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RESEARCH ARTICLE

Chemical analysis of blue corn base malt and the effect of malting and roasting of blue corn malts on color and antioxidant compounds associated with antioxidant capacity

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ABSTRACT

The objectives of this research were to carry out a chemical analysis of the base malts, to evaluate the effect of malting and roasting on both color and compounds associated with the antioxidant capacity (anthocyanins, phenolic compounds, melanoidins), and the capacity itself by ABTS and DPPH assays in base malt and caramel malt of blue corn. In order to provide styles of beers with different sensory characteristics as well as health benefits for the consumer, and since malt is the heart of beer, different cereals and pseudo-cereals have been malted. In addition to this, with the intention of taking advantage of the phenolic compounds present in the blue corn grain, and of developing color compounds as well as antioxidant activity through roasting, both blue corn base and caramel malts were developed. Results showed that the protein content was within the specified range for barley malt and, despite the malting not having a significant effect on the color parameters, a decrease in the anthocyanin content was observed. On the contrary, an increase in the content of phenolic acids as well as in the antioxidant capacity by DPPH assay in the base malt with respect to the grain was noticed. Additionally, the color of caramel malts increased in luminosity and chroma, and decreased in tone due to roasting. Likewise, the concentrations of anthocyanins and phenolic compounds depended on the roasting conditions. Moreover, the higher the roasting temperature was, the higher the concentrations of melanoidins were. Finally, malts that had a greater amount of phenolic compounds showed greater antioxidant capacity by ABTS assay, while malts that had a higher melanoidins content showed a greater antioxidant response by DPPH assay. In accordance with the results, it was concluded that the combined use of base and caramel malts to produce corn beer musts could provide significant amounts of compounds that will impact its color and antioxidant capacity, thereby favoring the chemical stability of the product.

Keywords: Antioxidant capacity, Base malt; Blue corn; Caramel malt; Phenolic compounds

INTRODUCTION

Some beers are exclusively made with pilsner malt, but others owe their typical flavor and characteristic color to the addition of a small amount of roasted or specialty malts (Yahya et al., 2014; Gruber, 2001). During its production, the thermal processes (drying and roasting) have the greatest impact on the color, flavor and antioxidants of malt (Yahya et al., 2014, Coghe et al., 2006). Depending on the time and temperature applied in the thermal processes, the malt color can vary, and the molecules responsible for the different colors are chromophores of different molecular weights, products of Maillard reactions (Coghe et al., 2006).

In relation to the study of compounds and antioxidant activity in barley malts, the dominant phenolic was ferulic acid in ten barley varieties and their corresponding malts (Dvořáková et al., 2008), the differences between the kilning regimens used in green malt suggest that further modification of the regimens could lead to greater release of bound phenolics with consequent beneficial effects on flavor stability in beer and, more generally, on human

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health (Inns et al., 2007), the malts with the highest degree of heat treatment in different malt types were characterized by the highest antioxidant activity, which was due to the content of Maillard reaction products with antioxidant capacity (Shopska et al., 2021), and that in kilned malts, phenolic compounds were the main identified contributors to antioxidant activity, and in roasted malts, Maillard reaction products were responsible for the majority of the antioxidant activity (Samaras et al., 2005).

On the other hand, with the intention of providing styles of beers with different sensory characteristics, as well as with other benefits to the consumer´s health, cereals and pseudo-cereals other than barley have been malted to obtain beer. For example, Mayer et al., obtained malts from rice (Mayer et al., 2016), Kordialik-Bogacka et al. (2014) studied malting of oat, buckwheat by Kolawole and Ebiloma, (2017), quinoa by Carciochi et al. (2016), sorghum by Rubio-Flores et al. (2020), and blue corn by Romero-Medina et al. (2020).

Corn contains a higher amount of phenols and antioxidant capacity than other cereals such as wheat, rice and oats (Escalante-Aburto et al., 2013). Specifically in blue corn, phenolic acids like p-coumaric, vanillic and protocatechuic (Pedreschi and Cisneros-Zevallos, 2007), and the anthocyanins cyanidin 3-glucoside, pelargonidin 3-glucoside and peonidin 3-glucoside have been identified (Abdel-Aal et al., 2006; Yang et al., 2009), these last compounds have been recognized as substances that improve health, due to different biological activities, such as antioxidant (Castilla et al., 2008), anti-inflammatory (Karlsen et al., 2007), and anti-cancer (Wang et al., 2007).

The objectives of the present study were to evaluate the effect of malting of blue corn through a proximal chemical analysis, and to evaluate the effect of malting and roasting on malt color, and the compounds associated with the antioxidant capacity (melanoidins, anthocyanins, polyphenols) and the capacity itself by ABTS and DPPH assays of base malt and caramel malt of blue corn.

MATERIALS AND METHODS

Solvents, reagents and standards

Deionized water and HPLC grade methanol were purchased from Honeywell (Muskegon, MI). Milli-Q ultrapure water was used in all experiments. Phosphoric acid for HPLC 85-90% Honeywell-Fluka. Glacial acetic acid, boric acid, hydrochloric acid (37% w/w), sulfuric acid (98%), sodium carbonate, D-glucose, hexanes, sodium hydroxide, and potassium persulfate from J.T. Baker (Xalostoc, Mexico). Kjeltabs CX (Gerhardt) catalyst tablet composed of 5g of potassium sulfate and 0.5g of copper sulfate. Folin-Ciocalteau reagent from Hycel (Zapopan, Jalisco). Glycine from Merck (Darmstadt, Hesse). Ethanol (96%) from Zapopan alcoholic plant (Zapopan, Jalisco). Methanol from Herschi Trading (Iztapalapa, Mexico City). DPPH (2,2-Diphenyl-1-picrilhydrazyl), ABTS (2,2'-azino-bis (3-ethylbenzothiazoline)- 6-sulfonic acid); and the standards: gallic acid (3,4,5-trihydroxybenzoic), syringic (4-hydroxy-3,5-dimethoxybenzoic), p-coumaric (predominantly trans-isomer), and cyanidin-3-glucoside chloride (chloride of kuromamin) from Sigma-Aldrich (St. Louis, MO), for the determination of phenolic compounds and anthocyanins by HPLC, respectively.

Corn and malt samples

Chalqueño (race) blue corn produced in Milpa Alta, Mexico City, was used. The blue corn base malt was obtained according to the malting process described in the Mexican Patent No. 365,910 (Verde-Calvo et al., 2019). To produce green malt, the clean grain was soaked for 24 hours by total immersion in drinking water, the water was removed and the corn was allowed to germinate for 3 days in a germination chamber at 80% humidity and at 25 °C. Subsequently, it was dried for 2 days at 50 °C in a gravity convection oven (Felisa, Mod. FE, Mexico), to obtain the base malt. The blue corn caramel malts were obtained by subjecting the green malt to a drying period at 70 °C for 3.5 h in a gravity convection oven. Finally, these malts were roasted in batches of 1.5 kg in a coffee roaster (100% MEX, Coatepec, Veracruz) under the conditions of temperature and time described in Table 1.

PHYSICOCHEMICAL ANALYSIS

Sample preparation

Prior to physicochemical analysis, the blue corn and malts were ground and passed through a No. 40 sieve.

Physicochemical analysis

Proximal chemical analysis in blue corn and base malt were carried out through the following determinations (AOAC, 2002): moisture 925.10, ashes 923.03, total nitrogen and crude protein 991.20, ether extract 920.35 and crude fiber (Weende Method).

Table 1: Temperature and time conditions used in the roasting of blue corn caramel malts

Caramel malts	Temperature (°C)	Time (min)
1	150	45
2	175	20
3	180	15
4	200	5
5	225	5
6	240	5

The following analyses were performed on all samples: blue corn, base malts and caramel.

Moisture

It was performed according to the AOAC method 925.10 (2002).

Color measurement

Color was determined according to Salinas-Moreno et al. (2012) in a Colorflex colorimeter (HunterLab, Virginia, USA), for which an ear with whole grains was built for each sample, on a gray base plasticine. The CIE-LAB chromatic parameters: L*, a* and b*, allowed the calculation of tint (h) and chromaticity © through the equations: $h=\arctan(b^*/a^*)$ and $C=(a^{*2}+b^{*2})^{1/2}$.

Total anthocyanins (TA)

The extraction and determination of total anthocyanins were performed according to Abdel-Aal and Hucl (1999). For each sample, 0.125 g of flour was mixed with 15 mL of 95% ethanol acidified with 1N HCl (85:15) and stirred for 60 min in darkness, at room temperature. The extract was centrifuged at 3000 rpm for 15 min, the supernatant was separated, and the volume was made up to 15 mL with 95% ethanol acidified with 1N HCl (85:15). Total anthocyanins were calculated using the following formula: $TA=(A/\varepsilon)*(Vol/1000)*MW*(1/\text{weight of the sample})*10^6$, where: A= absorbance, ε= molar absorptivity (cyanidin 3-glucoside= 25965 $cm^{-1}M^{-1}$), MW= molecular weight of cyanidin 3-glucoside(449.2 g/mol), Vol= final volume of the solution (15 mL). Results were expressed as equivalent mg of cyanidin 3-glucoside (ECG)/kg of sample in dry mass.

Anthocyanins by HPLC

For the determination of anthocyanins by HPLC, the same extract obtained for total anthocyanins was used. The solvent was evaporated under reduced pressure on a rotary evaporator at 35 °C, resuspended in methanol 0.01% HCl and passed through a 0.45µ polytetrafluoroethylene (PTFE) filter. HPLC anthocyanins analysis was performed by using an Agilent 1260 Infinity, (Agilent Technologies, Germany), equipped with a quaternary pump with integrated degasser (G1311B), autosampler (G1329B), column heater (G1316A), and diode array detector (G1315C). Chromatographic separation was carried out according to Hebrero et al. (1988) with slight modifications. A Zorbax SB column (250 x 4.60 mm, 5 µm) (Agilent, USA) at 25 °C was used. The eluents used were water-phosphoric acid mixture (96:4, pH 1.0), as eluent A, and acetonitrile, as eluent B.Gradient elution was performed as follows: 90-85% of A from 0 to10 min, 85-80% of A from minute 10 to 20, 80-70% of A from minute 20 to 38, and 70-90% of A from minute 38 to 40; the flow rate was 1 mL/min, injection volume of 10 µL, detector at 280 nm and 520 nm, and the total analysis time was 40 min. To quantify anthocyanins in samples, an external standard calibration curve, consisting of seven concentrations (from 0.03 to 0.25 µg/L) of cyanidin 3-glucoside (CG) in methanol at 0.01% HCl, was constructed.

Total phenolic compounds (TPC)

The extraction and quantification of total phenolic compounds by Folin-Ciocalteu reagent was carried out according to Velioglu et al. (1998). To perform this, 0.4 g of dehydrated flour was mixed with 4 mL of 80% methanol acidified to 1% with 1 N HCl, and this mixture was centrifuged at 3000 rpm for 15 min. The supernatant was poured in an amber flask, 4 mL of 80% MeOH acidified to 1% with 1 N HCl were added to the precipitate, and it was centrifuged again. The supernatants were pooled, and the volume of extract was measured. Then, 100 µL of extract were placed in test tubes, 750 µL of Folin's reagent (diluted 1:10 with distilled water) was added, and they were left to react for 5 min in darkness. Subsequently, 750 µL of Na_2CO_3 (60 g/L) were added, and their absorbance was read at 725 nm after 90 min. A calibration curve was made with six gallic acid concentrations between 25 and 250 mg/L. Results were expressed as mg of gallic acid equivalents (EAG)/g of sample in dry mass.

Phenolic compounds by HPLC

For the determination of phenolic compounds by HPLC, the same extract as for the quantification of total phenolic compounds was used. The solvent was evaporated under reduced pressure on a rotary evaporator at 40 °C, until a volume of about 0.8 mL was obtained, and it was passed through a 0.45µ PTFE filter.

The phenolic compounds HPLC analysis was carried out by using the Agilent 1260 Infinity equipment (Agilent Technologies, Germany) mentioned above. Chromatographic separation was achieved using a Zorbax SB column (250 x 4.60 mm, 5 µm) (Agilent, USA) at 30 °C. The eluents used were: water-acetic acid (98:2, pH 2.5), as eluent A, and methanol, as eluent B; gradient elution was: 90 to 85% of A from 0 to 10 min, 85 to 65% of A from minute 10 to 25, and 65 to 60% of A from minute 25 to 35; the flow rate was 0.8 mL/min, injection volume of 10 µL, detector at 275 nm, and the total analysis time was 35 min. To quantify phenolic compounds in samples, three external standard calibration curves, consisting of six concentrations of gallic acid, syringic acid, and p-coumaric acid (between 8.75-170 mg/L, 2.75-55 mg, and 5.25-105 mg, respectively), were constructed. Results were expressed as mg of the respective phenolic acid/g of sample in dry mass.

Melanoidins

Melanoidins content was determined according to Borrelli et al. (2002) and Wagner et al. (2002), for which the standard was prepared using the Cost Action 919 protocol for the glucose-glycine model. The calibration curve was constructed in a concentration range of 60-360 mg of melanoidins/L, read at 405 nm.

The extract obtained for the determination of TPC was used for the determination of melanoidins. Results were expressed as mg of melanoidins/kg of sample in dry mass.

Antioxidant capacity

To measure the antioxidant capacity of the samples, the extracts obtained for the determination of CFT were used. The methods used were ABTS (2,2'-azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid radical scavenging activity) and DPPH (2,2-diphenyl-1-picrihydracil) radical scavenging activity, according to Kuskoski et al., (2005).

At 980 µL of the dilution of the ABTS radical generated, the absorbance value was determined, and then 20 µL of the sample were added. The absorbance value was measured every minute up to 7 minutes.

A volume of 1.95 mL of 0.115 mL solution of DPPH radical dissolved in 80% methanol was mixed with 50 µL of sample dilution, homogenized and kept in darkness for 30 min. After this time, absorbance readings were made at 517 nm before adding the sample, and after 30 minutes absorbance was determined again.

In both methods, a curve of inhibition percentage versus concentration was constructed in a range of 20-320 mg of Trolox/L, and results were expressed as equivalent mg of Trolox/g sample in dry mass.

STATISTICAL ANALYSIS OF DATA

Data were expressed as mean ± standard deviation. A *Student's t-test* was used in the following analyses: 1) to compare the proximal chemical composition, color, anthocyanins and total phenolic compounds of the blue corn kernel used with respect to reported values; 2) to evaluate the effect of malting on the proximal chemical composition, the contents of the compounds associated with antioxidant capacity (total anthocyanins, HPLC anthocyanins, total phenolic compounds, HPLC phenolic compounds and melanoidins) and the antioxidant capacity by ABTS and DPPH assays.

The response variables to evaluate the effect of roasting on malts (color, total anthocyanins, HPLC anthocyanins, compounds total phenolics, phenolic compounds by HPLC, melanoidins and antioxidant capacity by ABTS and DPPH assays) were analyzed through two-factor analysis of variance (ANOVA) (temperature and time), as well as through Tukey's multiple comparison test. In all cases, it was considered that there was significant difference when $p<0.05$. The statistical analyses were developed using the XL-STAT program (Statistic for Windows, version 2012.5.02).

RESULTS AND DISCUSSION

Proximal chemical analysis of blue corn and base malt

Table 2 presents the results of the Proximal chemical Analysis (PCA). The moisture content and ethereal extract of the blue corn used to obtain the malts were not significantly different from those reported by Ortíz (2006), who analyzed different creole varieties of blue corn. On the contrary, the percentages of protein and ash showed a difference, since these percentages were lower in the present study. On the other hand, when compared with García-Campos et al. (2020), who analyzed blue creole corn from the State of Mexico, the protein and ethereal extract contents of the blue corn used were not significantly different, while the percentages of moisture and ash showed a difference. Regarding the content of ethereal extract, it was observed that the percentage of this component in the three studies did not show significant differences, and the differences between the other reported components may be due to the variety of corn, field management practices, climate, soil, harvest, and post-harvest methods (Robutti, 2004), as well as the use of different analysis methods.

Components derived from proteins and proteins are important due to their effect on the organoleptic character of beer and their importance for yeast nutrition. Biochemical evidence has established that α-amylase, endoprotease, and borderline dextrinase are produced de novo in aleurone layers stimulated by gibberellic acid (Palmer, 2006); according to this, an increase in enzyme content does not generate a significant increase in protein content of the base malt with respect to the blue corn grain.

The ethereal extract in the corn grain and the base malt represents an important percentage. In barley worts, from 3% of lipids present in barley, only 0.01% of them are extracted, mainly fatty acids (Palmer, 2006). This same situation can occur in worts made with blue corn malt, but further investigation is necessary to verify this. Corn ash includes minerals such as phosphorus, potassium, magnesium, and sodium, mainly (Ortíz, 2006). Minerals during brewing are necessary to maintain the structural integrity of the cell, flocculation, gene expression, cell division, nutrient intake, enzymatic activity, among others (Tenge, 2009). The content

of minerals expressed as ash in the chemical analysis did not show significant differences when comparing the corn grain content with that of the base malt.

The effect of malting on the PCA was observed as a decrease in the humidity percentage and an increase in the NFE (which represents sugars and starches), due to the thermal process (drying) to which the malt was subjected. The rest of the components have no variations, which indicates that the modifications presented during germination (to maintain embryonic growth and the limited modification of the endosperm) do not cause significant changes in the percentages of protein, ether extract, ash, and crude fiber.

Moisture contents and color parameters

Moisture content and color parameters of both blue corn and malts are presented in Table 3. The percentage of grain moisture decreased significantly from 10.7 to 4.5% in the base malt. De Meo et al. (2011) reported moisture contents in sorghum, buckwheat, quinoa, and amaranth malts of 6.86, 5.32, 8.13 and 5.48 %, respectively, and Mayer et al. (2014) reported humidity values between 4.3 and 6.7%, for rice malts. These reported values do not differ significantly from those obtained for the corn base malt.

The moisture contents in the caramel malts were between 1.4 and 3.3%. The effect of roasting, shown as a decrease in moisture content, was less in the 175 °C malt, followed by the 150 and 200 °C malts, and the effect was greater in the malts that were roasted at temperatures of 180, 225 and 240 °C, since they presented moisture contents between 1.6 and 1.4%. Yahya et al. (2014) reported a moisture content of 5% in crystal malt, which is significantly higher than those of the blue corn caramel malts.

Luminosity (L*) is an attribute related to the observed light transmission, the hue angle (h) is its qualitative expression, and chroma (C*) is the quantitative component of chromaticity, which is a two-dimensional parameter that is correlated to the colorful visual sensation attribute. When comparing the values of L*, h and C* between the grain and the blue corn base malt, they did not show a significant difference, which indicates that the malting of the corn had no significant effect on the color parameters.

The color results obtained for blue corn were within the ranges reported by Salinas-Moreno et al. (2012), who analyzed six populations of blue corn of the same variety used in the present study, and they reported L* values from 15.3 to 20.2, tone angle values from 299 to 335.9, and chroma values from 0.5 to 2.3. Also, they specified that, according to these values, the analyzed samples were dark blue/purple and not very bright, the same as the samples analyzed in the present study.

The color parameters in the caramel malts showed that their luminosity increased with respect to that of the grain, and it was significantly higher in the 175 and 200 °C malts. Regarding the tone, this parameter decreased in the caramel malts in relation to blue corn, and the values were between 274.9 and 284.6. These values are close to a blue tone whose h is 270°; this means that the initial dark blue/purple tone of the corn grains turned into a blue tone after producing the caramel malts. Finally, the C* values of roasted malts increased compared to corn grains, and they were significantly higher in 240 °C malt. The changes in color parameters reflect the effect of roasting on the concentration of the color compounds in the malt, namely the degradation of anthocyanins and the formation of melanoidins.

Table 2: Proximal chemical analysis of grain and blue corn base malt

	Moisture[a]	Protein	Ethereal extract	Ashes	Raw fiber	NFE[a]
Blue corn	10.7±1.2	9.1±0.1	4.8±0.3	1.3±0.05	0.13±0.05	73.9±1.7
Base malt	4.5±0.2	8.6±0.5	5.0±0.5	1.2±0.1	0.11±0.02	80.6±1.3

Ortíz (2006) reports moisture: 9.02±0.58, protein: 9.73±0.72, ether extract: 5.35±0.94, ash: 1.52±0.11. NFE: Nitrogen-free extract. García-Campos *et al.* (2020) report 13.94±0.018, protein 8.81±0.19, ether extract 5.15±0.05, ashes 1.43±0.0067. The letter ([a]) indicates a significant difference ($p<0.05$) between blue corn and base malt. Protein content in barley malt: 9.5-11, reported by Kreisz (2007)

Table 3: Moisture content and color parameters in blue

Sample	Moisture (%)	L (%)	Tone (h) (°)	Chroma
Blue corn	10.7* ± 1.2	14.7±0.71	333.7±10.2	1.73±0.25
Base malt	4.5±0.2	15.3±0.19	314.7±11.4	1.43±0.56
150°C-45 min	2.0±0.1 b	22.6±0.66 b	274.9±2.41 c	6.39±0.65 b
175°C-20 min	3.3±0.1 a	25.8±1.05 a	281.9±2.4 ab	7.62±1.41 ab
180°C-15 min	1.6±0.1 c	21.2±0.23 bc	283.2±2.53 ab	8.04±0.33 ab
200°C-5 min	1.7±0.2 bc	26.8±0.38 a	277.7±1.7 bc	7.25±0.24 ab
225°C-5 min	1.4±0.1 c	19.6±1.64 cd	284.6±3.22 a	7.97±0.45 ab
240°C-5 min	1.4±0.1 c	17.5±0.51 d	280.5±2.52 abc	8.58±0.77 a

Values are presented as mean and standard deviation. L: luminosity. The asterisk (*): indicates significant difference ($p<0.05$) when comparing blue corn with base malt. Different letters in the same column indicate significant difference ($p<0.05$) between caramel malts

Content of anthocyanins, phenolic compounds, and melanoidins in blue corn and base malt

Chalqueño blue corn presented a quantity of total anthocyanins (TA) (Table 4) significantly higher than those reported by Yang and Zhai (2010) for purple corn from China (558 mg/kg), by Zhao et al. (2008) for the varieties Jinheiyu, Perla negra and Shijiazhuang (127.4, 292.2, and 1493 mg/kg dm, respectively), as well as those determined by Salinas-Moreno et al. (2012) for blue corn of the same variety used in the present study (579.4 to 1046.1 mg ECG/kg dm). These differences in anthocyanin content among the varieties of corn mentioned can be due to several factors, including genotypes, developmental stages, growth conditions, and even the methods used for their quantification (2007).

A TA content decrease of 17.7% was observed in the base malt, in comparison to the grain, when the quantification was carried out using the spectrophotometric method; while in the determination by HPLC, the decrease was of 45.8%. This may be due to two factors: 1) Germination, since according to Paucar-Menacho et al. (2017), germination of purple corn causes a significant reduction of most of the anthocyanins present in grain. 2) Malt drying, since the stability of the anthocyanins is affected by several factors, one of which is temperature (Castañeda-Ovando et al., 2009).

As shown in Table 4, the anthocyanin content in corn and malt was higher with the spectrophotometric method than with HPLC. This same behavior was also reported by Abdel-Aal et al. (2006) and Lao and Giusti (2016), who indicated that it is due to the low specificity of the spectrophotometric method, since the single measurement at 535 nm considers that the answer is due solely to anthocyanins. However, other red pigments, such as phylobaphenes and their building block, 3-deoxianthocyanins, have been reportedly found in purple corn, in addition to monomeric and polymeric anthocyanins (Selinger and Chandler, 1999).

Abdel-Aal et al. (2006) indicated that deoxianthocyanins represented about 50% of the total anthocyanins, in addition to phylobaphenes which are alcohol soluble pigments. So, the total anthocyanin method could have overestimated the anthocyanins content in the grains.

The TPC content determined in blue corn was 1307 ± 62 mg GAE/kg dm (Table 4), which agrees with the range reported by Salinas-Moreno et al. (2012) (918.9 at 1479.2 mg/kg dm) for the same variety of corn used in the present study, and is significantly lower ($p<0.05$) than those reported by Paucar-Menacho et al. (2017), as well as by Jing et al. (2007) (16797, and from 9500 to 14310 mg/kg dm, respectively) for Peruvian varieties of purple corn (Paucar-Menacho et al., 2017; Jing et al., 2007).

During malting, the TF concentration in grains increased significantly ($p<0.05$), reaching a value of 2508 mg GAE/kg dm. Germination has been reported to increase the levels of total phenolic compounds in other grains such as barley, rye, sorghum, wheat, and rice (Donkor et al., 2012; Cáceres et al., 2014). The increase of phenolic acids content during malting, could be due to the action of endogenous esterases synthesized during germination, which can lead to the release of phenolic compounds originally bound to cell walls (Maillard et al., 1996; Acosta-Estrada et al., 2014), or due to novo synthesis (Tomková-Drábková et al., 2016).

With respect to melanoidins content, it also increased during malting due to drying. And, as shown in Table 4, the melanoidins content of base malt was 1071 ± 41 mg/kg dm.

In order to characterize phenolic acids in the samples, in addition to confirming the observed behavior of total phenolic compounds, a chromatographic analysis of the main phenolic compounds in corn, as well as in both base and caramel malts was performed (Tables 5 and 6). As can be seen in Table 5, the content of phenolic acids in the base malt (113.3 mg acids/kg d.m) increased, compared to the unmalted grain (37.9 mg acids/kg d.m). Also, the HPLC analysis indicated the presence of gallic and p-coumaric acids in both blue corn and the base malt; and, in this latter, syringic acid was also found, in addition to the two aforementioned acids. According to these results, gallic acid is the most abundant free phenolic compound found in the extracts of blue corn and base malt.

Many authors have reported the presence mainly of ferulic acid in blue corn (Lopez-Martínez et al., 2009; Del Pozo-Insfran et al., 2006). However, in the present study the acid mentioned was not identified, which may be due

Table 4: Content of anthocyanins, phenolic compounds and melanoidins in blue corn and base malt

Sample	Anthocyanins (mg cianidin-3-glucoside/ kg d.m)		Phenolic compounds (mg gallic acid eq./kg d.m)	Melanoidins (mg/kg d.m)
	Total	HPLC	Folin-Ciocalteau	
Blue Corn	1534[a]±10.1	189.7[a]±15.7	1307[a]±62	ND
Base malt	1263±45.8	102.9±17.3	2508±17	1071[a]±41

Values are presented as mean and standard deviation. d.m: dry mass. The letter ([a]) indicates significant difference ($p<0.05$) when comparing blue corn and base malt

to the extraction conditions, specifically due to the fact that hydrolysis was not carried out, since it is reported that ferulic acid is linked to lignin fragments by ether bonds, to arabinoxylans by ester bonds, and also forms diferulic bridges between heteroxylans that constitute the hemicellulose of the cell wall (Cabrera-Soto et al., 2009). In other research, Del Pozo-Insfran et al. (2006) reported the presence of both gallic and *p*-coumaric acid in white corn, and Adom and Liu (2002) reported syringic, p-coumaric, and ferulic acids, among others, in yellow corn

The content of phenolic acids was significantly higher in the base malt than in the grains, which, as previously mentioned, could be attributed to the fact that during the germination process, the concentration of phenolic compounds increases. As for the p-coumaric increase, it could be explained according to what was reported by Tomková-Drábková et al. (2016), who indicated that the enzyme 4-coumarate: CoA ligase catalyzes the conversion of three cinnamic acid derivatives into phenolic acids, one of them being 4-coumaric acid.

Content of anthocyanins, phenolic compounds and melanoidins in caramel malts

The TA content in caramel malts was between 130 and 271 mg CGE/kg d.m (Fig. 1), being significantly higher ($p<0.05$) in 200 °C malt, followed by 175 °C malt, and by the 180 and 225 °C malts, between which there was no significant difference; finally, the 150 and 240 °C malts presented the lowest anthocyanin content, and there was no significant difference between them.

The anthocyanin concentration in the caramel malts by HPLC was between 31.7 and 47.3 mg CG/kg d.m, being significantly higher ($p<0.05$) in the 200 and 225 °C malts (Fig. 1). The trend of the anthocyanin amounts in caramel malts determined by HPLC was very similar to that obtained by spectrophotometry at 535 nm, except for the 225 and 180 °C malts. Considering the results obtained through both methods, roasting at a lower temperature for a longer time had the same effect on anthocyanin content as roasting at a higher temperature for a short time.

TP content in caramel malts was between 2448 and 3256 mg GAE/kg d.m (Fig. 2), and it was significantly higher ($p<0.05$) in the malts 180 and 240 °C, followed by the malts 175, 200, 225, and finally, the malt 150 °C. According to these results, roasting at 180 °C for 15 min caused the same effect as roasting at 240 °C for 5 min; on the other hand, roasting at the lowest temperature for a longer time resulted in a lower content of phenolic compounds in the caramel malts.

The contents of phenolic acids in the caramel malts were between 104.0 and 124.9 mg acid/kg d.m. As can be observed in Table 6, the phenolic acids concentrations were higher in the malts roasted at higher temperatures (200, 225 and 240 °C), and, on the contrary, these values were lower for malts roasted for a longer time at a lower temperature (150 and 175 °C). According to Pérez-Martínez et al. (2010) the differences in concentrations between the TP contents and the phenolic acids contents could be due to the fact that the HPLC method is specific, namely three phenolic acids were identified and quantified in the extracts, while in the total phenols method, the Folin-Ciocalteau reagent can react with other reducing substances apart from phenolic compounds such as melanoidins.

Regarding the increase in the content of phenolic compounds in the caramel malts, Dewanto et al. (2002) and Chandrasekara and Shahidi (2011) reported that roasting could induce structural changes in the cell walls of plants, and consequently the release of phenolic compounds, previously glycosylated/esterified.

The HPLC analysis indicated the presence of gallic, syringic and p-coumaric acids in the samples. According to the results, except for the malt 200 °C, gallic acid was

Table 5: Content of phenolic acids in blue corn and base malt

Sample	Gallic acid (mg/kg d.m)	Syringic acid (mg/kg d.m)	*p*-coumaric acid (mg/kg d.m)	Total
Blue corn	26.1^{a}±1.6	ND	11.8^{a}±0.2	37.9^{a}
Base malt	41.4±6.0	39.7±0.9	32.2±0.7	113.3

Values are presented as mean and standard deviation. d.m: dry mass. ND: no detected. The letter (^{a}) indicates significant difference ($p<0.05$) when comparing blue corn and base malt

Table 6: Content of phenolic acids in caramel malts

Sample	Gallic acid (mg/kg d.m)	Syringic acid (mg/kg d.m)	p-coumaric acid (mg/kg d.m)	Total
150°C-45 min	55.0 ± 1.5^{b}	33.5 ± 0.2^{c}	16.4 ± 0.5^{d}	104.9
175°C-20 min	66.9 ± 5.0^{a}	20.6 ± 0.7^{d}	16.6 ± 0.8^{d}	104.0
180°C-15 min	65.4 ± 2.7^{a}	30.0 ± 1.9^{cd}	17.2 ± 0.3^{cd}	112.6
200°C-5 min	41.7 ± 0.6^{c}	63.9 ± 10^{a}	19.3 ± 1.4^{bc}	124.9
225°C-5 min	47.6 ± 0.9^{c}	49.2 ± 3.2^{b}	25.8 ± 2.1^{a}	122.6
240°C-5 min	62.2 ± 2.9^{a}	33.7 ± 2.1^{c}	21.7 ± 0.4^{b}	117.6

Values are presented as mean and standard deviation. d.m: dry mass. ND: not detected. Different letters in the same column indicate significant differences ($p<0.05$) between caramel malts

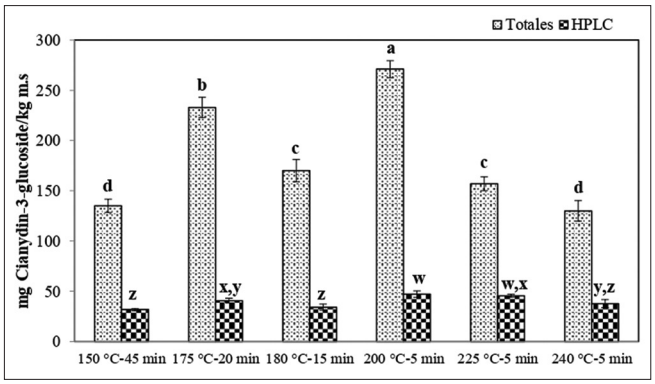

Fig 1. Anthocyanin content in blue corn caramel malts. The letters a-d indicate significant difference between the malts by the total anthocyanin method, and the letters w-z by HPLC.

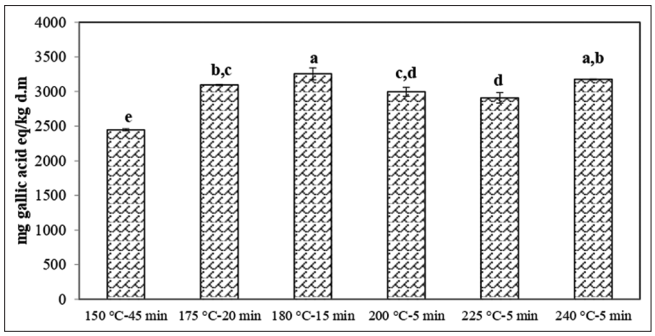

Fig 2. Content of total phenolic compounds(Folin-Ciocalteau method) in blue corn caramel malts. Different letters between the malts indicate significant difference (p<0.05).

the most abundant free phenolic compound in the extracts of caramel malts.

The content of gallic acid in the caramel malts was significantly higher, followed by syringic acid, and finally p-coumaric acid; the contents were between 41.7-66.9, 20.6-63.9 and 16.4-25.8 mg/kg d.m, respectively.

In roasted malts, the melanoidins content were between 4708 and 8929 mg/kg of d.m (Fig. 3). The highest content was presented by the malt 240 °C, followed by the malts 175, 180, 225, and finally the malts 200 and 150 °C, that had significantly lower concentrations. The 200 °C malt, in addition to presenting the lowest content of melanoidins, also showed the highest anthocyanin content, thus revealing a less intense roasting. According to the concentrations of the malts 240 and 150 °C, the effect of temperature on the melanoidins concentration increase was greater when a higher temperature was applied, in comparison to when a lower temperature for a long time was used.

Antioxidant capacity in corn and both base and caramel malts

The antioxidant capacity values of corn and base malt determined by ABTS and DPPH assays are presented

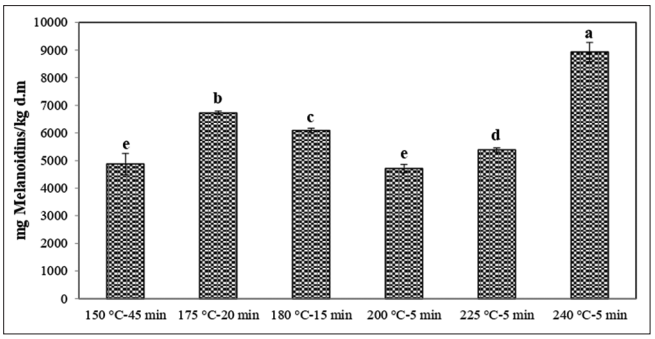

Fig 3. Melanoidin content in blue corn caramel malts. Different letters between the malts indicate significant difference (p<0.05).

Table 7: Antioxidant capacity by ABTS and DPPH assays in blue corn and base malt

Sample	ABTS (g Trolox equivalents/kg d.m)	DPPH (g Trolox equivalents/kg d.m)
Blue corn	78.3±4.1	51.7±7.6[a]
Base malt	83.8±7.4	± 4.4

Values are presented as mean and standard deviation. d.m: dry mass. The letter ([a]) indicates significant difference (p<0.05) when comparing the samples

in Table 7. And as can be seen, by ABTS assay, the antioxidant capacities between corn and base malt were not significantly different. On the contrary, by DPPH assay a significantly higher capacity (p<0.05) in the base malt than in the unmalted grain was observed, which indicates the compounds contribution to antioxidant capacity determined through this method, since the base malt presented a lower amount of anthocyanins and, conversely, a greater amount of both phenolic compounds and melanoidins. As can be observed in Fig. 4, the antioxidant capacity values, reported as g equivalents of Trolox, of the different caramel malts were higher when measured by DPPH assay (162.8-214.8 mg TE/kg d.m) than by ABTS assay (115.9-135.8 mg TE/kg d.m). In relation to this, Serpen et al. (2007), when evaluating the antioxidant activity in insoluble food materials, food ingredients, and Maillard reaction products (MRP), observed that ABTS assay is usually more sensitive to compounds containing phenols, while DPPH assay is more sensitive to the MRP. This could explain what was observed in the methanolic extracts of the malt, as the species that were found in greater quantity were melanoidins, followed by total phenolic compounds. Several authors have reported that melanoidins contribute greatly to the general antioxidant activity of certain foods, such as roasted coffee (Delgado-Andrade and Morales, 2005; Borrelli et al., 2002), barley caramel malts (Coghe et al., 2006), and in roasted quinoa malts (Carciochi et al., 2016).

As shown in Fig. 4, the antioxidant capacity determined by ABTS assay was significantly higher in the 240, 180 and

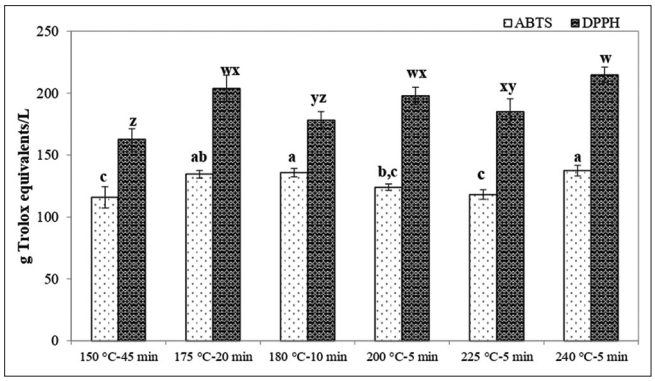

Fig 4. Antioxidant capacity by ABTS and DPPH assays of blue corn caramel malts. The letters a-c indicate significant difference between the malts by the ABTS method, and the letters w-z by the DPPH method.

175 °C malts; on the contrary, it was significantly lower in the 225 and 150 °C malts. These results agree with those reported by Serpen et al. (2007), since the malts 240 and 180 °C presented the highest concentrations of total phenolic compounds and the malts 225 and 150 °C presented the lowest.

As for the results by DPPH assay, the malts that presented significantly higher antioxidant capacity ($p<0.05$) were the 240, 200 and 175 °C malts, and significantly lower ($p<0.05$) was the 150 °C malt. This same trend was observed in the content of melanoidins, with the exception of 200 °C malt, which despite having a significantly lower content of melanoidins, as previously mentioned, also had a high antioxidant capacity. This situation could be related to the fact that this malt also had the highest anthocyanin content, so a synergistic effect between the two groups of compounds may have been presented.

CONCLUSIONS

The blue corn used to obtain both base and caramel malts, presented a proximal chemical analysis similar to other reported blue corn kernels, and the base malt showed a considerable protein content, which is within the specified range for barley malt. Although, in general the malting did not significantly affect the color, it caused the malts anthocyanin content to decrease, on the contrary, malting increased the content of both total polyphenols and phenolic acids, which favored a greater antioxidant capacity by DPPH assay. Regarding the caramel malts, toasting had an effect on color, due to both a decrease in anthocyanins and an increase in melanoidins; in addition, malt roasting also increased the concentration of phenolic acids. The conjunction of anthocyanins, melanoidins, and phenolic acids in the caramel malts produced a greater antioxidant capacity that was observed by ABTS and DPPH assays. Therefore, the combined use of both base and caramel malts to make corn beer musts could provide them with significant amounts of compounds that could impact their color as well as their antioxidant capacity, thereby favoring the chemical stability of the product throughout the processing stages.

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A.M.D. Flores-Calderón, H.B. Escalona-Buendía, and J.R. Verde-Calvo., conceptualization and design; A.M.D. Flores-Calderón, H. Luna, H.B. Escalona-Buendía, and J.R. Verde-Calvo, methodology, acquisition, formal analysis; A.M.D. Flores-Calderón, M.A. Hernández-Carapia, H. Luna, H. B. Escalona-Buendía and J.R. Verde-Calvo, data interpretation and writing-original draft; A.M.D. Flores-Calderón, M.A Hernández-Carapia, H. Luna, H.B. Escalona-Buendía and J.R. Verde-Calvo, review and editing.

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