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Characterization of White, Yellow, Red, And Purple Colored Corns (*Zea Mays İndentata L.*) According to Bio - Active Compounds and Quality Traits

Beyaz, Sarı, Kırmızı ve Mor Renkli Mısırların *(Zea Mays İndentata L.)* Biyo - Aktif Bileşenler ve Kalite Özellikleri Bakımından Karakterizasyonu

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CHARACTERIZATION OF WHITE, YELLOW, RED, AND PURPLE COLORED CORNS (*ZEA MAYS INDENTATA L.*) ACCORDING TO BIO - ACTIVE COMPOUNDS AND QUALITY TRAITS

ABSTRACT

The study was conducted to highlight differences of colored corns according to bio – active compounds and quality traits in Selcuk University, Agriculture Faculty, Crop Science Department, Konya/Turkey. At the study we attempted to explain the impacts of grain color (white, yellow, red, and purple) factor on bio – active compounds as total antioxidant activity, total phenolic compounds, total flavonoids, total anthocyanin content, total carotenoids, and some quality traits as grain fat content, grain protein content, grain starch content and amylose – amylopectin rate. All analysis was practiced at three coincidently chosen samples for each trait. Wide and significant variations were observed among the genotypes at all traits except grain fat content feature. While purple corn had the highest values at six (total antioxidant activity, total phenolic compounds, total flavonoids, total anthocyanin content, grain fat content and grain starch content) of nine characters; the red and the white ones had the highest values one of [total carotenoids (red corn), grain protein content (white corn)] nine characters in the study. Results of the study showed that grain color changes had remarkable effects on bio – activate contents and some quality features and exhibited possibility of using colored corns as bio – active resources in human and animal nutrition.

Keywords: Antioxidants, Phenolic Compounds, Flavonoids, Anthocyanins, Carotenoids.

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BEYAZ, SARI, KIRMIZI VE MOR RENKLİ MISIRLARIN (*ZEA MAYS INDENTATA L.*) BİYO - AKTİF BİLEŞENLER VE KALİTE ÖZELLİKLERİ BAKIMINDAN KARAKTERIZASYONU

ÖZ:

Çalışma renkli mısırların bio – aktif bileşenler ve kalite özellikleri bakımından farklılıklarını belirlemek amacıyla Selçuk Üniversitesi, Ziraat Fakültesi, Tarla Bitkileri Bölümü, Konya/Türkiye' de yürütülmüştür. Bu çalışmada mısırda tane rengi faktörünün (beyaz, sarı, kırmızı ve mor) total antioksidanlar, total fenolik bileşenler, total flavanoidler, total antosiyaninler, total karotenoidler gibi biyo – aktif bileşenler ile, tane yağ içeriği, tane protein içeriği, nişasta içeriği ve amiloz –

amilopektin oranı gibi kalite özelliklerine etkileri belirlenmiştir. Tüm analizler her bir analiz için üç kez tesadüfen seçilmiş örneklerde yapılmıştır. Tane yağ içeriği karakteri dışındaki tüm özelliklerde istatistik olarak önemli varyasyonlar görülmüştür. Mor mısır dokuz karakterin altısında (total antioksidan aktivitesi, total fenolik bileşenler, total flavonoid, total antosiyanin içeriği, tane yağ içeriği ve tane nişasta içeriği) en yüksek değerlere sahip olurken; kırmızı ve beyaz renkli mısırlar dokuz karakterin birinde en yüksek değere sahip olmuşlardır [total karotenoid (kırmızı mısır); tane protein içeriği (beyaz mısır)]. Çalışmanın sonuçları tane rengindeki farklılığın biyo — aktif bileşenler ve bazı kalite özelliklerinde önemli değişikliklere neden olduğunu; renkli mısırların insan ve hayvan beslenmesinde biyo — aktif kaynaklar olarak da değerlendirilmesinin mümkün olabileceğini göstermiştir.

Anahtar Kelimeler: Antioksidanlar, Fenolik Bileşenler, Flavanoidler, Antosiyaninler, Karotenoidler.

1. INTRODUCTION

About 2 billion people suffer from micronutrient deficiency around the world. Although cereals are responsible 50% of human nutrition; they are deficient by essential minerals and vitamins. While yield increased nearby 530% with green revolution; new generation corn genotypes are still poor in micronutrients according to local populations and traditional varieties (Ashokkumar et al., 2020). The economic and nutritional value of maize grains is mainly due to its high starch (73%), protein (9%) and oil (4%) contents (Özdemir and Sade, 2019). Maize is also known with its wide range of grain color variation. Purple corn trade had been increased near by 467% between 1998 — 2002 years according to "United Nations Bio-Trade Facilitation Program". Purple corn usage as a natural colorant is very common particularly in Germany, France, Italy and Japanese. Anthocyanins that are basic compounds of purple corn, decrease risky of cardiovascular diseases, obesity, diabetes, cancer and some chronic diseases (Fei et al., 2017). Oxidative stress is defined as corruption of redox balance against antioxidants (Lobo et al., 2010; Magaña-Cerino et al., 2020). Antioxidative defense is effective enough during normal metabolic conditions; but defense system sometimes must be supported artificially under unfavorable situations. Vitamin C, E, carotenoids, anthocyanins and flavonoids can balance redox cycle of plants and animals (Kasote et al., 2015). Human consumed anthocyanins rich fruits and vegetables have lower risks of having cancer, diabetes and cardiovascular diseases was reported in many epidemiological studies (Pandey and Rizvi, 2009). Anthocyanins get attention of especially food industry's producers because of being colorants from natural resources and have a wide range of color spectrum. They are responsible of red, blue, and violet colors of plant tissues and foods. Pharmacological and therapeutic effects of anthocyanins gained them properties as related humans' health beside their food colorant usage (Yang and Zhai, 2010). In many studies conducted so far anthocyanin's detoxifying effects of free radicals that cause chronically illnesses as atherosclerosis, aging, diabetes, and hypertension were reported as well (Fei et al., 2017; Kim et al., 2013; Long et al., 2013; Urias-Lugo et al., 2015). Anthocyanins are water soluble flavonoids of polyphenolic pigments. Corns that have colored grains are known as rich antioxidant resources as well. Being natural and reliable of plant sourced anthocyanins increased their popularity day by day. Anthocyanins from natural antioxidants have ability of detoxifying ROS (reactive oxygen species) that cause cell damage. It was reported that antioxidant enzymes in milk and plasma of ruminants that consumed anthocyanin rich purple corn were higher (Tian et al., 2019).

Carotenoids are important components for human nutrition. All these yellow and orange pigments are tetraterpenes. Those with C and H atoms in their structure are known as carotenes, those with C and H together with O atoms are collectively known as xanthophylls. The long ─ term conjugate bonds and elemental structures of all carotenoid forms determine their color, revealing their biological activities and antioxidant capacities. Previous research has identified a relationship between the intake of these ingredients and the prevention of cancer, heart disease, and age-related degeneration (Kahrıman et al., 2019).

The aim of the study is explaining the impacts of grain color (white, yellow, red, and purple) factor on bioactive compounds as total antioxidants, total phenolic compounds, total flavonoids, total anthocyanins, total carotenoids, and some quality traits as grain fat content, grain protein content, grain starch content and amylose-amylopectin rate.

2. MATERIALS AND METHODS

2.1. Materials

White, yellow, red, and purple seeds of dent corn variety group were used as materials that were obtained from "Sakarya Maize Research Institute". The seeds of each genotype were from long term self-pollinated populations and produced in Sakarya/Turkey at 2018 growing season.

2.2. Methods

Enough seed samples from each genotype were grinded with the grinder "BOSCH TSM6A013B" at approximately 15% grain moisture. The moisture contents of the seed samples were detected by "Kett Grain Moisture Tester, Model PM 600".

The powder was sieved with 0.5 mm sieve. All analysis was practiced at three coincidently chosen samples.

Total Antioxidant Activity (TAA)

DPPH (2,2 — Diphenyl — 1 — picrylhydrazyl) radical scavenging activity of phenolics was assessed by measuring the capacity of bleaching a black colored methanol solution of DPPH radical according to (Khampas et al., 2013). A 4.5 ml DPPH solution was added on 0.5 ml phenolic extract. The mixture was vortexed and left to stand for 30 minutes in dark. The absorbance values of samples were measured at 517 nm against solvent blank.

The scavenging rate on DPPH radicals was calculated according to the formula as follows:

$$Scavanging\ rate\ (\%) = \left[\frac{A_0 - A_1}{A_0}\right] \times 100$$

where A_0 is the absorbance of the control (0.5 ml extraction solvent with 4.5 ml DPPH solution) and A is the absorbance in the presence of phenolic extracts solution.

Total Anthocyanin Content (TAC)

Anthocyanins were analyzed following procedure of Cervilla et al. (2012). A 0.1 g DW (dry weight) sample was homogenized in 5 ml propanol and HCl solution. Homogenates were centrifuged at 5000 rpm. All samples were left at room conditions for 24 hours. Afterwards tubes were centrifuged at 6500 rpm. Absorbance readings at 535 nm were taken and corrected for background absorbance at 700 nm in a UV Spectrophotometer (Leticia et al., 2009).

Total Phenolic Compounds (TPC)

The total phenolic compounds of grinded corn grains were extracted using methanol according to Mohsen and Ammar (2005) with some modifications. Extraction was carried out using a shaking incubator at room temperature, followed by filtration through Whatman No.1 filter paper. The total phenolic compounds was determined according to the Folin — Ciocalteu method (Konrade and Klava, 2017) using gallic acid standard calibration curve.

Total Flavonoids (TF)

Grain total flavonoids concentrations were determined according to Jothy et al. (2011). 0.5 ml seed extract (1 mg ml^{-1}) (Konrade and Klava, 2017) was added falcon tubes and mixed with 2 ml distilled water. Subsequently 0.15 ml aluminum trichloride was added and allowed to stand for 6 min; then 2 ml sodium hydroxide was added to reaction mixture. The final volume was completed up to 5 ml with distilled water. After 15 minutes absorbance of pink color was measured at 510 nm with UV spectrophotometer against blank. Total flavonoids were calculated according to Quercetin standard calibration curve.

Total Carotenoids (TC)

Total carotenoids were detected according to Rocha et al. (2015). Briefly, 2 g grain samples were ground with cold acetone (25 ml). The mixture was agitated for 10 min, followed by filtration using Whatman No. 1 filter paper. The filtrate was transferred into a separation funnel and partitioned with petroleum ether (20 ml). To remove the acetone, the filtrate was washed with distilled water (100 ml) and the lower phase was discarded. The procedure was repeated twice. The petroleum ether layer was filtrated by using Whatman No. 1 filter paper covered with 5 g of anhydrous sodium sulphate to remove residual water. The petroleum ether extracts were pooled, and the volume was adjusted to 25 mL with petroleum ether. The absorbance was measured at 450 nm to determine the total carotenoids content using the following formula:

$$Total\ carotenoids\ (\mu g\ \beta - carotene\ g^{-1}) = \frac{[A \times V\ (25\ ml) \times 10^4]}{[E\%1cm\ \times P\ (2g)]}$$

Grain Starch Content (GSC)

Each sample was extracted with HCl (1%) then sedimented with phosphorus wolfram acid. Optical rotations of solutions were determined with polarimeter. Results were concerted and recorded according to Alan et al. (2015).

Amylose/Amylopectin

Amylose and amylopectin rate was determined according to Galicia et al. (2008) by using spectrophotometry. The calibration curve of standard potato amylose was used to modify the results.

Grain Fat Content (GFC)

Grain fat content was determined according to Khan et al. (2014) with soxhlet extractor. Fifteen gram of each sample was treated with n — hexane; after extraction, oil and n — hexan was evaporated from each other. Oil content was calculated and recorded as % oil content.

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Grain Protein Content (GPC)

Grain protein content rate was detected according to Radha et al. (2013) with Lowry method then results were converted and recorded as % protein rate.

2.3. Statistical Analysis and Evaluation

All data shown are the mean values. Data were statistically analyzed according to "Completely Randomized Plot Design" with the analysis of variance [ANOVA (One – Way)] in MINITAB software, means were grouped by Tukey' s multiple range test at the 0.05 level of significance. Correlation analyses were done in the MINITAB software at the 0.01 and 0.05 level of significance as well.

3. RESULTS AND DISCUSSION

This study was conducted to characterized different colored maize genotypes according to bioactive compounds and some quality traits. White, yellow, red, and purple corn grains were used as materials in the trial. Total antioxidant activity, TPC, TF, TAC, TC, GFC, GPC, GSC and amylose/amylopectin features were determined for this aim. According to the results of variance analysis, wide variations were observed among all genotypes at all traits except GFC (Table 1). While the highest TAA (10.24%) was obtained from purple corn; the lowest value was from the white one (1.57%). The red corn had more TAA (10.12%) than the yellow (6.43%) one but not as higher as the purple corn. It may be concluded that TAA results indicated a correlation between antioxidant levels and pigment concentrations because of increasing TAA level while grain's color becomes darker (Figure 1, Table 2). Correlations of the traits were investigated as well. Total antioxidant trait had significant and positive correlations with TPC (0.63*), TF (0.83**) and GSC (0.58*) characters (Table 3). Antioxidants prevent oxidative degeneration and protect health in biological systems (Halliwell et al., 1992). Purple corn is rich in antioxidants; so detoxifying effects of it has been tried so far also in vivo and in vitro conditions. Results of these studies verified it' s strong detoxifying effects (Fei et al., 2017). Results of this trial are also compatible with literature; Khampas et al. (2013) detected TAA levels between 13.2% – 68.9%.

Total anthocyanin contents of the genotypes were determined as well. According to the results purple corn (373.78 mg $100g^{-1}$ C3G) had the highest TAC level and followed by red (16.14 mg $100g^{-1}$ C3G), white (7.51 mg $100g^{-1}$ C3G), and yellow (6.26 mg $100g^{-1}$ C3G) ones respectively (Table 1, Figure 1, Table 2). While grain color concentration increased, TAC level increased as well like TAA, but the yellow corn left behind of the white one at this trait. Total anthocyanin content had significant and positive correlations with TPC (0.88**), TF (0.83**), GFC (0.59*) and GSC (0.61**) traits. Martínez-Martínez et al. (2019) stated TAC between 0.73

and 36.12 mg 100g^{-1} C3G while Zilic et al. (2012) reported 2.50 — 696.7 mg C3G kg^{-1} TAC levels. Navarro et al. (2018) detected in wide range of varying amounts anthocyanin levels; between 0.018 – 1600 mg 100g^{-1} C3G in purple corn. Purple corn is known with its higher anthocyanin contents that indicates higher antioxidant potentials such that anthocyanin level of purple corn is higher than blueberry that is popular with high anthocyanin concentrations (1.3 – 3.8 mg g^{-1} FW) (Cevallos-Casals and Cisneros-Zevallos, 2003; Li et al., 2008; Wu et al., 2006).

Total phenolic contents changed due to color concentrations; purple corn (188.99 mg 100g^{-1} GAE) has garnered attention with its highest TPC as well (Table 1, Table 2, Figure 1). Méndez-Lagunas et al. (2020) determined average 307.57 mg 100g^{-1} GAE TPC at purple corn samples; at another study Cuevas Montilla et al. (2011) detected TPC ranged from 311.00 mg 100g^{-1} GAE to 817.60 mg 100g-1 GAE. Martínez-Martínez et al. (2019) reported TPC between 68.19 and 137.39 mg 100g^{-1} GAE at purple corn samples as well. Total phenolic concentrations represent significant and positive correlations with other traits as TF (0.82**) and GSC (0.59*) (Table 3). Phenolic compounds are second metabolism productions in plants and animals. There are soluble and non-soluble forms of them.

Table 1. Variance analysis results of all traits

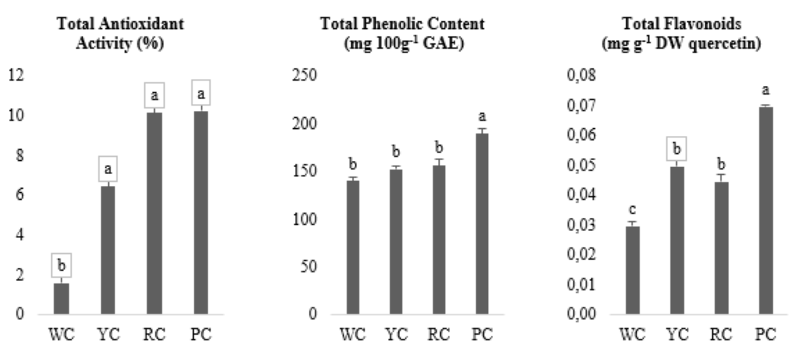
Çizelge 1. *Tüm özelliklere ait varyans analiz sonuçları*

Source	DF	TAA	TAC	TPC	TC	TF	GFC	GPC	GSC	Amylose/Amylopectin
Genotype	3	285.67**	297968**	4041.50**	670.28**	0.0024**	1.90	9.00**	95.22**	0.09**
Error	8	28.69	1846	713.00	20.02	0.0001	2.56	0.35	46.91	0.01
Total	11	314.36	299814	4754.50	690.30	0.0025	4.64	9.35	142.13	0.09

$^{**}P<0.01$

$^{*}P<0.05$

TAA	: Total Antioxidant Activity (%)	**TC**	: Total Carotenoids (µg ß — carotene g^{-1})	**GPC**	: Grain Protein Content (%)
TAC	: Total Anthocyanin Content (mg 100g^{-1} C3G)	**TF**	: Total Flavonoids (mg g^{-1} DW Quercetin)	**GSC**	: Grain Starch Content (%)
TPC	: Total Phenolic Compounds (mg 100g^{-1} GAE)	**GFC**	: Grain Fat Content (%)		



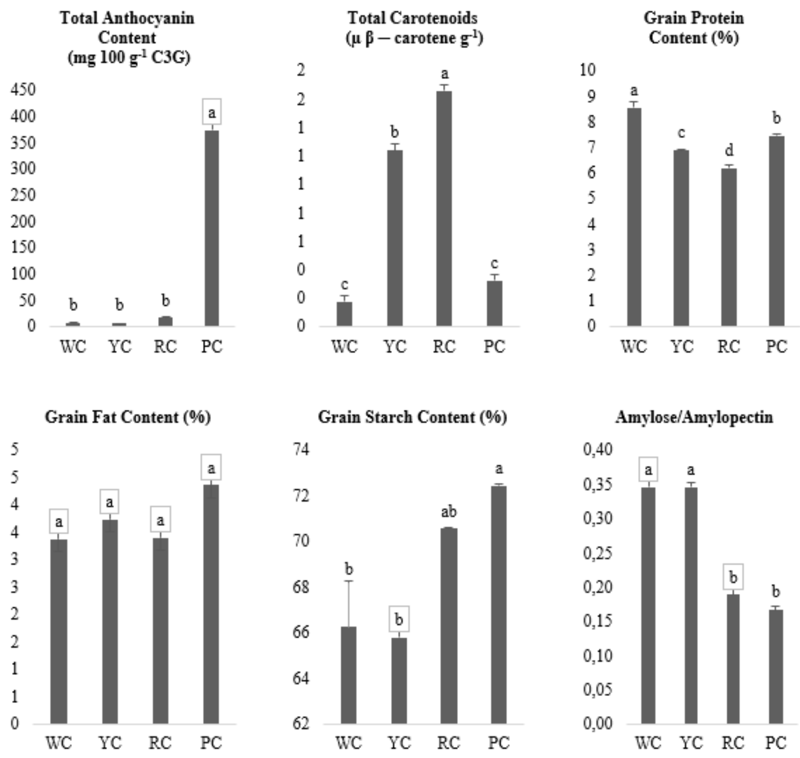


Figure 1. Means of TAA, TPC, TF, TAC, TC, GPC, GFC,GSC and Amylose/ Amylopectin features

Şekil 1. *TAA, TFB, TF, TAİ, TK, TPİ, TYİ, TNİ ve Amiloz/Amilopektin özelliklerinin ortalamaları*

Table 2. Comparison means of all features of each genotype

Çizelge 2. *Tüm genotiplerin denemeye konu özeliklere ait ortalamalarının karşılaştırması*

TAA	Purple > Red > Yellow > White
TAC	Purple > Red > White > Yellow
TPC	Purple > Red > Yellow > White
TC	Red > Yellow > Purple > White
TF	Purple > Yellow > Red > White
GFC	Purple > Yellow > Red > White
GPC	White > Purple > Yellow > Red
GSC	Purple > Red > White > Yellow
Amylose/Amylopectin	White = Yellow > Red > Purple

Table 3. Correlation coefficients of all traits with each other

Çizelge 3. *Denemeye konu özelliklerin korelasyonları*

Features	TAA	TAC	TPC	TC	TF	GFC	GPC	Amylose/ Amylopectin
TAC	0.54							
TPC	0.63*	0.88**						
TC	0.52	-0.22	0.03					
TF	0.83**	0.83**	0.82**	0.04				
GFC	0.49	0.59*	0.43	-0.16	0.70**			
GPC	-0.65*	0.10	-0.21	-0.85**	-0.29	-0.01		
Amylose/Amylopectin	-0.74**	-0.64*	-0.66**	-0.50	-0.63*	-0.19	0.40	
GSC	0.58*	0.61*	0.59*	0.30	0.53	0.03	-0.24	-0.07**

****P<0.01**
*** P<0.05**

Soluble phenolics that show a wide range of variation are effective on grain color formation (Liyama et al., 1994; Takanori et al., 1994). Total phenolic compounds are antioxidants, besides their responsibility of grain color formation; they are also effective on grain hardness of white corns. Effects of phenolic concentrations on grain hardness of white corn depends on ferulic acid concentrations of cell wall (Chalker – Scott, 1999). Purple corn is very rich in phenolic compounds and include anthocyanins and flavonoids as phenolics (Gonzalez – Manzano et al., 2008). Red grain corns are also very rich in phenolic compounds compared with uncolored genotypes (Chalker – Scott, 1999).

According to the results of TF concentration, purple corn (0.07 mg g^{-1} DW Quercetin) had higher TF than all other genotypes of the trial. Yellow one followed purple corn with 0.05 mg g^{-1} DW Quercetin flavonoid (Figure 1; Table 2). Martínez-Martínez et al. (2019) reported TF values ranged from 0.02 to 0.10 mg g^{-1} DW Quercetin. Similar results were obtained from the study as previous. Total flavonoid feature has significant and positive correlations with GFC (0.70*) as summarized at Table 3. Plant resources natural compounds get attention of producers with being economic, bioavailability, reliability and minimum side effects (Navarro et al., 2018). Flavonoids have potential of acting as anti-cancer agents that triggers cytotoxic cancer cell apoptosis therewithal get attention of scientist with antioxidant and neuroprotective effects (Abotaleb et al., 2019).

A remarkable relation was not observed between color and carotenoid content because carotenoid concentration was lower at purple corn (0.32 µg β – carotene g^{-1}) that has the highest pigmentation. Red (1.66 µg β – carotene g^{-1}) and yellow (1.24 µg β – carotene g^{-1}) genotypes had higher TC concentrations although lower pigmentation than purple one (Figure 1; Table 2). Total carotenoids have significant and negative relations with GPC (-0.85**) trait as represented at Table 3. There are limits to major and minor components in the literature to specify maize genotypes. These limits indicate that maize genotypes can be classified. For example, maize genotypes containing more than 6 — 7% fat are called high-fat maize, while those with carotenoids over 50 micrograms per gram are high carotenoids (Kahrıman et al., 2021). Messias et al. (2014) reported TC ranged from 10.03 to 61.50 µg g^{-1} ß — carotene while Khampas et al. (2013) revealed TC between 1.00 — 35.60 µg g^{-1} ß — carotene. Trono (2019) reported widely varying TC values and declared carotenoid concentrations between 1.60 and 156.14 µg g^{-1} ß — carotene at corn grain samples. Main carotenoids that are essential for human nutrition are α, ß — caroten, ß – cryptoxanthin, lutein, zeaxanthin and lycopene and are metabolized to provitamin-A in human metabolism. Carotenoids cannot be produced in human body thus must be taken regularly in human diet otherwise deficiency of them can cause blindness and this is very common in some countries in the world (Ashokkumar et al., 2020; Davey et al., 2009; Fraser and Bramley, 2004). Phytochemicals of cereals are localized at outer part of the grains more than inner. Those kind of bio activated compounds have ROS detoxification, minimizing peroxidase forms and activating antioxidant enzymes (Smuda et al., 2018). To be rich in those kinds of bio-activated compounds of a genotype can gain it nutrition values as well.

A wide variation was observed among genotypes according to GSC in the trial (Figure 1; Table 2). Purple corn (72.43%) has the highest starch and followed by red (70.55%), white (66.26%), and yellow (65.79%) genotypes. It was supposed that grain massiveness is more effective on formation of this trait more than grain color because purple corn is the one that has the biggest grains in the trial.

Fluctuations were observed at GPC rates. The highest values were obtained from white corn (8.55%) and followed by purple (7.46%), yellow (6.90%) and red (6.18%) ones (Figure 1, Table 2). Purple corn (4.36%) was the genotype with the highest GOC and followed by yellow (3.73%), red (3.40%) and white (3.37%) genotypes (Figure 1, Table 2). Özdemir and Sade (2019) reported that a standard corn grain contains 73% starch, 9% protein and 4% oil. Findings of this trial are compatible with the previous knowledge as well (Table 1).

While amylose/amylopectin results were investigated it was observed that purple (0.17) and red corns (0.19) that are in the same color scale has lower rates than yellow (0.35) and white (0.35) ones. Amylose/amylopectin feature had significant and negative relations with some other traits as TAA (-0.74**), TAC (-0.64*), TPC (-0.66**) and TF (-0.63**) and GSC (-0.07**) (Table 3). This situation could indicate relation of color and amylose/amylopectin trait (Table 2). While common corn amylose/amylopectin rate is near by 0.38; this value goes up 1.94 at amylose maize according to Xie et al. (2020). Chen et al. (2021) revealed that amylose/amylopectin rates can increase till 4. Yalçın et al. (2020) reported that amylose/

amylopectin values were between 25 — 28% / 72 — 75%. Previous literatures support results of this study. Common starch has higher glycemic index because of easy digestion property. Starch digestion can be decreased with higher amylose content hence glycemic index of starch can be managed with this way. Amylose/amylopectin rate and structure; arrangement, form and position of starch molecule and interactions with other molecules determined physicochemical and functional properties of starch (Shevkani et al., 2017; Yalçın et al., 2020). Hogg et al. (2015) stated that high amylose pasta is richer in enzyme resistant starch that decreases glycemic reaction and prevent cardiovascular diseases compatible with this Yalçın et al. (2020) expressed that amylose/amylopectin is one of the main factors that effects starch digestion as well.

4. CONCLUSION

Results of the study showed distinct advantages of purple corn compared with others. Purple corn had the highest values at 6 (TAA, TAC, TPC, GFC, TF and GSC) of 9 traits. The red one also had higher values than others after the purple one. These results indicated a relation between color factor and features subjected of this study therefore it is possible claiming that colored corns can be used as source of bio — active compounds in animal and human nutrition. According to the results of the trial yellow and white corns amylose/amylopectin values were higher than the purple and red ones. These findings indicated higher amylopectin rates of purple and red corns. These genotypes can be used in breeding programs with the aim of decreasing starch digestion as well.

Conflict of Interest:

The authors declare that there is no conflict of interest.

Ethics:

This study does not require ethics committee approval.

Authors Contribution Rates:

Design of the Study: EÖ (60%), RC (20%), BS (20%)

Data Acquisition: EÖ (70%), RC (15%), BS (15%)

Data Analysis: EÖ (70%), RC (15%), BS (15%)

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