floury). The Morelia region showed genotypes 2, (33 x 58) 23 (352 x 38), 24 (356 x 152) and 25 (364 x 224) as those with the highest vitreous endosperm. Differences between genotypes among both environments show genotypes 2 (33 x 58) and 3 (63 x 26), as having mostly floury or mostly vitreous endosperm depending on what region they were harvested from. Genotype 2 (33 x 58) showed 1.62 and 4.90, for Bajío and Morelia region, respectively. And, genotype 3 (63 x 26) showed contrasting differences with 4.43 and 2.15 for Bajío and Morelia, respectively. This suggests that both genotypes are affected by G x E interactions ($p<0.0001$). And type of texture is dependent on what region it was harvested from. A low endosperm texture heritability (0.4847) suggests phenotypic effects are not carried onto future generation as readily, corroborated by the strong environmental effects and significant differences exercised by the G x E interactions.

Table 4.10 Flotation index of 25 elite hybrid crosses from blue maize grown in two environments

	%		
Genotype	Bajío	Morelia	Genotype mean
1	71.00 ± 0.58 f	96.63 ± 0.03 ab	83.81
2	92.94 ± 0.47 bc	89.50 ± 0.50 cd	91.25
3	13.01 ± 0.16 tu	16.33 ± 0.33 st	14.78
4	20.33 ± 0.33 rs	40.50 ± 0.50 no	30.41
5	86.39 ± 0.25 d	53.42 ± 0.08 jk	69.97
6	10.00 ± 0.00 u	23.27 ± 0.07 r	16.63
7	81.00 ± 0.58 e	63.32 ± 0.02 gh	72.20
8	23.44 ± 0.18 r	10.00 ± 0.00 u	16.71
9	33.88 ± 0.56 pq	29.50 ± 0.50 q	31.74
10	29.79 ± 3.45 q	46.68 ± 0.02 l	38.27
11	40.00 ± 0.58 no	56.28 ± 0.38 ij	48.18
12	52.91 ± 0.41 jk	53.32 ± 0.02 jk	53.10
13	99.33 ± 0.67 a	63.42 ± 0.08 gh	81.44
14	50.00 ± 1.15 kl	89.50 ± 0.50	69.89
15	53.21 ± 0.16 jk	93.27 ± 0.07 bc	73.28
16	41.33 ± 1.86 mn	46.68 ± 0.02 l	44.02
17	31.00 ± 0.58 q	46.33 ± 0.33 lm	38.73
18	49.67 ± 0.88 kl	76.73 ± 0.07 e	63.20
19	36.49 ± 0.30 op	30.00 ± 0.00 q	33.24
20	70.00 ± 0.58 f	56.78 ± 0.12 ij	63.47
21	66.79 ± 0.55 fg	90.50 ± 0.50 cd	78.60
22	41.00 ± 0.58 no	40.10 ± 0.10 no	40.62
23	79.00 ± 0.58 e	76.63 ± 0.03 e	77.86
24	59.67 ± 0.88 hi	40.50 ± 0.50 no	50.17
25	66.49 ± 0.30 fg	63.27 ± 0.07 gh	64.92
Mean	51.95	55.70	
LSD			0.68
Heritability			0.8037

*Values within columns with no letters in common are significantly different ($p<0.05$)

Table 4.11 Endosperm texture of 25 elite hybrid crosses from blue maize grown in two environments

Genotype	Bajío		Morelia		Genotype mean
1	1.79 ± 0.13	fghijkl	1.92 ± 0.08	fghijk	1.91
2	1.62 ± 0.14	ijkl	4.90 ± 0.10	a	3.38
3	4.43 ± 0.30	a	2.15 ± 0.15	efghij	3.31
4	1.58 ± 0.17	ijklm	2.10 ± 0.10	efghij	1.83
5	2.52 ± 0.11	efgh	1.40 ± 0.10	ijklmn	2.02
6	1.72 ± 0.11	ghijkl	2.18 ± 0.02	efghij	2.01
7	1.71 ± 0.10	hijkl	1.73 ± 0.07	fghijkl	1.74
8	2.29 ± 0.07	efghi	2.17 ± 0.17	efghij	2.27
9	1.70 ± 0.21	hijkl	1.25 ± 0.25	jklmn	1.55
10	1.52 ± 0.16	ijklmn	1.25 ± 0.25	jklmn	1.42
11	1.53 ± 0.15	ijklmn	2.08 ± 0.08	efghij	1.82
12	2.03 ± 0.10	efghij	1.54 ± 0.24	ijklmn	1.81
13	0.68 ± 0.01	n	0.87 ± 0.03	klmn	0.84
14	2.61 ± 0.26	def	0.62 ± 0.12	mn	1.65
15	2.37 ± 0.19	efghi	1.02 ± 0.02	klmn	1.76
16	2.57 ± 0.18	defgh	1.72 ± 0.12	fghijkl	2.13
17	2.58 ± 0.14	defg	0.84 ± 0.00	lmn	1.70
18	1.34 ± 0.00	jklmn	2.20 ± 0.20	efghij	1.87
19	0.68 ± 0.01	n	3.01 ± 0.00	cde	1.85
20	1.29 ± 0.03	jklmn	1.72 ± 0.12	fghijkl	1.51
21	1.73 ± 0.12	ghijkl	1.86 ± 0.02	ghijkl	1.84
22	2.08 ± 0.09	efghij	1.48 ± 0.03	ijklmn	1.89
23	4.04 ± 0.23	ab	4.30 ± 0.30	ab	4.26
24	3.43 ± 0.23	bcd	4.62 ± 0.28	a	4.03
25	3.49 ± 0.25	bc	3.87 ± 0.03	abc	3.72
Mean	2.13		2.11		
LSD					0.13
Heritability					0.4847

*Values within columns with no letters in common are significantly different ($p<0.05$). Where: 1=(completely floury), 2.5=(intermediate), 5=(completely vitreous).

4.3 Crosses analysis (Hybrid analysis by environment)

4.3.1 Free phenolic acid quantification

Results from cross analysis of 25 hybrid blue corn genotypes by two environments are shown in **Table 4.12** it can be observed there was a statistically significant difference ($P<0.05$) between the tested genotypes for free phenolic compounds measured by the Folin-Ciocalteu method. As observed, the range of free phenolic compounds was from cross 4 (87 x 160) harvested from the Bajío region with 85.55 mg GAE/100g to cross 2 (33 x 58) from Bajío region with 131.07 mg GAE/100g.

Free phenolic acid concentrations previously reported by Lopez-Martinez and colleagues (2009) show free phenolic content for two black (Mm04c1- from Colegio de Postgraduados), (NO04C2-from Colegio de Postgraduados) and two purple (purple-Local market, Mexico City), (O337-CIMMyT) corn genotypes, with 123, 101, 83.7 and 124 mg GAE/100g respectively. Additional results from the same laboratory show free phenolic content for blue genotype (Blue-Local market, Mexico, City) and white (White-Local market, Veracruz) with 73.1 and 33.4 mg GAE/100g respectively. Other results show a range of free phenolic acid content of 13.5 to 28.0 mg GAE/100g for white, yellow, blue and red maizes (Mora-Rochin *et al*., 2010).

Overall the blue corn with the highest average for the two regions for phenolic acids concentration was genotype 2 (33 x 58) with 134.8 mg GAE/100g followed by genotype 18 (285 x 67) with 118.06 mg GAE/100g. This would suggest that genotypes 2 (33 x 58) and 18 (285 x 67) are not affected by both regions as is observed in **Table 4.20**. Free phenolic acid content, shows no significant differences between both environments evaluated. Genotype however, ($p<0.0001$) suggests a significant difference exists between those evaluated and thus is exhibited in free phenolic acid content as does the genotype-environment interaction ($p<0.0001$). Differences can also be attributed to the genetic background, grain physical properties and to the ratio of anatomical parts of the kernel since the pericarp and endosperm's aleurone layer are the structures richer in phenolic compounds (Adom *et al*., 2005; Mora-Rochin *et al*., 2010).A ($p<0.6326$) suggests that

phenolic content is not changed or affected by environment factors. However, the genotype-environment interaction suggests a significant difference between the tested genotypes and must be studied extensively This may in part be due to the low free phenolic acid content in the grain and its impact by the environment when compared to bound phenolic acids.

Broad heritability is defined as the proportion of phenotypic variance that is attributable to an effect for the whole genotype, comprising the sum additive, dominance, and epistatic effects (Piepho *et al*., 2007). The heritability (0.0128) in free phenolic acid composition suggests an interaction for the trait and the environment (Hallauer *et al.,* 1981). The heritability is said to be affected by both the genotype and the environment, in this particular trait, the interaction is weak. Differences in soil composition, precipitation, temperatures, salinity, harvesting times, flowering time among others play critical roles in determining the G X E interactions. And thus, manifest in a low heritability value which renders this particular trait not readily inherited to future generations. This particular value indicates free phenolic acids are affected by genotype-environment interactions heavily, leading to free phenolic acid content which is primarily determined by this interaction and not by any environmental stresses affecting kernel content alone.

Table 4.12 Free phenolic acid content in 25 elite hybrid crosses from blue maize grown in two environments

	Free phenolic acid (mg GAE/100g)		
Genotype	Bajío	Morelia	Genotype mean
1	90.62 ± 3.13 cd	104.24 ± 6.12 abcd	96.07
2	131.08 ± 9.12 a	140.39 ± 2410 abcd	134.80
3	105.22 ± 6.48 abcd	102.91 ± 5.24 abcd	104.29
4	85.56 ± 5.01 d	133.45 ± 8.22 abc	104.71
5	105.25 ± 9.35 abcd	115.60 ± 13.58 abcd	109.39
6	128.41 ± 10.12 ab	93.40 ± 4.48 abcd	114.40
7	114.20 ± 7.10 abcd	101.80 ± 10.14 abcd	109.24
8	112.70 ± 13.22 abcd	93.31 ± 5.26 bcd	104.94
9	89.22 ± 4.05 cd	114.35 ± 23.69 abcd	99.27
10	114.01 ± 7.17 abcd	86.19 ± 2.25 cd	102.89
11	91.03 ± 5.98 cd	111.92 ± 9.77 abcd	99.39
12	109.98 ± 5.31 abcd	104.17 ± 7.15 abcd	107.66
13	107.60 ± 8.05 abcd	87.02 ± 4.98 cd	99.36
14	99.89 ± 9.08 abcd	100.98 ± 7.12 abcd	100.32
15	98.81 ± 6.01 bcd	113.20 ± 4.79 abcd	104.56
16	106.67 ± 4.39 abcd	108.03 ± 3.25 abcd	107.21
17	106.97 ± 3.48 abcd	105.00 ± 7.15 abcd	106.18
18	115.33 ± 7.01 abcd	122.16 ± 5.01 abc	118.06
19	116.25 ± 5.18 abcd	117.19 ± 4.36 abcd	116.62
20	107.95 ± 4.33 abcd	119.95 ± 6.17 abcd	112.75
21	108.09 ± 2.28 abcd	98.79 ± 3.28 abcd	104.37
22	92.77 ± 4.73 cd	97.36 ± 3.94 abcd	94.60
23	108.56 ± 5.92 abcd	101.58 ± 1.25 abcd	105.77
24	110.73 ± 3.74 abcd	92.77 ± 3.64 bcd	103.54
25	110.78 ± 6.95 abcd	119.44 ± 5.68 abcd	114.24
Mean	106.71	107.41	
LSD			7.72
Heritability			0.0128

*Values within columns with no letters in common are significantly different ($p<0.05$)

4.3.2 Bound phenolic acid quantification

Results obtained from the analysis of bound phenolic acids show that >80% of total phenolic acids were bound as previously discussed by bulk analysis results. As observed in **Table 4.13**, the range of bound phenolics was 173.69 to 308.34 mg GAE/100g for genotype 7 (136 x 197) harvested in Morelia to genotype 10 (171 x 131) from Bajío region respectively. Previous evaluations by Del Pozo-Insfran (2007) show total soluble phenolics for Mexican white, Mexican blue and American blue genotypes with 343, 41 and 121 mg GAE/100g respectively.

Bound phenolic acid concentrations previously reported by Lopez-Martinez and colleagues (2009) show bound phenolic acid content for two black (Mm04c1- from Colegio de Postgraduados), (NO04C2-from Colegio de Postgraduados) and two purple (purple-Local market, Mexico City), (O337-CIMMyT) corn genotypes, with 421, 463, 381 and 414 mg GAE/100g, respectively. Additional results from the same laboratory show bound phenolic acid contents for blue (Blue-Local market, Mexico, City) and white (White-Local market, Veracruz) corns of 271 and 136 mg GAE/100g, respectively.

Results suggest that the blue corn hybrids developed for this particular study contained a relatively higher content when compared to white and other blue corns. The overall crosses with the highest concentration is genotype 10 (171 x 131) with 294.1 mg GAE/100g followed by genotype 8 (144 x 124) and genotype 11 (193 x 210) with 280.16 and 279.75 mg GAE/100g, respectively. Mora-Rochin *et al*., (2010) reported total phenolic acid contents in the range of 137.7-167.4 mg GAE/100g.

As was proposed in the bulk section of the present study, total phenolic content shows that >80% of the phenolics occured as bound or attached to cell walls. Such findings are consistent with previous studies which indicate that 80% of polyphenolic content in maize and other cereal grains are primarily bound to hemicelluloses in cell walls of the pericarp, aleurone layer and germ (Adom *et al*., 2005; Cabrera-Soto *et al.,* 2009;De la Parra *et al*., 2007; Del Pozo-Insfran *et al*., Mora-Rochin *et al.,* 2010).

Analysis of variance results for bound phenolic acids indicated that the environment was a key factor affecting phenolic acid composition (p-0.0060). Additionally, genotype and G x E (p-0.0001) also affected the 25 genotypes. Environment may be eliciting a

change, which would suggest as secondary metabolites, phenolic acids in the grain are reacting to a harsh abiotic stress.

Studies involving quantitative trait loci (QTL) associated with phytochemical composition state that G x E interactions have a large influence on phenolic composition of pericarp, cell wall and endosperm (Garcia-Lara *et al*., 2010). Such studies would suggest that the genotype-environment interaction affected the total concentration of a variety of polyphenols present in the grain. Considering that phenolic components are mainly present in the bound form, the extent of the G x E interaction is necessarily needed to be clarified. The high heritability (0.8780) value indicates that the selection for this trait in this population would be most effective for its expression in any succeeding generations.

Table 4.13 Bound phenolic acid content in 25 elite hybrid crosses from blue maize grown in two environments

	Bound phenolic acid (mg GAE/100g)		
Genotype	Bajío	Morelia	Genotype mean
1	281.74 ± 14.00 abc	258.48 ± 6.07 abcdef	272.44
2	236.35 ± 17.48 abcdef	224.37 ± 13.12 abcdef	231.56
3	253.70 ± 20.95 abcdef	256.14 ± 7.02 abcdef	254.68
4	213.25 ± 11.21 bcdef	267.21 ± 20.24 abcde	234.83
5	252.01 ± 9.10 abcdef	237.95 ± 7.02 abcdef	246.39
6	255.49 ± 15.14 abcdef	191.98 ± 4.05 def	230.09
7	199.88 ± 28.14 def	173.69 ± 17.25 f	189.41
8	282.07 ± 14.71 abc	277.31 ± 14.98 abcd	280.16
9	240.47 ± 11.84 abcdef	240.87 ± 8.04 abcdef	240.63
10	308.35 ± 16.01 a	272.73 ± 10.01 abcde	294.10
11	272.26 ± 15.69 abcd	290.98 ± 10.58 abc	279.75
12	286.12 ± 15.14 ab	241.33 ± 26.36 abcdef	268.20
13	239.73 ± 14.98 abcdef	293.84 ± 12.98 ab	261.37
14	273.06 ± 5.51 abcd	213.37 ± 30.21 bcdef	249.18
15	261.76 ± 8.08 abcde	226.30 ± 13.25 abcdef	247.58
16	279.59 ± 3.06 abc	253.03 ± 9.08 abcdef	268.97
17	254.41 ± 15.21 abcdef	268.21 ± 22.12 abcde	259.93
18	250.65 ± 13.00 abcdef	202.83 ± 5.03 bcdef	231.52
19	257.00 ± 7.05 abcdef	240.06 ± 15.21 abcdef	250.22
20	249.76 ± 10.97 abcdef	238.77 ± 29.95 abcdef	245.36
21	251.07 ± 5.05 abcdef	282.51 ± 1.07 abcd	263.64
22	268.80 ± 12.69 abcd	236.52 ± 18.13 abcdef	255.89
23	286.58 ± 8.03 ab	224.27 ± 22.68 abcdef	261.65
24	188.37 ± 11.21 ef	199.64 ± 16.12 cdef	192.87
25	235.34 ± 13.22 abcdef	275.26 ± 9.08 abcd	251.30
Mean	255.11	243.51	
LSD			15.90
Heritability			0.8780

*Values within columns with no letters in common are significantly different ($p<0.05$)

4.3.3 Antioxidant capacity quantification

Antioxidant capacity values as affected by genotype and environment measured by the ORAC method for free and bound phenolic compounds are depicted in **Tables 4.14** and **4.15**, respectively. The cross with the highest antioxidant capacity was genotype 23 (352 x 38) harvested from the Bajío region with 9,306.9 µM TE/100g. The genotype with the least antioxidant capacity was 5 (121 x 84) with 3,555.9 µM TE/100g from the same region. Materials planted in the Morelia region showed a range of 3,916.0 to 9,468.4 from genotypes 5 (121 x 84) and 24 (356 x 152), respectively.

Analysis of variance indicated that in general as it was observed in the bulk analysis, the environment factor affected the antioxidant capacity exerted by the free phenolics ($p<0.5277$). The materials from the Bajío and Morelia regions had an average of 6,769.1 and 6,853.9 µM TE/100g, respectively. The G x E interaction ($p<0.0001$) suggests nutraceutical function was affected by environment affecting genotypic output in the materials harvested from both regions. High heritability (0.8513) for free phenolic acid antioxidant capacity suggests that this particular trait is carried onto any subsequent crosses. Once again, the selection of this nutraceutical trait is of vital importance for the advancement of this hybrid crop. Determining heritability enables the investigator to develop a qualitative choice on traits that have positive functions such as sequestering of free radicals, which cause oxidative stress.

Table 4.14 Free phenolic acid antioxidant capacity in 25 elite hybrid crosses from blue maize grown in two environments

	Free phenolic acid antioxidant capacity (μM TE/100g)						
Genotype	Bajío			Morelia			Genotype mean
1	5101.7	± 34.2	ghij	4954.3	± 15.3	ghij	5028.0
2	4849.4	± 15.1	hij	4823.3	± 21.8	hij	4836.3
3	7040.2	± 430.2	abcdef	7416.4	± 21.6	abcdef	7228.3
4	7486.1	± 10.1	abcde	7476.0	± 32.1	abcde	7481.0
5	3555.9	± 246.2	j	3916.0	± 261.0	j	3735.9
6	5911.9	± 277.4	defgh	6802.4	± 649.1	defgh	6357.2
7	5658.6	± 458.6	fghi	5339.0	± 352.2	fghi	5498.8
8	5826.9	± 451.2	defgh	6950.1	± 606.1	defgh	6388.5
9	4266.0	± 247.4	ij	4216.8	± 302.2	ij	4241.4
10	6956.5	± 546.6	bcdef	6952.8	± 856.5	bcdef	6954.7
11	6345.8	± 573.6	efghi	5164.2	± 572.9	efghi	5755.0
12	6211.3	± 736.0	cdefg	7152.7	± 447.2	cdefg	6682.0
13	6733.9	± 688.5	abcd	9363.7	± 251.7	abcd	8048.8
14	9199.4	± 223.9	a	8694.0	± 47.1	a	8946.7
15	7134.3	± 1042.3	cdefg	6395.3	± 530.0	cdefg	6764.8
16	4626.2	± 240.4	ghij	5579.6	± 525.3	ghij	5102.9
17	5326.6	± 316.0	efghi	6255.2	± 144.9	efghi	5790.9
18	8283.3	± 488.6	a	9151.2	± 282.5	a	8717.3
19	7393.5	± 555.1	abcde	7406.7	± 568.8	abcde	7400.1
20	8332.7	± 554.9	abcd	6943.2	± 778.5	abcd	7637.9
21	8708.5	± 461.7	abcde	5907.1	± 339.9	abcde	7307.8
22	8492.0	± 453.5	a	8990.5	± 426.6	a	8741.2
23	9306.9	± 226.0	abc	7203.5	± 604.3	abc	8255.2
24	7619.6	± 500.8	ab	9468.4	± 139.2	ab	8544.0
25	8861.6	± 91.1	a	8825.6	± 117.3	a	8843.6
Mean	6769.1			6853.92			
LSD							511.4
Heritability							0.8513

*Values within columns with no letters in common are significantly different ($p<0.05$)

Regarding bound phenolic acid antioxidant activity, previous investigations (De la Parra *et al.,* 2006) report that bound phenolic acids are the primary contributors to antioxidant activity; the results in the present study are consistent with these observations. As observed in **Table 4.15**, the range of antioxidant activity in the Bajío region was from

genotype 10 (171 x 131) with 38,243.5 µM TE/100g to 22,861.5 µM TE/100g from genotype 7 (136 x 197).

Harvested from the Morelia region genotype 25 (364 x 224) had the highest activity of (41,637.3 µM TE/100g) whereas genotype 7 the lowest (136 x 197) with (12,268.5 µM TE/100g). This variance can be attributed to genotypic differences ($p<0.0010$) among materials harvested in the region. In a previous study, Mora-Rochin *et al*., (2010) reported similar antioxidant activities for bound phenolics for white (19,312 µM TE/100g), red (19,191µM TE/100g), yellow (19,180 µM TE/100g) and blue (12,286 µM TE/100g) corns.

Antioxidant capacity values compared to their respective phenolic acid fractions showed a direct relationship with a positive correlation (0.3533). For instance genotype 3 (63 x 26) which had a low antioxidant capacity (17,565.0 µM TE/100g) also contained one of the lowest total phenolic acid content (294.7 mg GAE/100g). In contrast, genotype 10(171 x 131) which contained a high antioxidant capacity from bound phenolic acid fractions (34,228.0 µM TE/100g) also had a high total phenolic acid content (390.64 mg GAE/100g (*See: correlation studies*).

The average antioxidant capacity for genotypes across both regions showed a range from 38,404.20 to 17,565.05 µM TE/100g for genotypes 25 (364 x 224) and 7 (136 x 197), respectively. The particular difference between both genotypes indicates the potential for plant breeding focused to achieve higher nutraceutical properties. Selection for genotypes based on phytochemical composition or nutraceutical values indicate that genotypes 4 (87 x 160), 7 (136 x 197), 8 (144 x 124) and 9 (160 x 87) showed low content. And thus can be excluded from any further breeding programs centered on AOX capacity from bound phenolics. Additionally, according to the results from the analysis of variance from environment ($p<0.0864$), indicate that there was no significant difference between regions. This demonstrates that the genotypes were stable, making the genotypic selection based on nutraceutical properties more plausible. Similar to free phenolic acid AOX capacity, bound phenolics show differences between genotypes. Environment may not be affecting antioxidant capacity from phenolic acid content due to stability and good crop management. Additionally, the genotype and environment interaction ($p<0.0501$) suggests that a factor(s) embedded within the G x E interaction does not affect AOX capacity.

Heritability (0.8572) for bound phenolic acids showed a high effective transmission of traits from one generation to the other. The main purpose for estimating heritability is to make predictions about selection response under varying scenarios in order to design the optimum selection strategy. Based on these findings, bound phenolic acid antioxidant capacity is one of the main components in determining the value of hybrid blue corns.

Table 4.15 Bound phenolic acid antioxidant capacity in 25 elite hybrid crosses from blue maize grown in two environments

	Bound phenolic acid antioxidant capacity (μM TE/100g)			
Genotype	Bajío		Morelia		Genotype mean
1	29616.6 ± 2441.2	abcdefghij	32749.2 ± 4851.0	abcdefghij	31182.9
2	24680.9 ± 3873.1	cdefghijk	20804.3 ± 3438.6	efghijk	22742.6
3	21348.3 ± 2550.0	ghijk	21816.2 ± 1711.8	defghijk	21582.2
4	18606.0 ± 2128.9	jk	19611.4 ± 1640.7	hijk	19108.7
5	21817.2 ± 1206.2	fghijk	21120.2 ± 772.2	defghijk	21468.7
6	21716.6 ± 1451.8	fghijk	18566.5 ± 2168.1	hjk	20141.6
7	22861.5 ± 3882.1	defghijk	12268.5 ± 660.0	k	17565.0
8	18269.0 ± 617.0	jk	21233.2 ± 2020.9	defghijk	19751.1
9	19677.2 ± 478.1	hjk	18467.0 ± 2262.8	hjk	19072.1
10	38243.5 ± 1436.4	abc	30212.5 ± 3099.1	abcdefghij	34228.0
11	33604.4 ± 3191.9	abcdefgi	33109.7 ± 1552.2	abcdefghij	33357.0
12	32140.5 ± 2248.8	abcdefghij	25957.2 ± 5844.6	abcdefghijk	29048.8
13	25896.7 ± 3348.6	bcdefghijk	19912.0 ± 3854.9	ghijk	22904.3
14	26445.9 ± 3129.3	abcdefghijk	25448.9 ± 3191.0	abcdefghijk	25947.4
15	26789.6 ± 3999.2	abcdefghijk	29101.8 ± 505.4	abcdefghijk	27945.7
16	30180.3 ± 2329.3	abcdefghij	22963.0 ± 1696.4	cdefghijk	26571.7
17	26536.1 ± 1829.7	abcdefghijk	24495.8 ± 1636.2	bcdefghijk	25515.9
18	30560.6 ± 1797.2	abcdefghij	22637.2 ± 2266.8	defghijk	26598.9
19	24108.8 ± 946.7	defghijk	26709.3 ± 1178.6	abcdefghijk	25409.1
20	34204.0 ± 1594.8	abcdefgi	33203.3 ± 4294.6	abcdefghij	33703.7
21	30825.1 ± 4504.2	abcdefghij	41096.0 ± 1452.3	ab	35960.5
22	36446.5 ± 1208.0	abcd	37118.8 ± 2098.7	abcdef	36782.6
23	36141.5 ± 1265.1	abcde	31908.6 ± 3921.4	abcdefghij	34025.1
24	33002.8 ± 2785.4	abcdefghi	33735.7 ± 2255.1	abcdefghij	33369.2
25	35171.0 ± 2827.6	abcdefg	41637.3 ± 1437.4	a	38404.2
Mean	27955.6		26635.3		
LSD					2745.0
Heritability					0.8572

*Values within columns with no letters in common are significantly different ($p<0.05$)

4.3.4 Bound ferulic acid quantification

Ferulic acid content in maize has been shown to be mainly associated with arabinoxylans (Abdel-Aal *et al*., 2003) and other polysaccharides or proteins present within the cell walls of the aleurone layer for structural support (Bunzel *et al*., 2001; Mora-Rochin, *et al*., 2010;). **Figure 4.2** depicts a HPLC chromatogram where ferulic acid was detected at 280 nm.

As seen on **Table 4.16**, the materials of the Bajío region had a concentration range from 92.00 to 228.23 mg ferulic acid/100g these values corresponded to genotypes 24 (356 x 152) and 3 (63 x 26) respectively. Likewise, the materials planted in Morelia region had a range from 77.80 to 212.93 mg of ferulic acid/100g corresponding to genotypes 24 (356 x 152) and 3 (63 x 26) respectively.

Genotype 24 had a phenotypically red color and the lowest total phenolic acid content and a relatively high antioxidant capacity. The ferulic acid for this genotype was relatively low across both regions with an average of 86.32 mg ferulic acid/100g, this is almost half of observed in the other genotypes. This would suggest that neither total phenolic acid content nor ferulic acid content are completely responsible for antioxidant activity.

The genotype containing the highest concentration of bound ferulic acid was 3 (63 x 26) with an average across both regions of 222.11 mg of ferulic acid/100g followed by genotypes 1 (23 x 53), 10 (171 x 131) and 4 (87 x 160) with 191.23, 192.12 and 180.37 mg of ferulic acid/100g, respectively. Results show high levels of ferulic acid content when compared to previous studies where a blue corn genotype contained 127.85 mg of ferulic acid/100g (De la Parra *et al*., 2006). A study by Gutierrez-Uribe and colleagues (2010) reported that blue and white corn genotypes contained 127.5 and 134.1 mg of ferulic acid/100g, respectively. Additionally, Lopez-Martinez and colleagues (2009) reported a content for two black (Mm04c1- from Colegio de Postgraduados), (NO04C2-from Colegio de Postgraduados) and two purple (purple-Local market, Mexico City), (O337-CIMMyT) corn of 151, 151, 154 and 153 mg ferulic acid/100g, respectively. Additional results from the same laboratory showed that ferulic acid content for blue (Blue-Local market, Mexico, City) and white (White-Local market, Veracruz) of 152 and 148 mg ferulic acid/100g

respectively. All these studies clearly indicate that the conversion of white to blue corn does not affect ferulic acid.

The most appropriate genotype for selection based on ferulic acid content was genotype 3 (63 x 26). Additionally, genotype 10 (171 x 131) had a high ferulic acid content and contained the highest total phenolic acid content. Also, this particular genotype (10) had a high antioxidant capacity when compared to the rest.

Analysis of variance results showed a significant difference among genotypes ($p<0.0009$) and between environments ($p<0.0001$). Results indicated that bound ferulic acid was the main phytochemical associated to the corn kernel and was influenced by the environment. Plant stress is caused both by presence or absence of any given causal factors. Environmental stresses like low soil moisture, low soil N, and low or high ambient temperature may affect grain metabolite production (Tollenaar *et al*., 2000). This indicates that both genotype and environment individually affect ferulic acid composition. In contrast, the G x E interaction showed ($p<0.1889$) suggesting that corn genotypes were not affected by environmental effects. Any future breeding efforts for the genotypes with the highest FA content would suggest that content will not be affected regardless of where they are harvested from. Ferulic acid in the maize grain had a high heritability (0.8783), suggesting that trait is effectively transmitted from generation to generation. No significant G x E interactions along with a high heritability signifies the materials utilized for this study may show stability across future generations in distinct environments.

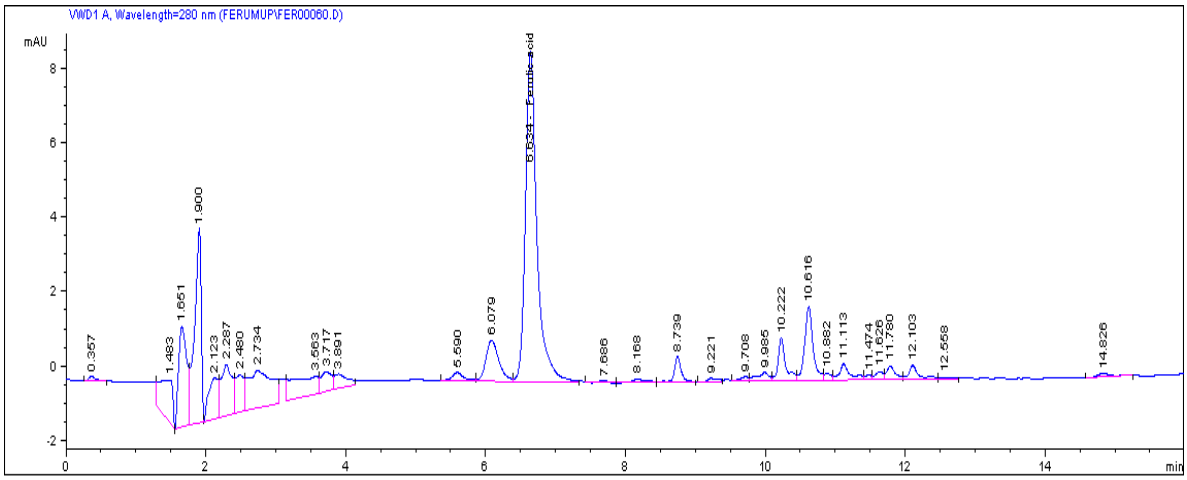


Figure 4.2 Bound ferulic acid chromatogram with detection at 280nm

Table 4.16 Bound ferulic acid content in 25 elite hybrid crosses from blue maize grown in two environments

	(mg ferulic acid/ 100g)				
Genotype	Bajío		Morelia		Genotype mean
1	196.82 ± 10.79	abcd	182.84 ± 14.38	abcdefg	191.23
2	152.20 ± 12.14	cdefgh	142.17 ± 1.72	defghij	148.19
3	228.23 ± 17.42	a	212.93 ± 6.22	abc	222.11
4	181.85 ± 10.19	abcdef	178.15 ± 3.21	abcdefg	180.37
5	166.03 ± 12.63	bcdefgh	177.57 ± 6.77	abcdefg	170.65
6	168.59 ± 9.33	bcdefgh	143.30 ± 6.04	cdefghij	158.47
7	162.26 ± 22.38	bcdefgh	137.26 ± 14.73	defghij	152.26
8	185.13 ± 7.66	abcde	156.46 ± 1.82	bcdefgh	173.67
9	162.07 ± 11.88	bcdefgh	164.74 ± 7.24	abcdefgh	163.14
10	213.18 ± 9.89	ab	160.53 ± 6.46	bcdefgh	192.12
11	170.04 ± 11.69	bcdefg	159.01 ± 11.65	bcdefgh	165.63
12	158.86 ± 10.17	bcdefgh	146.17 ± 4.22	cdefghij	153.78
13	143.58 ± 9.44	defghi	171.89 ± 14.15	abcdefgh	154.91
14	152.33 ± 2.12	cdefgh	140.53 ± 10.77	defghij	147.61
15	144.61 ± 8.74	defghi	144.93 ± 10.18	cdefghij	144.74
16	156.75 ± 7.67	bcdefgh	151.15 ± 5.28	bcdefghi	154.51
17	160.27 ± 13.13	bcdefgh	158.24 ± 7.88	bcdefgh	159.46
18	163.70 ± 6.77	bcdefgh	142.68 ± 8.34	cdefghij	155.29
19	142.36 ± 9.65	defghi	119.84 ± 6.87	fghij	133.35
20	154.68 ± 11.10	cdefgh	133.59 ± 18.67	defghij	146.24
21	112.53 ± 17.91	hij	137.92 ± 1.31	defghij	122.69
22	132.67 ± 9.30	efghij	115.55 ± 14.31	ghij	125.82
23	166.04 ± 4.30	bcdefgh	133.93 ± 3.20	defghij	153.19
24	92.00 ± 10.10	ij	77.80 ± 3.10	j	86.32
25	158.14 ± 7.00	bcdefgh	168.96 ± 4.40	abcdefgh	162.47
Mean	161.00		150.33		
LSD					11.37
Heritability					0.8783

*Values within columns with no letters in common are significantly different ($p<0.05$)

4.3.5 Total monomeric anthocyanin quantification

Results obtained from the quantification of total monomeric anthocyanin content in the 25 blue maize genotypes are shown on **Table 4.17**. The anthocyanin content from the Bajío hybrids ranged from 181.68 mg eq. cianidin-3-glucoside/kg of genotype 24 (356 x 152) to 769.48 mg eq. cyanidin-3-glucoside/kg of genotype 2(33 x 58). The counterparts planted in the Morelia region contained anthocyanins in the range of 307.26 mg/kg to 796.20 mg/kg of genotypes 10 (171 x 131) and 2 (33 x 58), respectively.

Studies by Cortes and colleagues (2006) show 271.77 mg/kg from a commercial blue corn from the state of Queretaro. The anthocyanin content determined in the present study shows a higher yield when compared to previous studies. Additional comparable results showed that Mexican and American blue corns ranged from 307 to 321 mg/kg (Del Pozo-Insfran *et al.,* 2006). Mora-Rochin and colleagues (2010) recently determined and anthocyanin content of 306.9 mg /kg for a blue corn harvested in Sinaloa.

Overall, the genotype means for tested materials showed higher concentration than previous reports. Genotypes 2 (33 x 58) and 22 (324 x 190) show the highest content and are stable among both regions. As observed, genotypes 7, 10, 23, 24 and 25 show important changes in anthocyanin content in both regions. This may be attributed to genotype variation because both environment ($p<0.3760$) and genotype x environment interactions ($p<0.0762$) do not show significant differences affecting content. The ANOVA (**Table 4.20**) showed that anthocyanin content remains stable among both studied regions. As mentioned before, anthocyanins serve as protectors against photoinhibition and photooxidation, the damaging effects of excess quanta (Gould, 2004). Anthocyanin content in the genotypes did not react to environmental and stress factors. Thus, the effects of different stresses would yield important information for further studies.

However, as observed, differences in genotypes suggest the breeding program yielded important differences among the 25 hybrids evaluated. This variability may be due to genetic loss from the conversion of white inbred to the blue color converted hybrid used for this particular study. Also, the loss of activity of regulatory genes like the *R* gene family involved in determining timing, distribution and amount of anthocyanin synthesis has been reported to occur and may the cause of the lower anthocyanin content (Holton *et al*., 1995).

Anthocyanin content in maize varieties is sensitive to environmental conditions like drought and temperature (Arellano-Vazquez *et al*., 2003).

Table 4.17 Total monomeric anthocyanin content in 25 elite hybrid crosses from blue maize grown in two environments

	(mg cyanidin-3-glucoside equivalents/Kg)		
Genotype	Bajío	Morelia	Genotype mean
1	511.2 ± 60.2 abcde	472.9 ± 9.4 abcdef	492.1
2	769.4 ± 25.9 a	796.2 ± 42.2 abc	782.8
3	484.4 ± 54.2 abcdef	395.4 ± 38.2 def	440.0
4	466.6 ± 41.1 abcdef	406.1 ± 60.9 def	436.4
5	397.2 ± 27.1 def	387.4 ± 47.1 def	392.3
6	511.2 ± 12.7 abcde	510.3 ± 9.4.3 abcdef	510.8
7	514.7 ± 36.9 abcde	325.9 ± 53.4 def	420.4
8	422.1 ± 33.8 def	502.3 ± 60.5 abcdef	462.2
9	507.6 ± 21.6 abcde	446.1 ± 5.7.8 abcdef	476.9
10	459.5 ± 59.9 abcdef	307.2 ± 9.4.3 ef	383.4
11	667.9 ± 36.1 abcd	480.9 ± 64.0 abcdef	574.4
12	452.4 ± 14.2 cdef	678.6 ± 68.1 abcd	565.5
13	407.9 ± 26.8 def	427.4 ± 49.2 abcdef	417.7
14	416.8 ± 42.5 def	467.5 ± 17.5 abcdef	442.2
15	482.7 ± 21.6 abcdef	414.1 ± 21.6 def	448.4
16	322.4 ± 3.3.7 ef	424.8 ± 1.9.6 abcdef	373.6
17	411.4 ± 26.2 def	358.0 ± 72.8 def	384.7
18	457.7 ± 20.1 bcdef	358.0 ± 34.1 def	407.9
19	395.4 ± 29.2 def	392.7 ± 51.0 def	394.1
20	391.8 ± 32.7 def	384.7 ± 34.3 def	388.3
21	418.5 ± 17.9 def	379.4 ± 49.6 def	399.0
22	767.7 ± 69.3 ab	788.1 ± 85.7 abc	777.9
23	504.0 ± 63.1 abcde	374.0 ± 49.5 def	439.1
24	181.6 ± 11.4 f	366.0 ± 66.7 def	273.9
25	391.8 ± 38.8 def	499.6 ± 13.3 abcdef	445.7
Mean	468.6	453.7	
LSD			45.96
Heritability			0.7931

*Values within columns with no letters in common are significantly different ($p<0.05$)

4.3.6 Soluble carbohydrate quantification

Quantification of soluble carbohydrates leads to a rapid estimation of total carbohydrate content present in the grain. Soluble carbohydrates correspond to glucose, fructose and galactose monomers and disaccharides such as sucrose present in the maize grain. Most of the soluble carbohydrates are associated to the germ tissue, particularly the scutellum (Serna-Saldivar *et al*., 2010).

The range for soluble carbohydrate content for the materials planted and harvested in the Bajío region was from 2.07% to 4.95% these corresponded to genotype 9 (160 x 87) and genotype 23 (352 x 38), respectively (**Table 4.18**). The materials of the Morelia region showed a range from 2.11% of genotype 10 (171 x 131) to 5.38% of genotype 18 (285 x 67).

The purpose for determining soluble carbohydrate content was to verify the characteristically floury endosperm property of blue corn. Studies have shown that blue corn tortilla consumption and preference is rising in the United States and Mexico mainly due to their sweeter flavor and softness when compared to white or yellow maize tortillas (Cortes *et al*., 2006). Mean content for each corn indicated that genotypes 23(352 x 38), 18 (285 x 67) and 3 (63 x 26) with 4.6, 4.7 and 4.2 %, respectively were the highest. The lowest amounts of soluble carbohydrates per mean average were observed in genotypes 10 (171 x 131), 7 (136 x 197) and 9 (160 x 87) with 2.20, 2.20 and 2.23%, respectively.

Variability within environment shows genotypes 14 (225 x 242) and 18 (285 x 67) react differently in both regions. This change in content may be attributable to climatic stress placed on the grain during germination. Analysis of variance (**Table 4.20**) indicates that both environment and genotypes play a crucial role in determining carbohydrate content in the maize grain. With ($p<0.0005$) and ($p<0.0001$) for environment and genotype respectively, carbohydrate content suggests differences among all genotypes in both contrasting regions. The genotype-environment interaction also affects significantly, contributing to changes among corns in both regions.

Table 4.18 Soluble carbohydrate content in 25 elite hybrid crosses from blue maize grown in two environments

	Soluble carbohydrate (%)						
Genotype	Bajío			Morelia			Genotype mean
1	3.48	± 0.29	bcdefghijk	2.91	± 0.35	cdefghijk	3.20
2	2.98	± 0.18	cdefghijk	3.44	± 0.18	bcdefghijk	3.21
3	4.25	± 0.31	abc	4.15	± 0.42	abcdef	4.20
4	2.66	± 0.26	efghijk	2.46	± 0.11	fghijk	2.56
5	2.26	± 0.13	ijk	2.54	± 0.16	defghijk	2.40
6	2.52	± 0.14	ghijk	3.07	± 0.12	bcdefghijk	2.80
7	2.26	± 0.13	ijk	2.16	± 0.14	ijk	2.21
8	2.93	± 0.25	cdefghijk	2.81	± 0.17	cdefghijk	2.87
9	2.07	± 0.13	k	2.41	± 0.19	ghijk	2.24
10	2.30	± 0.10	ijk	2.11	± 0.12	ijk	2.21
11	2.90	± 0.27	cdefghijk	3.61	± 0.13	bcdefghijk	3.26
12	3.01	± 0.41	cdefghijk	3.06	± 0.01	bcdefghijk	3.03
13	2.12	± 0.14	jk	2.87	± 0.44	cdefghijk	2.50
14	2.39	± 0.15	hijk	3.99	± 0.35	abcdefg	3.19
15	3.85	± 0.27	abcdefg	4.22	± 0.80	abcd	4.03
16	3.52	± 0.32	bcdefghi	2.90	± 0.23	cdefghijk	3.21
17	3.59	± 0.32	bcdefghi	4.19	± 0.65	abcde	3.89
18	3.57	± 0.21	bcdefghi	5.38	± 0.34	a	4.47
19	2.67	± 0.09	efghijk	2.95	± 0.11	cdefghijk	2.81
20	3.00	± 0.25	cdefghijk	3.88	± 0.23	abcdefgh	3.44
21	3.38	± 0.06	bcdefghijk	3.47	± 0.05	bcdefghijk	3.42
22	3.62	± 0.23	bcdefghi	3.00	± 0.11	cdefghijk	3.31
23	4.59	± 0.55	ab	4.67	± 0.52	ab	4.63
24	2.94	± 0.12	cdefghijk	3.57	± 0.12	bcdefghijk	3.25
25	2.52	± 0.22	ghijk	2.33	± 0.11	ghijk	2.42
Mean	3.01			3.29			
LSD							0.29
Heritability							0.8071

*Values within columns with no letters in common are significantly different ($p<0.05$)

4.3.7 Albumin and globulin quantification (soluble protein)

Soluble protein quantification of albumins and globulins present in the maize grain suggest a rapid estimation of total protein content because it represents more than 30% of total protein found in the maize grain (Serna-Saldívar *et al*., 2008; Paulis *et al*., 1969). Albumin and globulin protein fractions are mainly associated to the germ (Serna-Salivar *et al*., 2010). It has been found that *opaque-2* genotypes contain 20.2% albumin/globulin and 14.6% prolamins compared to 7.8% and 37.6% of total protein found in regular maize (Paulis *et al*., 1969). Genotypes evaluated show a high content of 4.15 and 4.12% for genotypes 12 (207 x 192) and 13(222 x 323), respectively (**Table 4.19**). The lowest content were observed in genotypes 2 (33 x 58) and 8 (144 x 124) with 2.13 and 2.26%, respectively. On the other hand the genotypes 12 and 13 contained 4%. Values higher than these genotypes contained similar levels of albumin and globulin to QPM and experimental QPM genotypes HEC 768642 with 4.41% and H-368-C with 3.81% evaluated in Celaya, Mexico (Serna-Saldívar *et al*., 2008). The same study shows normal endosperm corn genotypes contained from 3.1 to 3.4% albumin/globulin (Serna-Saldívar *et al*., 2008).

Based on results, the protein content evaluated in these blue maize hybrids is not affected by the white corn conversion into blue genotypes and thus remained as a positive nutritional property. The estimate of soluble proteins showed an increased probability that some genotypes evaluated in the present study might combine blue pigment and high quality protein in the endosperm. Additionally, heritability (0.8465) predictions suggest soluble protein content is transmitted to future generations. Result which contributes to future breeding efforts seeking high protein content and stability among environments. Analysis of variance (**Table 4.20**) indicated that environment does not affect protein content ($p<0.6027$) in this particular study. Results showed that soluble protein content (albumin/globulin) is not affected by environmental stresses, though considerable differences exist between some genotypes. Especially between genotypes 2 (33 x 58) and 12 (207 x 192) which exhibit considerable differences among genotype and among environment. This difference attributable to the genotype-environment interaction ($p<0.0004$) that suggest a significant difference for this trait.

Table 4.19 Albumin and globulin content in 25 elite hybrid crosses from blue maize grown in two environments

	(%)		
Genotype	Bajío	Morelia	Genotype mean
1	2.71 ± 0.20 efghijklm	1.91 ± 0.24 lm	2.31
2	2.51 ± 0.23 ghijklm	1.75 ± 0.32 m	2.13
3	2.80 ± 0.22 defghijklm	3.13 ± 0.36 bcdefghijkl	2.96
4	2.56 ± 0.41 fghijklm	2.02 ± 0.23 jklm	2.29
5	2.98 ± 0.28 cdefghijkl	2.66 ± 0.11 efghijklm	2.82
6	3.12 ± 0.12 bcdefghijk	2.58 ± 0.17 efghijklm	2.85
7	3.00 ± 0.14 cdefghijkl	3.01 ± 0.18 bcdefghijklm	3.00
8	2.42 ± 0.25 hijklm	2.10 ± 0.39 ijklm	2.26
9	2.12 ± 0.16 jlm	2.59 ± 0.22 efghijklm	2.35
10	2.86 ± 0.41 defghijklm	3.16 ± 0.21 bcdefghijkl	3.01
11	3.27 ± 0.10 bcdefgh	3.84 ± 0.32 abcde	3.56
12	3.71 ± 0.12 abcde	4.60 ± 0.40 a	4.15
13	3.98 ± 0.25 abc	4.27 ± 0.31 ab	4.13
14	3.68 ± 0.24 abcde	3.05 ± 0.10 bcdefghijkl	3.37
15	3.72 ± 0.16 abcde	3.90 ± 0.48 abcd	3.81
16	3.75 ± 0.29 abcde	3.36 ± 0.30 abcdefghi	3.55
17	3.83 ± 0.23 abcd	3.72 ± 0.21 abcdef	3.78
18	3.33 ± 0.12 bcdefgh	3.42 ± 0.13 abcdefgh	3.37
19	3.60 ± 0.11 abcdef	3.26 ± 0.14 bcdefghijk	3.43
20	3.36 ± 0.11 bcdefgh	3.80 ± 0.37 abcde	3.58
21	3.18 ± 0.18 bcdefghik	3.45 ± 0.29 abcdefgh	3.31
22	3.33 ± 0.27 bcdefgh	3.60 ± 0.23 abcdefg	3.47
23	3.40 ± 0.13 bcdefgh	3.49 ± 0.12 abcdefgh	3.44
24	3.36 ± 0.12 bcdefgh	3.58 ± 0.22 abcdefgh	3.47
25	3.43 ± 0.10 bcdefgh	3.03 ± 0.27 bcdefghijklm	3.23
Mean	3.20	3.17	
LSD			0.21
Heritability			0.8465

*Values with no letters in common are significantly different ($p<0.05$)

4.4 Analysis of variance and compound correlation

4.4.1 Analysis of variance

Analysis of variance of the evaluated genotypes across both contrasting regions is shown on **Table 4.20**. The phytochemical, nutraceutical and biophysical traits evaluated for materials harvested from the Bajío and Morelia regions showed considerable genotypic variance as discussed in previous sections. Results show genotypes 22 (324 x 190), 11 (193 x 210) and 10 (171 x 131) contained high phytochemical content and antioxidant capacity when compared to other genotypes. This variability within the selected crosses harvested in both regions show the importance of determining trait stability for the selection of appropriate crosses for further studies involving breeding of these crops. Considering both genotypic variations and environmental variations, only a portion of the 25 genotypes evaluated show stability among the factors discussed. These inconsistencies of certain genotypes to perform differently in certain regions showed the implications with which a breeding program is faced with.

It is important to add that the environment itself, did not affect the vast majority of traits analyzed but it depends on the genotype to observe differences in specific traits, such as phenolics, anthocyanin content and biophysical properties of the kernel. Those affected by the environment were bound phenolic acid and bound ferulic acid concentration which make up most of the phytochemical content present in the bound form. Additional properties affected by the environment were soluble carbohydrate content and flotation index. All others remained unaffected by environmental factors which indicate that the materials harvested in both regions show stability. A characteristic which makes the genotypes harvested in both regions stable and thus attractive for further studies.

Table 4.20 Analysis of variance for 25 elite hybrid crosses from blue maize grown in two environments

		Free phenolic acids		Bound phenolic acids		Free phenolic acid AOX capacity		Bound phenolic acid AOX capacity	
Origin	df	F	P	F	P	F	P	F	P
Environment	1	0.2292	0.6326	7.7091	0.0060	0.4003	0.5277	2.9695	0.0864
Genotype	24	3.1908	0.0001	5.4263	0.0001	20.3939	0.0001	11.0017	0.0010
Genotype *Environment	24	3.1501	0.0001	2.6487	0.0001	3.0322	0.0001	1.5715	0.0501

		Monomeric anthocyanins		Soluble carbohydrates		Soluble protein		Bound ferulic acid	
Origin	df	F	P	F	P	F	P	F	P
Environment	1	0.7918	0.3760	12.6432	0.0005	0.2718	0.6027	11.3539	0.0009
Genotype	24	7.5220	0.0001	13.0771	0.0001	15.9394	0.0001	10.4245	0.0001
Genotype *Environment	24	1.5566	0.0762	2.5225	0.0003	2.4469	0.0004	1.2691	0.1889

		a*		b*		L		E value	
Origin	df	F	P	F	P	F	P	F	P
Environment	1	6.9005	0.0093	0.7445	0.3893	4.9414	0.0273	3.9329	0.0487
Genotype	24	21.2354	0.0001	7.2377	0.0001	2.9265	0.0001	2.3504	0.0007
Genotype *Environment	24	3.9533	0.0001	1.8610	0.0114	2.3103	0.0009	2.1695	0.0020

		Chroma		HUE		Flotation index		Endosperm texture	
Origin	df	F	P	F	P	F	P	F	P
Environment	1	1.0095	0.3162	2.5059	0.1150	228.5000	0.0001	0.2395	0.6260
Genotype	24	15.4427	0.0001	6.4220	0.0001	1333.8370	0.0001	54.1917	0.0001
Genotype *Environment	24	2.3864	0.0006	2.2019	0.0017	261.8441	0.0001	27.9232	0.0001

4.4.2 Correlation studies

The phytochemical and nutraceutical content of the 25 genotypes evaluated are presented in **Table 4.21**. Correlation analysis showed a significant correlation for HUE and anthocyanin content (0.2909). This relationship between colorimetric analysis and anthocyanin concentration has been previously discussed by Cevallos-Casals and colleagues (2004). As stipulated before, there exists to a moderate extent a relationship between kernel color and anthocyanin concentration. However, based on the approximate location of the mean a*and b* values represented in the color gamut observed on **Figure 4.1** indicate that a calculation considering distances between a* and b* is much more appropriate than a calculation utilizing HUE angles. As discussed before, the calculation of chroma considers distances between colorimetric values a* and b*. Results showed a strong negative correlation between anthocyanin content and chroma (-0.3157). Chroma values are calculated based on a* and b*, results showed a strong correlation between a* and anthocyanin content (-0.3522) thus, making a* values a strong indicator of content. Genotype selection based on anthocyanin content would suggest determining genotypes which show low a* values. As observed on **Figure 4.3**, where lower a* values correspond to higher anthocyanin content.

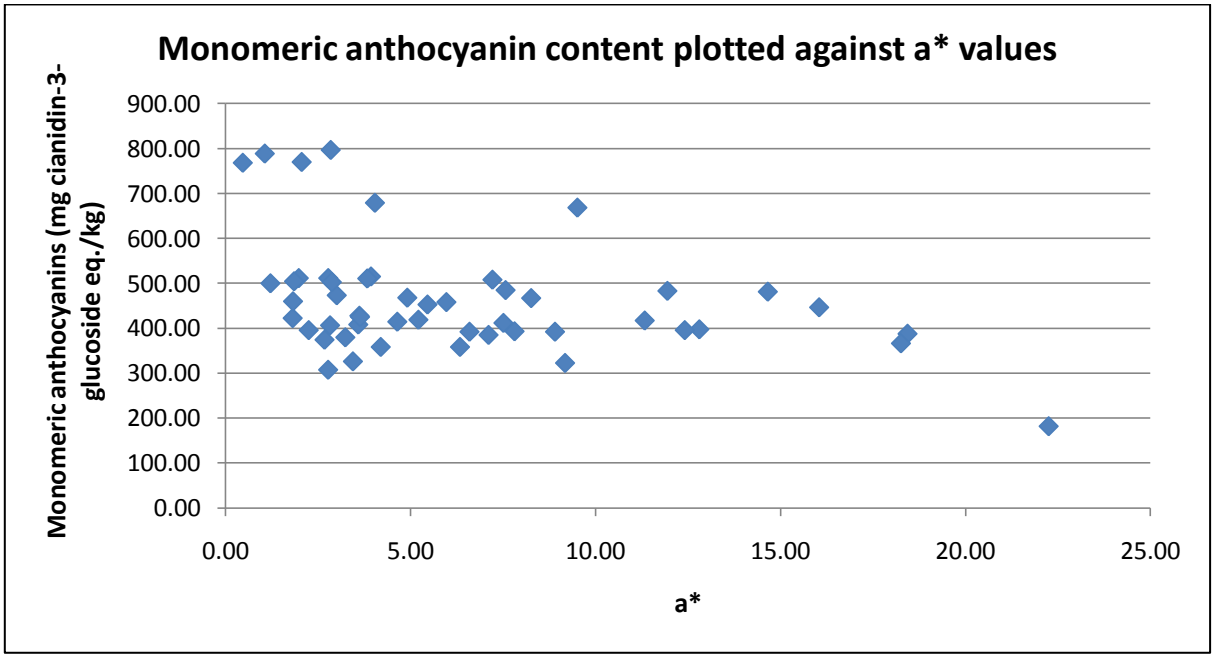


Figure 4.3 Monomeric anthocyanin content plotted against a*.

Also, pertaining to the evaluation of an accurate measurement method for phytochemical content in the grain, a bound phenolics/anthocyanins ratio was significantly correlated with chroma (0.3165). This suggests that there is an association between phytochemical content and colorimetric methods by combining a ratio for both traits. Thus this positive correlation indicates an increase in chroma values would indicate higher phytochemical content in the kernel. This is mainly due to a* values which are associated to anthocyanin content, phenolics and any other components measured in function of a* values. The bound phenolics/anthocyanin ratio increases proportionally with a*. **Figure 4.4** shows a scatter plot with the relationship between the bound phenolics/anthocyanins ratio and chroma value.

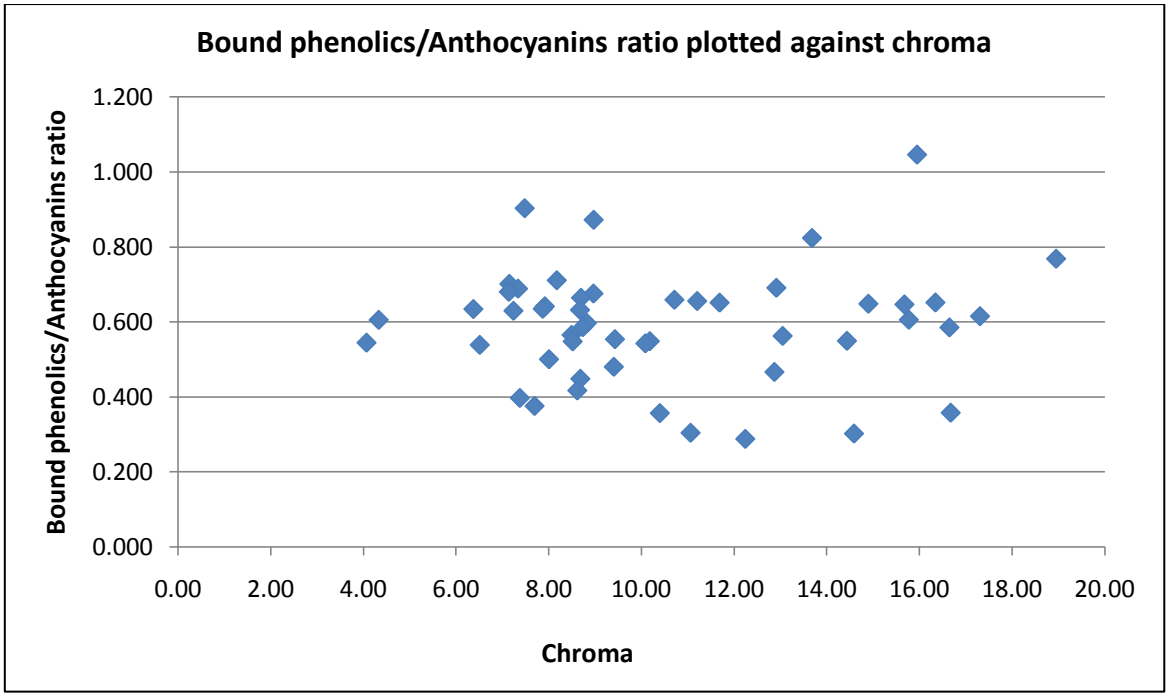
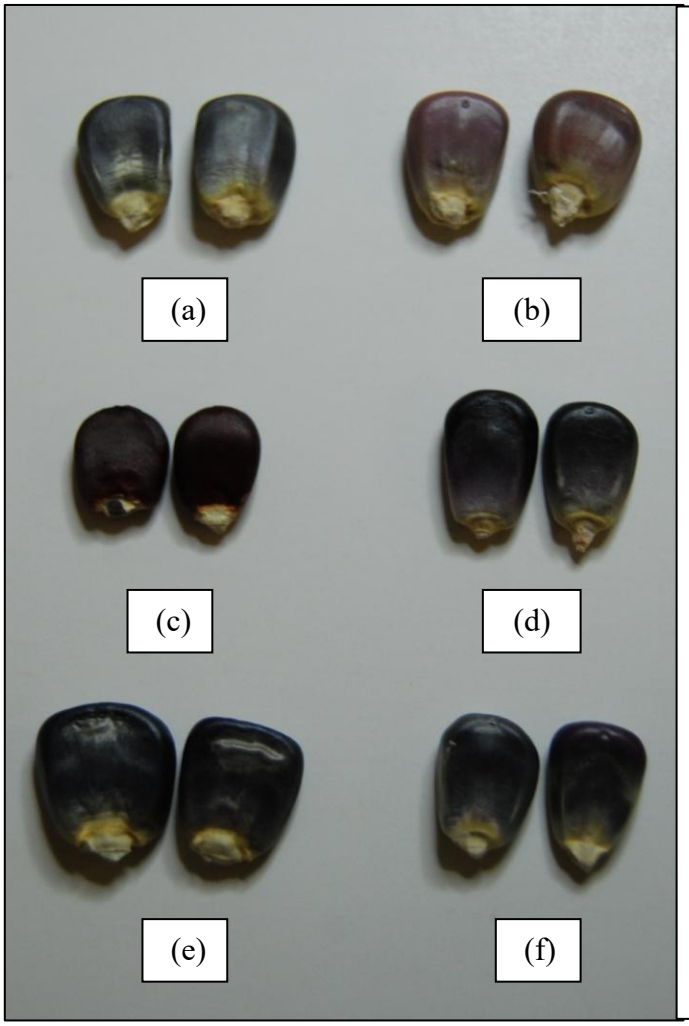


Figure 4.4 Bound phenolics/Anthocyanin ratio plotted against chroma..



(a) Genotype 21 (293x227)-Morelia
Chroma= 19.00

(b) Genotype 25 (364x224)-Morelia
Chroma= 4.10

(c) Genotype 24 (356x152)-Bajío
a*= 22.25

(d) Genotype 22 (324x190)-Bajío
a*= 0.46

(e) Genotype 1 (23x53)-Morelia
b*= 14.12

(f) Genotype 19 (292x160)-Morelia
b*= 1.08

Figure 4.5 Kernel image of highest and lowest chroma, a* and b* values for blue maize hybrids.

As observed on **Figure 4.5**, highest chroma value of 19.00 from genotype 21 (293 x 227) is phenotypically different than the lowest chroma value 4.10 from genotype 25 (364 x 224). The same is noticeable for a* and b* values, indicating high and low values as well as genotype number and region of harvest. Bound phenolic content plotted against a * values is shown in **Figure 4.6**, as previously discussed, low a* values are associated to bound phenolic acid content

Noticeable correlation coefficients include bound phenolic acid significant association (0.3533) with bound phenolic acid antioxidant capacity. This correlation is expected being that the fraction utilized to analyze bound phenolic acid AOX is the same as that used to quantify phenolic acid content by the Folin method. This indicating that bound

phenolics are the main contributors to antioxidant capacity in the grain. Additional correlation coefficients identify a moderate correlation between bound ferulic acid content present in the maize kernel and bound phenolic acid content (0.5433). As previously stated, ferulic is the main phenolic acid in the bound form. This positive association fortifies the usefulness of the method utilized for bound components extraction and quantification of the cereal kernel.

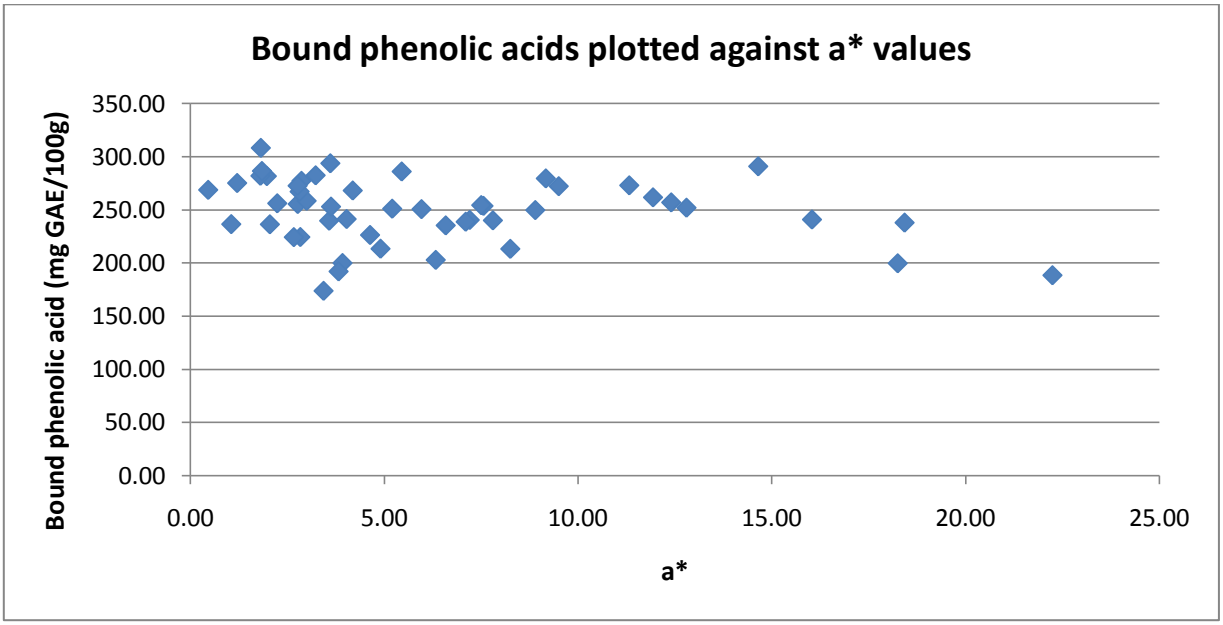


Figure 4.6 Bound phenolic acid content plotted against a*.

The weak correlation between free phenolic acid antioxidant capacity and bound phenolic acid antioxidant capacity (0.3121) suggests an overlapping of phenolic acid fractions and their respective antioxidant capacities. This positive association among both antioxidant capacity values indicates that both are responses to phenolics content causing this activity. The same nutraceutical component (AOX capacity of free phenolic acids) presents a positive correlation with soluble carbohydrates (0.2583) and soluble protein (0.2817) content. This correlation may be indicating that the phenolics causing the AOX activity are associated to carbohydrates. As discussed previously, a vast amount of phenolics are commonly found glycosilated (Abdel-Aal *et al*., 2006). The association between free phenolics AOX activity and soluble protein may be caused by an interaction

between the soluble protein content and soluble phenolics when functioning as reducing agents (Kahkonen *et al*., 1999).

Positive associations exist between carbohydrate content and endosperm texture (0.2619), indicating that evaluation of soluble carbohydrate content present in the kernel (mainly in the germ) may be interacting with any carbohydrates located in the endosperm.

Table 4.21 Correlation analysis of phytochemical and nutraceutical content for 25 elite hybrid crosses from blue maize grown in two environments

	Free phenolic acids	Bound phenolic acids	Free phenolic acid antioxidant capacity	Bound phenolic acid antioxidant capacity	Bound ferulic acid	Monomeric anthocyanins	Soluble protein	Soluble carbohydrates	L	a*	b*	Chroma	HUE	E	Bound phenolic acids/Anthocyanin ratio	1000 kernel weight	Flotation index	Endosperm texture
Free phenolic acids	1																	
Bound phenolic acids	-0.0310 0.7318	1																
Free phenolic acid antioxidant capacity	-0.1284 0.1535	0.0080 0.9296	1															
Bound phenolic acid antioxidant capacity	-0.0832 0.3562	0.3533 0.0001	0.3121 0.0004	1														
Bound ferulic acid	0.0054 0.9520	0.5433 0.0000	-0.1924 0.0316	-0.0485 0.5908	1													
Monomeric anthocyanins	-0.0227 0.8015	0.0895 0.3211	-0.0374 0.6787	0.0465 0.6065	0.5090 0.5732	1												
Soluble protein	-0.0150 0.8683	0.0320 0.7229	0.2701 0.0023	0.2817 0.0015	-0.2454 0.0058	-0.1987 0.0263	1											
Soluble carbohydrates	-0.0029 0.9740	-0.0043 0.9620	0.2583 0.0036	0.1152 0.2008	-0.0580 0.5209	-0.0208 0.8180	0.2469 0.0055	1										
L	0.0416 0.6451	0.0713 0.4297	-0.1733 0.0533	-0.1151 0.2159	0.2156 0.0157	0.0939 0.2974	-0.1807 0.0437	-0.1431 0.1114	1									
a*	0.0014 0.9872	-0.1927 0.0313	-0.0423 0.6397	-0.0384 0.6711	-0.2694 0.0024	-0.3522 0.0001	0.1301 0.1481	-0.0137 0.8795	-0.2337 0.0087	1								
b*	0.0108 0.9051	-0.0944 0.2952	-0.0618 0.4939	0.0905 0.3154	-0.1211 0.1785	-0.0863 0.3387	-0.1929 0.0311	0.0088 0.9228	0.5108 0.0000	0.1770 0.0483	1							
Chroma	0.0067 0.9407	-0.1970 0.0276	-0.0644 0.4753	0.0172 0.8491	-0.2699 0.0023	-0.3158 0.0003	0.0006 0.9947	-0.0060 0.9472	0.0837 0.3532	0.8613 0.0000	0.6525 0.0000	1						
HUE	0.0330 0.7127	0.1352 0.1327	0.0237 0.7934	0.1675 0.0618	0.1174 0.1923	0.2909 0.0010	-0.2588 0.0036	-0.0540 0.5496	0.4284 0.0000	-0.6070 0.0000	0.4947 0.0000	-0.2121 0.0176	1					
E	0.0394 0.6624	0.0177 0.8448	-0.1733 0.0532	-0.0978 0.2780	0.1363 0.1295	0.0109 0.9039	-0.1701 0.0579	-0.1383 0.1241	0.9711 0.0000	-0.0278 0.7587	0.6420 0.0000	0.3100 0.0004	0.3689 0.0000	1				
Bound phenolic acids/Anthocyanin ratio	0.0308 0.7331	0.3019 0.0006	0.0494 0.5846	0.1729 0.0539	0.0815 0.3660	-0.8374 0.0000	0.1903 0.0335	0.0198 0.8269	-0.0592 0.5123	0.2940 0.0009	0.1744 0.0517	0.3165 0.0003	-0.1221 0.1750	0.0287 0.7510	1			
1000 kernel weight	0.0236 0.7936	-0.0398 0.6592	-0.1475 0.1007	-0.1356 0.1316	0.0795 0.3782	0.0402 0.6566	0.0639 0.4789	0.2129 0.0171	0.1049 0.2445	0.0650 0.4714	0.0719 0.4257	0.0871 0.3343	-0.0628 0.4867	0.1241 0.1680	-0.0622 0.4906	1		
Flotation index	0.1176 0.1914	-0.1505 0.0940	-0.0319 0.7243	0.2288 0.0103	-0.2413 0.0067	0.0263 0.7712	0.1126 0.2114	0.0828 0.3585	0.0764 0.3971	-0.0758 0.4008	0.1564 0.0815	0.0223 0.8052	0.1381 0.1245	0.0758 0.4005	-0.0582 0.5193	0.1666 0.0633	1	
Endosperm texture	0.0465 0.6069	-0.0825 0.3605	0.1468 0.1023	0.1488 0.0959	-0.0641 0.4775	0.0011 0.9906	-0.1146 0.2033	0.2619 0.0032	-0.2020 0.0239	0.1183 0.1889	0.1784 0.0466	0.1832 0.0408	0.0578 0.5221	-0.1457 0.1049	0.0498 0.5811	-0.0445 0.6219	-0.037 0.6821	1

4.4.3 Genotype-environment interaction

The genotype-environment interaction (**Table 4.20**) affected all measured traits except anthocyanin, bound ferulic acid content and bound phenolic acid AOX capacity. These traits appeared to be independent of any interactions between genotype and environment. Possible explanations could be that as secondary metabolites, environmental stresses in both regions did not elicit any serious increase or decrease in content. This interaction which still has not been conclusively studied has been known to affect the behavior or outcome of genotypes in different environments. The interaction represents the inconsistency of genotypes from one environment to another (Alejos *et al*., 2006).

As discussed previously, traits showed significant differences among genotypes, but mainly remained stable among environments. Phytochemical content for bound phenolic acid, bound ferulic acid and carbohydrate content do vary among both regions, this response indicates that ferulic acid and bound phenolic acids serve as both cell wall constituents and as a response for abiotic stress.

The overall results from the analysis of variance suggest that the 25 genotypes differed as it was expected. However, the environment did not affect most of the phytochemical traits evaluated. This suggests genetic stability, a positive outcome for any future analysis on these particular maize genotypes.

4.5 Conclusions and recommendations

Based on the results, a number of traits exist for developing an improvement regiment for blue corn production assisted by phytochemical selection. In this particular study, phytochemical selection based on nutraceutical properties like antioxidant capacity provide a sound basis for crop improvement. Results from this study indicated bound phenolic acids showed an association with antioxidant capacity, which as discussed provides health promoting properties. Hybrid blue corn development showed high ferulic acid content, a concentration which equals or surpasses previous findings in white and blue. Considering that anthocyanin content is preserved in subsequent crosses, these particular genotypes showed great potential for development as commercial seeds.

Corn kernel studies evaluated by the determination of a*, b* and L values indicated a possible change in measurement calculations to properly correlate anthocyanin content to a phenotypic color determination. Previous findings state that HUE was utilized for color determination and correlation with anthocyanin content. In this study, a significant negative correlation was found between anthocyanin content and chroma (-0.2909). Also, a correlation between a* values and anthocyanin content showed that anthocyanin content determination can be evaluated by means of chroma or a* determination. A strong correlation between a bound phenolic acid/anthocyanin ratio and chroma suggested anthocyanins were not the only phytochemicals measured in function of a* values. The color properties determined in the present study suggest that HUE may not be the most appropriate parameter for color measurement in the kernel.

Genotype selections of the results obtained from the present study are based on positive phytochemical content and/or nutraceutical activity. Among the 25 blue corn hybrids evaluated, those which showed low levels of total phenolic, ferulic acid, anthocyanin and antioxidant capacity should be discarded from any participation in breeding programs. In particular, genotype 7 (136 x 197) showed low phenolics content and low AOX capacity along with variability among environments. Also, genotype 10 (171 x 131) showed low anthocyanin content and variability among regions for soluble protein and carbohydrates. Genotype 24 (356 x 152) showed low phenolics, ferulic acid and anthocyanin content.

However, this genotype showed high AOX capacity and can be considered for this particular trait. If selection were to be based on any of the particular traits evaluated in the present study, then a number of crosses have the phytochemical and nutraceutical potential for the continuation of crop development based on phytochemical content. Breeders should combine these phytochemical traits with agronomic performance to successfully develop crops with high productive potential.

Based on the findings of this research the major conclusions are summarized below:

- Free and bound phenolic acid content in 25 blue or pigmented genotypes showed high content when compared to other white, yellow and blue genotypes.

- These particular corn hybrids contained high anthocyanin content and antioxidant capacity. These two traits impact the nutraceutical potential of the corn crop and thus can be further developed as commercial hybrid seeds.

- Bound ferulic acid content in the maize grain was consistent with values observed in white corn. Thus the conversion of white into blue did not affect this important trait.

- Analysis of variance showed that most phytochemical and nutraceutical traits were not affected by the two tested environments. This represented a positive outlook for future uses of the evaluated genotypes in other environments.

- The particular differences among increases the potential for new crosses to seek for new materials containing higher nutraceutical properties.

- The selection for genotypes based on phytochemical composition or nutraceutical values was effective and mainly affected by the genotypic background.

- The concentration of key nutraceuticals was positively correlated with soluble protein and carbohydrate contents.

- The medium to high heritability values for decisive and important traits like phenolic acids, antioxidant capacity, ferulic acid and anthocyanin contents was positive for the transmission of genes to future generations.

This study reflects the increasing necessity for development of blue maize hybrids for commercial use. The resulting high phytochemical content for the genotypes evaluated show the hard work which was put into the development of the hybrids evaluated. The selection for blue donor color and the subsequent development of the experimental converted lines shows a carefully planned method for a hybrid blue corn with excellent phytochemical properties. Any future breeding based on hybrid blue corn should look into determination of key phytochemical content in the grain. Such procedures should implement specific analysis of anthocyanin content in order to discriminate among high and low content corns. Evaluation of properties with high nutraceutical potential includes ferulic acid and bound phenolics content. Both traits are central for AOX potential and their corresponding benefits to human health. To our judgment Anthocyanin/Ferulic acid/AOX activity content should be considered as the main contributors to an overall quality of a blue corn hybrid. A special emphasis should be placed on the determination of carbohydrate and protein content in order to properly select corns with high productive potential, and to emphasize on nutritional value. Breeding practices should implement determination of anthocyanin content by a*, b* and L measurements, as to obtain a reference for anthocyanin content by chroma calculation.

4.6 References

Abdel-Aal, E.-S. M., Huel, P. **1999**. A rapid method for quantifying total anthocyanins in blue aleurone and purple pericarp wheats. *Cereal Chemistry*. 76: 350-354

Abdel-Aal, E.-S. M., Hucl, P. **2003**. Composition and stability of anthocyanins in blue-grained wheat. *Journal of Agricultural and Food Chemistry*. 51: 2174-2180.

Abdel-Aal, E.-S, Young, J.C., Rabalski I. **2006**. Anthocyanin composition in black, blue, pink, purple, and red cereal grains. *Journal of Agricultural and Food Chem*istry. 54: 4696-4704

Adom, K. K., Liu, R. H. **2002**. Antioxidant activity of grains. *Journal of Agricultural and Food Chemistry*. 50: 6182–6187.

Adom, K. K. Sorrells, M., Liu, R.H. **2005**. Phytochemicals and antioxidant activity of milled fractions of different wheat varieties. *Journal of Agricultural and Food Chemistry*, 53: 2297-2306

Alejos, G., Monasterio, P., Rea, R. **2006**. Analisis de la interacción genotipo-ambiente para rendimiento de maíz en la región maicera del estado Yaracuy Venezuela. *Agronomia Trop*. 56(3): 369-384

Andrade, F. H., Cirilo, A. G., Echarte, L. **2000**.Factors affecting kernel number in maize, in *Physiological bases for maize improvement*, Otegui, M. E., Slafer, G. A., Ed.; Food products press, Binghampton, NY, (CH5)

Antonio-Miguel, M., Arellano-Vazquez, J.L., Garcia-Santos G., Miranda-Colin, S., Mejia-Contreras, J. A., Gonzalez-Cossio, F. V. **2004**. Variedades criollas de maíz azul raza chalqueño. Características agronómicas y calidad de semilla. *Revista Fitotecnia Mex*icana Vol. 27 (1): 9-15

Arellano-Vázquez, J. L., Tut-Couoh, C., Maria-Ramirez, A., Salinas-Moreno, Y., Taboada-Gaytan, O. R. **2003**. Maiz azul de los valles altos de México. I. Rendimiento de grano y caracteres agronómicos. *Revista Fitotecnia Mex*icana Vol. 26 (2): 101-107

Bakan, B., Bily, A.C., Melcion, D., Cahagnier, B., Regnault- Roger, C., Philogene, B.J.R., Richard-Molard, D. **2003**. Possible role of plant phenolics in the production of trichothecenes by *Fusarium graminearum* strains on different fractions of maize kernels. *Journal of Agricultural and Food Chem*istry, 51: 2826-2831.

Bello-Perez., L.A., **2009**. Blue maize: morphology and starch synthase characterization of starch granule. *Plant Foods in Human Nutr*ition. 64:18-24

Bergvinson, D.J., Hamilton, R.I., Arnason, J.T. **1995**. Leaf profile of maize resistance factors to European corn borer, *Ostrinia nubilalis*. *Journal of Chem. Ecol*. 21: 343 – 354.

Betran, F.J., Bockholt A.J., Rooney L.W. **2001**. Blue corn, in *Specialty corns*, 2nd ed., Hallauer A.R., Eds., CRC press LLC, Boca Raton, Florida, (CH10)

Blum, A. **1988**. Plant breeding for stress environments. CRC press LLC, Boca Raton, Florida. pp.18-28.

Bradford, M. M. **1976**. A rapid and sensitive for the quantitation of microgram quantitites of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry 72:* 248-254.

Bunzel, M., Ralph, J., Marita, M.J., Hatfield, R.D., Steinhart, H., **2001**. Diferulates as structural components in soluble and insoluble cereal dietary fibre. *Journal of the Science of Food and Agriculture,* 81: 653-660.

Cabrera-Soto, M.L., Salinas-Moreno, Y., Velazquez-Cardelas, G.A., Espinoza, E. **2009**. Contenido de fenoles solubles e insolubles en las estructuras del grano de maíz y su relación con propiedades físicas. *Agrociencia* 43: 827-839

Cao, G., Alessio, H.M., Culter, R., **1993**. Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biology and Medicine.* 1: 303**-** 311.

Cevallos-Casals, B. A., Cisneros-Zevallos, L. **2003a**. Stochiometric and kinetic studies of phenolic antioxidants from andean purple corn and red-fleshed sweet potato. *Journal of Agricultural and Food Chemistry*, 51: 3313–3319.

Cevallos-Casals, B. A., Cisneros-Zevallos, L. A. **2003b**. A comparative study of antimicrobial, antimutagenic and antioxidant activity of phenolic compounds from purple corn and bilberry colorant extracts. In Book of Abstracts of the Institute of Food Technologists Technical Program Abstracts (pp. 29). Chicago, IL: Poster 14E-1.

Cevallos-Casals, B. A., Cisneros-Zevallos, L. **2004**. Stability of anthocyanin-based aqueous extracts of Andean purple corn and red-fleshed sweet potato compared to synthetic and natural colorants. *Food Chemistry*. 86: 69-77

Christie, P.J, Alfenito, M.R, Walbot V. **1994.** Impact of low-temperature stress on general phenylpropanoid and anthocyanin pathways: Enhancement of transcript abundance and anthocyanin pigmentation in maize seedlings. *Planta.* 194:541–549.

Cisneros-Zevallos, L. **2003**. The use of controlled postharvest abiotic stresses as a tool for enhancing the nutraceutical content and adding value of fresh fruits and vegetables. *J. Food Sci.* 68:1560-1565.

Coe, E. H., Neuffer, M. G., and Hoisington, D. A. **1998**. The genetics of corn, in *Corn and Corn Improvement*, 3rd ed., Sprague, G.F. and Dudley, J. W., Eds., Agron. Monograph 18, Am. Soc. Agron., Madison, WI

Cone, K.C. **2007**. Anthocyanin synthesis in maize aleurone tissue. *Plant cell Monographs*, Vol. 8: 121-139

Córdova, H. S. **1991**. Estimación de parámetros de estabilidad para determinar la respuesta de híbridos de maíz (*Zea mays L.*) a ambientes contrastantes de centro América, Panamá y México. *Agronomía Mesoamericana* 2: 01-10

Cortes, G.A., Salinas, M.Y., San Martın-Martinez, E., Martınez-Bustos, F. **2006**. Stability of anthocyanins of blue maize (*Zea mays L.*) after nixtamalization of separated pericarp-germ tip cap and endosperm fractions. *Journal of Cereal Science,* 43: 57–62

Dávila, J. **2007**. Procedimiento de preparación de reactivo DNS. Perez-Carrillo, E. (Ed). En: Manual de procedimientos del grupo de Bioetanol del Centro de Biotecnología FEMSA. Monterrey, México.

De la Parra, C., Serna-Saldivar, S.O., Liu, R.H. **2007**. Effect of processing on the phytochemical profiles and antioxidant activity of corn production of masa, tortillas and tortilla chips. *Journal of Agricultural and Food Chemistry*, 55: 4177-4183

Del Pozo-Insfran, D., Serna-Saldivar, S.O., Brenes, C., Talcott, S., **2007**. Polyphenolics and antioxidant capacity of white and blue corns processed into tortillas and chips. *Cereal Chemistry,* 84: 162-168.

Del Pozo-Insfran, D., Brenes, C. H., Serna-Saldívar, S. O., Talcott, S. T. **2006**. Polyphenolic and antioxidant content of White and blue corn (*Zea mays L*) products. *Food Research International*, 39: 696-703

Della Penna, D .**1999**. Nutritional genomics: manipulating plant micronutrients to improve human health. *Science* 285:375-379.

De Munter, J.S.L., Hu, F.B, Spiegelman, D, Franz, M., Van Dam, R.M. **2007**. Whole grain, bran, and germ intake and risk of type 2 diabetes: A prospective cohort study and systematic review. *PLoS Med* 4(8): e261. doi:10.1371/journal.pmed.0040261

Dickerson, G. W., **1990**. Blue Corn Production and Marketing in New Mexico, New Mexico Co-op Ext. Serv. Guide H-226

Dickerson, G.W. **2003**. Nutritional analysis of New Mexico blue corn and dent corn kernels. College of Agriculture and Home Economics. New Mexico State University.

Egesel, C. O., Wong, J. C., Lambert, R. J., Rocheford, T. R. **2003**. Combining ability of maize inbreds for carotenoids and tocopherols. *Crop Science,* 43: 818-823.

Espinosa-Trujillo, E., Mendoza-Castillo, Ma. Del C., Castillo-González, F., Ortiz-Cereceres, J., Delgado-Alvarado, A. **2010**. Aptitud combinatoria del rendimiento de antocianinas y de características agronómicas en poblaciones nativas de maíz pigmetnado. *Revista Fitotecnia Mex*icana, 33 (1): 11 – 19

Espinosa-Trujillo, E., Mendoza-Castillo Ma. Del C., Castillo-González, F., Ortiz-Cereceres, J., Delgado-Alvarado, A., Carrillo-Salazar, A., **2009**. Acumulación de antocianinas en pericarpio y aleurona del grano y sus efectos genéticos en poblaciones criollas de maíz pigmentado. *Revista Fitotecnia Mexicana,* 32 (4): 303 – 309

Food and agriculture organization (FAO). **2008**. Maize production statistical database.

Recuperado en: Junio 30, 2010, www.fao.org

Garcia-Alonso, M., De Pascual-Teresa, S., Santos-Buelga, C., Rivas-Gonzalo, J.C. **2004**. Evaluation of the antioxidant properties of fruits. *Food Chem*istry, 84: 13-18.

García-Lara, S., Bergvinson, D. J., Burt, A. J., Ramputh, A. I., Díaz-Pontones, D. M., Arnason, J. T. **2004**. The role of pericarp cell wall components in maize weevil resistance. *Crop Science*, 44(5): 1546-1552

García-Lara, S., Burt, A. J., Arnason, J. T., Bergvinson, D. J. **2010**. QTL mapping of tropical maize grain components associated with maize weevil resistance. *Crop Science,* 50(3): 815-825

Giusti, M.M., Wrolstad, R.E. **2001**. Anthocyanins. Characterization and measurement with UV-visible spectroscopy. Current Protocols in *Food Analytical Chemistry*, F 1.2- F 1.13. John Wiley & Sons, New York.

Gonzalez, R., Reguera, E., Mendoza, l., Figueroa, J., Sanchez, F. **2004**. Physiochemical changes in the hull of corn grains during their alkaline cooking. *Journal of Agricultural and Food Chemistry,* 52: 3831-3837

Gould, K. S. **2004**. Nature's swiss army knife: the diverse protective roles of anthocyanins in leaves. *Journal of Biomed Biotechnology*, (5): 314-320

Gutierrez-Uribe, J. A., Rojas-Garcia, C., Garcia-Lara, S., Serna-Saldivar, S. O. **2010**. Phytochemical analysis of wastewater (nejayote) obtained after lime-cooking of different types of maize kernels processed into masa for tortillas. *Journal of Cereal Science*, In Press, Corrected Proof, Available online 3 August 2010, ISSN 0733-5210, DOI: 10.1016/j.jcs.2010.07.003.

Hagiwara, A., Miyashita, K., Nakanishi, T., Sano, M., Tamano, S., Kadota, T., Koda, T., Nakamura, M., Imaida, K., Ito, N., Shirai, T. **2001**. Pronounced inhibition by a natural anthocyanin, purple corn color, of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-associated colorectal carcinogenesis in male F344 rats pretreated with 1,2-dimethylhydrazine. *Cancer Letters,* 171(1):17-25

Hallauer, A. R, Miranda, J.B. **1981**. Quantitative genetics in maize breeding. Ames: Iowa State Univ. Press

Harrigan, G.G., Stork, L. G., Riordan, S. G., Reynolds, T. L. **2007**. Impact of genetics and environment on nutritional and metabolite components of maize grain. *Journal of Agricultural and Food Chemistry*, 55: 6177-6185

Hernández-Uribe, J.P., Agama-Acevedo, E., Islas-Hernández, J.J., Tovar, J., Bello-Pérez, L.A. **2007**. Chemical composition and invitro starch digestibility of pigmented corn tortilla. *Journal of the Science of Food and Agriculture,* 87:2482-2487

Holton, T. A., Cornish, E. C. **1995**. Genetics and biochemistry of anthocyanin biosynthesis. *The Plant Cell*, 7: 1071-1083

Irani, N. G., Hernandez M.J., Grotewold E. **2003**. Regulation of anthocyanin pigmentation. *Recent Advances in Phytochemistry*, 37:59-78

Irani, N. G., Grotewold, E. **2005**. Light-induced morphological alteration in anthocyanin-accumulating vacuoles of maize cells. *BMC Plant Biology*, 5:7

Jing, P., Noriega, V., Schwartz, S.J., Giusti, M.M. **2007a**. Effects of growing conditions on purple corncob (*Zea mays L.*) anthocyanins. *Journal of Agricultural and Food Chem*istry, 55: 8625-8629

Jing, P., Giusti, M. M. **2007b**. Effects of Extraction Conditions on Improving the Yield and Quality of an Anthocyanin-Rich Purple Corn (*Zea mays L.*) Color Extract. *Journal of Food Science: Chemistry and Toxicology*, 72(7):363-368

Jones, K. **2005**. The Potential Health Benefits of Purple Corn. *HerbalGram.* 65: 46-49

Kahkonen, M. P., Hopia A. I., Vuorela, H. J., Rauha J. P., Pihlaja,K., Kujala, T. S., Heinonen, M. **1999**. Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. *Journal of Agricultural and Food Chemistry, 47:* 3954-3962

Kang, M.S., Balzarini, M.G., Guerra, J.L.L. **2004**. Genotype-by-environment interaction. In (A.M. Saxton ed.) Genetic Analysis of Complex Traits Using SAS. Cary, NC: SAS Institute Inc.

Kitamura, S. **2006**. Transport of Flavonoids, In: *The Science of Flavonoids*. Grotewold, E. Ed., Springer science, New York, pp. 133-135 (Ch 5)

Liu, R.H. **2007**. Whole grain phytochemicals and health. *Journal of Cereal Science* 46: 207–219

Liu, Z., Li, X. O., Weber, C., Lee, C.Y., Brown, J., Liu, R.H. **2002**. Antioxidant and antiproliferative activities of raspberries. *Journal of Agricultural and Food Chemistry*, 50: 2996-2930

López, A. F., Gómez, P.A. **2004**. Comparison of color indexes for tomato ripening. *Horticultura Braileira*, 22 (3): 534-537

Lopez-Martinez, L. X., Oliart-Ros, R. M., Valerio-Alfaro, G., Lee, C. H., Parkin, K. L. Garcia, H. S. **2009**. Antioxidant activity, phenolic compounds and anthocyanins content of 18 strains of Mexican maize. *LWT-Food Science and Technology*, 42(2): 1187-1192

Martinez-Tome, M., Murcia, M. A., Frega, N., Ruggieri, S., Jimenez, A. M., Roses, F., Parras, P. **2004**. Evaluation of Antioxidant Capacity of Cereal Brans, *Journal Agricultural and Food Chemistry, 52:* 4690-4699

May, J. B. **1987**. Wet Milling: Process and Products. In *Corn: Chemistry and Technology* S. A. Watson, and Ramstad, Paul E. (Ed.), (pp. 377-397). St. Paul, MN: American Association of Cereal Chemists.

Mora-Rochín, S., Gutiérrez-Uribe, J.A., Serna-Saldívar, S.O., Sánchez-Peña, P., Reyes-Moreno, C., Milán-Carrillo, J. **2010.** Phenolic content and antioxidant activity of

tortillas produced from pigmented maize processed by conventional nixtamalization or extrusion cooking. *Journal of cereal Science.* Article in press: doi:10.1016/j.jcs.2010.08.010

Moreno, Y. S., Sánchez, G. S.; Hernández, D. R.; Lobato, N.R. **2005**. Characterization of anthocyanin extracts from maize kernels. *J. Chromatogr. Sci*., 43: 483-487.

Ortega, P. R., Sanchez J.J., Castillo F., Hernandez, J.M. **1991**. Estado actual de los estudios sobre maíces nativos de México. En: *Avances en el Estudio de los Recursos Fitogenéticos de México*; Ortega, P., Palomino, G., Castillo, H., Gonzales, V. A., Livera, M. Ed.; Avances en el estudio de los recursos fitogenéticos de México. Sociedad Mexicana de Fitogenética A. C. (SOMEFI). Chapingo México, pp: 161-185

Pascual-Teresa, S., Sánchez-Ballesta. **2008**. Anthocyanins: from plant to health. *Phytochemistry Reviews*. 7: 281-299

Paulis J.W., James, C., Wall, J.S. **1969**. Comparison of glutelin proteins in normal and high lysine corn endosperm. *Journal of Agriculrutal and Food Chemistry,* 17:1301–1305

Pedreschi, R., Cisneros-Zevallos, L. **2007**. Phenolic profiles of Andean purple corn (*Zea mays L*.). *Food Chemistr,.* 100: 956–963

Piepho, H. P., Mohring, J. **2007**. Computing heritability and selection response from unbalanced plant breeding trials. *Genetics*. 177: 1881-1888

Preciado, R. E. **2010**. Hibridos de maíz pigmentado de alto potencial de rendimiento para regiones subtropicales de riego. En libro de memorias de XXIII Congreso nacional de fitogenética (pp.80). Nuevo Vallarta, Nayarit. Ponencia 18.

Puupponen-Pimia, R., Nohynek, L., Meier, C., Kahkonen, M., Heinonen, M., Hopia, A., Oksman-Caldentey, K.-M. **2001**.Antimicrobial properties of phenolic compounds from berries. *Journal of Applied Microbiology*. 90: 494-507

Ribaut, J.M., **2006.** Drought tolerance in maize. In *Handbook of Maize: It's Biology*. Bennetzen, J.L., Hake, S.C. Eds., Springer, New York (Ch:16)

Rojas, C., Tinoco, B. **2010**. Obtención de películas biopolimericas flexibles con acción antioxidante a partir de harina de maíz azul**.** Patente WO/2010/024657, 4 Marzo 2010

Rooney, L., W., Dykes, L. **2007**. Phenolic compounds in cereal grains and their health benefits. *Cereal food world*, 52(3):105-111

Rosado, J.L., Diaz, M., Rosas, A., Griffit, I., Garcia, O.P. **2005**. Calcium absorption from corn tortilla is relatively high and is dependent upon calcium content and liming in Mexican women. *J. Nutr*. 135:2578-2581

SAGARPA. **2010**. Servicio de información y estadística agroalimentaria y pesquera. Secretaria de agricultura, ganadería, desarrollo rural, pesca y alimentación. Producción anual por cultivo: maíz. Recuperado en 19 de Agosto de 2010 en www.siap.sagarpa.gob.mx.

Sakakibara, H., Honda, Y., Nakagawa, S., Ashida, H., Kanazawa, K. **2003**. Simultaneous determination of all polyphenols in vegetables, fruits and teas. *Journal of Agricultural and Food Chem*istry, 51: 571-581

Salinas-Moreno, Y., Saavedra-Arellano, S., Soria-Ruíz, J., Espinosa-Trujillo, E. **2008**. Características fisicoquímicas y contenido de carotenoides en maíces (*Zea mays L.*) amarillos cultivados en el Estado de México. *Agricultura Técnica en México.* 34(3): 357-364

Salinas-Moreno, Y., Vázquez, G. **2006**. Metodologías de análisis de la calidad nixtamalera-tortillera en maíz. INIFAP. Folleto Técnico No. 24. 98

Salinas-Moreno, Y., Martínez-Bustos, F., Soto-Hernández, M., Ortega-Paczka, R., Arellano-Vázquez, J. L. **2003**. Effect of alkaline cooking process on anthocyanins in pigmented maize grain. *Agrociencia.* 37: 617-628.

Salinas-Moreno, Y., Martínez, F., Gómez, J. **1992**. Comparación de métodos para medir la dureza del maíz (*Zea mays* L.). *Arch. Latin. Nutr*. 42: 59-63.

Sanchez, G. J. J., Goodman, M. M., Stuber, C. W. **2000**. Isozymatic and morphological diversity in the races of maize of México. *Econ. Bot*. 54:43-59

Scanlon, M.J., Schneeberger, R.G., Myers, A.M., Freeling, M. **1997**. The empty pericarp-2 gene is required for leaf development during maize embryogenesis. *Plant J.*12: 901–909

Serna-Saldívar, S. O. **2010**. Chemical composition of cereal grains. In *Cereal Grains: Properties, Processing, and Nutritional Attributes.* Serna-Saldívar, S.O. Ed.; CRC Press LLC., Boca Raton, Florida (CH 3)

Serna-Saldívar, S.O. **1996**. Química, Almacenamiento e Industrialización de los Cereales. AGT Editor, México, D.F., México

Serna-Saldívar, S.O., Gómez, M. H., Rooney, L. W. **2001**. Food uses of regular and specialty corns and their dry-milled fractions. In *Specialty corns*, 2nd ed., Hallauer, A.R., Ed..; CRC press LLC., Boca Raton, Florida (CH11)

Serna-Saldívar, S. O., Gómez, M. H., Rooney, L. W. **1990**. The Technology, Chemistry, and Nutritional Value of Alkaline Cooked Corn Products. In *Advances in Cereal Science and Technology*; Pomeranz, Y., Ed.; American Association of Cereal Chemists: St. Paul, MN. Vol. 10. 243-307

Serna-Saldívar, S. O., Amaya, C.A., Herrera, P., Melesio, J.L., Preciado, R. E., Terron, A. D., Vazquez, G. **2008**. Evaluation of the lime-cooking and tortilla making properties of quality protein maize hybrids grown in Mexico. *Plant Foods in Human Nutrition,* 63:119-125

Shih, P.H., Yeh, C.T., Yen, G.C. **2005**. Effects of anthocyanidin on the inhibition of proliferation and induction of apoptosis in human gastric adenocarcinoma cells. *Food Chem Toxicol.* 55:1557–1566

Slavin, J. L., Jacobs, D., Marquart, L., Wiemer, K. **2001**. The role of whole grains in disease prevention. *Journal of the American Dietetic Association.*101:780-785.

Talcott, S. T., Percival, S. S., Pittet-Moore, J., Celoria, C. **2003**. Phytochemica composition and antioxidant stavility of fortified yellow passion fruit (*Passiflora edulis*). *Journal of Agricultural and Food Chem*istry, 51: 935-941.

Temple, N.J. **2000**. Antioxidant and disease: More questions than answers. *Nutr. Res*: 20: 449-459.

Tollenaar, M., Wu, J. **2000**. Improving maize grain yield potential in a cool environment, in *Physiological Bases for Maize Improvement*, Otegui, M. E., Slafer, G. A., Ed.; Food products press, Binghampton, NY, (CH2)

Tsuda, T., Horio, F., Osawa, T. **2002.** Cyanidin 3-O-β-D-glucoside suppresses nitric oxide production during zymosan treatment in rats. *J Nutr. Sci. Vitaminol*. 48(4):305-310.

Tsuda, T., Horio, F., Uchida, K., Aoki, H., Osawa, T. **2003**. Dietary cyanidin 3-*O*-β-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice. *Journal of Nutrition.* 133: 2125–2130.

Tsuda, T., **2008**. Regulation of adipocyte function by anthocyanins; possibility of preventing the metabolic syndrome. *Journal of Agricultural and Food Chemistry*, 56(3): 642–646

Ultrilla-Coello, R.G., Agama-Acevedo, E., Barba de la rosa, A.P., Martínez –Salgado, J.L., Rodriguez-Ambriz, S.L., Bello-Perez, L.A. **2009**. Blue maize: morphology and starch synthase characterization of starch granule. *Plant Foods Hum Nutr*. 64: 18-24

Vavilov, N. I. 1931. Mexico and Central America, as the principal centre of origin of the cultivated plants of the New World. *Bull. Of Appl. Botany.* 26:179-199

Vielle-Calzada, J.-P., Padilla, J. 2009. The mexican landraces: description, classification and diversity, In *Handbook of maize: Its Biology.* Bennetaen, J.L., Hake, S.C. Eds. Springer Science, New York. pp. 543-561 (Ch: 27)

Wang, L.S., Stoner, G.D. **2008**. Anthocyanins and their role in cancer prevention. *Cancer Lett*. 269(2): 281–290.

Warburton, M.L., Reif, J.C. , Frisch, M., Bohn, M., Bedoya, C., Xia, X.C., Crossa, J. Franco, J., Hoisington, D., Pixley, K., Taba, S., Melchinger, A.E. **2008**. Trends in genetic diversity in CIMMYT non-temperate maize germplasm. *Crop Science*, 48: 617-624

Welch, C. R., Wu, Q. Simon, J. E. **2008**. Recent advances in anthocyanin analysis and characterization. *Curr. Anal. Chem*. 4(2): 75-101

Westcott, B. **1986**. Some methods of analyzing genotype-environment interaction. *Heredity*, 56: 243-253

Wildman, R. E. C. **2000**. Handbook of Nutraceuticals and Functional Foods CRC Press: Boca Raton, London, New York, Washington D.C.

Wrolstad, R.E. **1976**. Color and pigment analyses in fruit products. Oregon Agronomic Expt. Sta. Bul. 624.

Wyszecki, G..Stiles, W.S. **1982**. *Color Science*. John Wiley & Sons: New York

Yang, Z., Chen, Z., Yuan, S., Zhai, W., Piao, X., Piao, X. **2009**. Extraction and identification of anthocyanin from purple corn (*Zea mays L.*). International Journal of Food Science and Technology, 44:2485-4292

Zhao, X., Corrales, M., Zhang, C., Hu, X., Ma, Y., Tauscher, B. **2008.** Composition and thermal stability of anthocyanins from Chinese purple corn (*Zea mays* L.). *Journal of Agricultural and Food Chem*istry, 56: 10761–10766