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PROXIMATE CHEMICAL COMPOSITION AND TOCOPHEROL LEVELS IN MAIZE GENOTYPES GROWN IN PUNJAB, PAKISTAN

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In present study, proximate chemical composition and tocopherol content were evaluated for estimation of food quality. The maize genotypes with promising agronomic parameters were analyzed for their biochemical composition and further guidance for maize crop improvement with respect to quality traits. Twelve (12) genotypes (n= 60) of maize (*Zea mays* L.) cultivated at Maize and Millet Research Institute, Yousafwala, Sahiwal, Punjab, Pakistan were evaluated for diversity based on protein, crude fiber, moisture contents, oil contents and tocopherol. The results showed that the crude protein contents were ranged from 1.75 to 5.25%, crude fiber 2.12 to 2.55%, fat contents 2.83 to 5.20%, moisture contents 9.75 to 11.95% and total tocopherol 1971 to 9382 µg/g γ-tocopherol was found in maximum concentration and crude fiber in minimum percentage. These findings will be helpful to produce quality products from the maize and valuable to protect human beings against degenerative processes, such as cancer and cardiovascular diseases.

Keywords: Maize, proximate chemical composition, fiber, fat, moisture content, protein, tocopherol, food quality

INTRODUCTION

Maize (*Zea mays* L.) or corn, belongs to the family *Poaceae* and it is the one of the most important staple crops for feeding and for industrial processing (Qamar *et al*., 2016; Akram *et al*., 2010). Maize has multiple uses as food for human population, feed for livestock and poultry and primary raw material for industries. Maize history is very old and about 9000 years ago it was recognized as wild grass in Mesoamerican region, area of origin and domestication (Abbasi *et al.*, 2012; Haq *et al.*, 2012). Maize is a good source of protein, fiber, saturated and unsaturated fatty acids. Maize contains little or no ascorbic acids and is short in niacin, riboflavin and some essential amino acids but contains significant concentration of phytic acid (Sattar *et al*.,1985).
Globally, maize, rice and wheat are major source for staple food due to rich in carbohydrates, protein and fiber. According to FAO data, Asian countries are the main growers and consumers of cereals per capita per year. The production of cereal grain nearly 2.3 billion tons each year, 1 billion tons are intended for humans uses, 0.75 billion tons for animals and 0.50 billion tons are processed by industry (FAO, 2013). Maize is the third most important crop after rice and wheat in Punjab, Pakistan. In Punjab province, maize has been grown twice in a year, spring (January-March) and autumn (June-August) covering an area of 1.14 million hectares producing 4.94 million tons with an average yield of 4321 kg/hectare (Agricultural Statistics of Pakistan, 2014-15).

Tocopherols and tocotrienols are tocochromanols known as vitamin E. Four different tocopherols (α, β, γ and δ) can be recognized on their chromanol ring with number and position of methyl groups (Ryynanen *et al*., 2007). It is comparatively easy for young people to get adequate amount of tocopherols and tocotrienols from food pre-requisite to prevent vitamin E deficiency symptoms. Processed foods from plants, particularly fruits and vegetables, having reasonable contents of vitamin E; but daily consumption of plant-based food supply ample and consistent amount of vitamin E (Chun *et al*., 2006). Additionally, high consumption of vitamin E may reduce the threat of various chronic diseases associated to oxidative damage, e.g., heart diseases and multiple type of cancer. Most of these health effects in human are connected to behavior of fat-soluble antioxidants (Sanagi *et al*., 2005). The biological functions of these molecules are mostly recognized to antioxidant activity in hindering lipid peroxidation in organic membranes. They function as antioxidant by contributing to reduction of peroxyl radicals of unsaturated lipid molecules, producing radical of tocopheroxyl along with a hydroperoxide, which interact with other radicals of peroxyl or tocopheroxyl converting them into stable adducts (Schwenke, 2002). Actually, Tocopherol performs a primary role in preventing the formation of radicals in human system i.e. membranes, plasma, tissues, and disruption of the free radical chain reactions (Lampi *et al.*, 1999).

After 1950s, many reports have described the tocopherol content of vegetables and fruits (Eitenmiller and Lee, 2004; Piironen *et al.,* 1986). The level of tocopherol in vegetables and fruits is influenced by several factors like growing conditions (sowing season, amount of sunlight, soil status, and weather), irregular tocopherols distribution, maturity, species, method and time of harvesting and variety (Bauernfeind, 1980). After harvesting, there is still a chance of variation in the tocopherol concentration affected by many factors such as storage time and conditions, preparation of sample, difference in analytical methods and processing procedures (Piironen *et al.,* 1986; Ruperez *et al.,* 2001).

Maize contains major concentration of tocopherols (Vitamin E) which arises in eight forms of stereoisomers, four tocopherols (α, β, γ, δ) and four other tocotrienols. Conversely, the significant tocols existed in maize are: α-tocopherol, γ-tocopherol and α-tocotrienol (Heinemann *et al.,* 2008). Tocopherols have enormous antioxidant activity to lessen lipid peroxidation, demonstrate anti-inflammatory properties and cardiovascular protective effects (Pascual *et al.,* 2013). Many factors like (DNA, environmental factors, phytic acid, anti-oxidants etc.) are responsible to vary the concentration of tocopherol may vary contents of tocopherols in genotypes (Brankovic *et al.,* 2015). The under taken study is intended to probe the level of tocopherol and its relationship with the proximate measurement in maize genotypes. The research work carried out is innovative since will assist the improvement of the current maize genotypes. The data will be also supportive to protect humans against degenerative processes, such as cancer and cardiovascular diseases.

MATERIALS AND METHODS

***Plant material*:** Sixty (n = 60) samples of twelve maize (*Zea mays* L.) genotypes (FH-988, YH-1898, FH-949, FH-922, YH-5482, YH-1899, 30Y87 Pioneer, NT-6621 Syngenta, FH-1046, YH-5490, FH-1036, YH-5521) were collected from Maize and Millet Research Institute, Yousafwala, District Sahiwal, Punjab, Pakistan. The seeds were air-dried without exposure to sunlight and stored at the Food Toxicology Laboratory, NIAB, Faisalabad at 4°C for biochemical analysis.

***Determination of proximate analysis moisture content*:** The air-dried seeds of maize genotypes were ground using high speed grinding mill (ZM 200, Retsch, Germany) and passed through a sieve of 0.25 mm mesh size. The ground sample (10 g) in triplicate were kept at 105°C for overnight, cooled in desiccator and weighed again to determine moisture content (AOAC, 1990; Qamar *et al.,* 2016). The moisture percentage was estimated by the following formula.

$$\text{Moisture content} = \frac{\text{weight loss ofmaize}}{\text{weight of the original maize}} \text{ X } 100$$

***Crude fiber*:** The fiber content was assessed using Soxhlet extraction method (AOAC, 2000) with some modifications. Maize ground powder (2 g) was extracted with solvent (petroleum ether). The maize powder was taken in a beaker (1 L), added 200 mL of 1.20% boiled sulfuric acid, one gram asbestos, glass chips and diluted antifoam. The whole mixture was digested with hot plate and boiled for 30 minutes. After completion of the reaction, beaker was taken off, cooled and contents were filtered using filter paper Whatman No. 1 and dried in oven (Memmert, Germany) for 2 h at 135°C, cooled and weighted, to finally being ignited at 550°Cfor one hour in the furnace (Labtech GmbH, Germany) and crude fiber was calculated using the following formula.

Crude Fiber in maize (%) = (Loss of wt. on ignition - Loss in wt. of asboestos blank) × 100

***Ash content*:** Dried maize powder (2 g) was taken in crucible and ignited in a muffle furnace (Labtech GmbH, Germany) at 590°C for 8 hours, cooled and noted the weight (AOAC, 1990; Bremner *et al.,* 1982). Ash content was determined using the following formula:

$$\% \text{ Ash} = \frac{\text{weight of Ash}}{\text{weight of the original maize}} \text{ X } 100$$

***Crude protein*:** The protein content in maize genotypes was studied following the procedure of AOAC (1995, 2000) with little modifications while using Kjeldahl apparatus and crude protein was determined using the following formula:

Crude Protein (%)=Nitrogen content × 6.25 (*factor for cereals)

***Extraction of oil*:** The oil content was determined by weighing samples of ground maize (30 g) in cellulose thimble by Soxhlet extraction apparatus at 60°Cfor 8hwith 135 mL n-hexane (Gliszczynska-Swigło and Sikorska, 2004). The oil (0.12 g) was mixed in 2-propanol, vortexed and analyzed for tocopherol by HPLC equipped with fluorescence detector.

***Analytical parameters for HPLC*:** Tocopherol in maize genotypes was performed by HPLC-FLD system equipped with silica based C_{18} column (25 cm x 4.6 mm, 5 µm particle size), Discovery, Supelco MA, USA. The mobile phase was a mixture of (acetonitrile + methanol; 1:1) adjusting to the flow rate of (1.8 ml. min^{-1}) and injection loop of 20 µL. The analyte was assayed by a fluorescence detector (FLD-530) with excitation and emission wavelengths of 295 nm and 325 nm, respectively. The column was operated in an oven (CTO-10A) at 30°C (Iqbal *et al*., 2014).

***Statistical analysis*:** The data of proximate and tocopherol in maize genotypes were subjected statistical analysis and displayed as average ± Standard Deviation (SD) and performed the regression analysis to find regression coefficient (R^2). The analysis of variance (ANOVA) was used to find the significant differences in maize genotypes and vitamin E levels, by software of Statistical Package for the Social Sciences (SPSS) (IBM, PASW Statistics 19, USA).

RESULTS

***Proximate chemical composition*:** In the present study, data of proximate chemical composition of different maize

genotypes are presented in Table 1. The results showed that highest moisture content was found in YH-5521 (11.95% ± 0.10) while the lowest in YH-1898 (9.75±0.15%). For crude protein content the highest mean value in four maize genotypes (NT-6621 Syngenta, FH-1046 & YH-5490 & YH-5521) i.e. 5.25% whereas the lowest mean value of crude protein was present in FH-1036 (1.75±0.02%). Crude fiber of the studied maize genotypes was evaluated and found that NT-6621 Syngenta contained high value (2.55±0.60%) as compared to other genotypes. The lowest fiber content was recorded in YH-1898 i.e. 2.12±0.60%. The highest fat content as determined by Soxhlet extraction apparatus with n-Hexane solvent was recorded in YH-1898 as compared to the other maize genotypes.

***Level of tocopherol*:** The analyses of tocopherol for maize genotypes were performed by high performance liquid chromatograph (LC-10, Shimadzu, Japan) in reverse phase mode. The resolution of individual tocopherol is presented in Fig. 1. Tocopherol content in maize genotypes are presented in Table 2. The data have recognized the high concentration of sum of tocopherols in YH-5521 (9382 µg/g) and lowest in YH-5482 (1971 µg/g). Alpha-tocopherol content was highest in NT-6621 Syngenta i.e. 1869.17 ± 9.06 µg/g and lowest in YH-5521, i.e. 25.03 ± 0.58 µg/g. The concentration of γ-tocopherol was found highest in all maize genotypes as compared to α- and δ-tocopherol. The highest concentration of γ-tocopherol was detected in YH-5521 (9354.85 ±3.59 µg/g). From the data, it is also evident that δ-tocopherol was found in least concentration in all studied genotypes as compared to α- and γ-tocopherol. The lowest concentration of δ-tocopherol was however recorded in YH-1898 (1.40 ± 0.05 µg/g). The concentration of tocopherol and sum of tocopherol are given in Fig. 1. The results have revealed significant differences ($p < 0.05$) of vitamin E levels found in different maize genotypes and exhibited a negative correlation (r) between proximate chemical composition and tocopherol levels.

Table 1. Proximate chemical compositions of maize genotypes.

Sr.	Maize germplasm	Moisture Content (%)	Crude Protein (%)	Crude Fiber (%)	Fat content (%)
1	FH-988 (n =5)	11.60 ± 0.40	3.15 ± 0.03	2.25±0.05	3.87 ± 0.11
2	YH-1898 (n = 7)	9.75 ± 0.15	4.55 ± 0.07	2.12±0.01	5.20 ± 0.08
3	FH-949 (n = 10)	11.55 ± 0.10	4.55 ± 0.06	2.25±0.05	3.93 ± 0.05
4	FH-922 (n = 6)	11.40 ± 0.10	3.85 ± 0.05	2.40±0.10	2.87 ± 0.07
5	YH-5482 (n = 4)	11.80 ± 0.08	4.90 ± 0.08	2.34±0.04	3.37 ± 0.05
6	YH-1899 (n = 5)	11.50 ± 0.15	3.85 ± 0.08	2.25±0.03	4.27 ± 0.07
7	30Y87 Pioneer (n =3)	11.55 ± 0.07	3.15 ± 0.06	2.48±0.10	4.17 ± 0.06
8	NT-6621 Syngenta (n = 4)	11.35 ± 0.07	5.25 ± 0.10	2.55±0.05	2.83 ± 0.03
9	FH-1046 (n = 4)	11.40 ± 0.18	5.25 ± 0.09	2.40±0.05	4.33 ± 0.09
10	YH-5490 (n = 6)	11.75 ± 0.13	5.25 ± 0.05	2.48±0.02	3.23 ± 0.06
11	FH-1036 (n = 3)	11.60 ± 0.15	1.75 ± 0.02	2.32±0.02	3.80 ± 0.10
12	YH-5521 (n = 3)	11.95 ± 0.10	5.25 ± 0.11	2.40±0.10	3.40 ± 0.50

Data is mean of triplicate analysis ± standard deviation.

Table 2. Level of tocopherol (µg/g) in maize genotypes.

Sr.	Maize germplasm	α-tocopherol	δ-tocopherol	γ-tocopherol	∑-tocopherol
1	FH-988 (n =5)	1051.26 ± 12.20	2.54 ± 0.05	1900.46 ± 9.33	2954
2	YH-1898 (n = 7)	1289.11 ± 09.10	1.40 ± 0.05	1551.04 ± 8.99	2842
3	FH-949 (n = 10)	1021.11 ± 09.79	2.68 ± 0.08	1739.77 ± 7.26	2764
4	FH-922 (n = 6)	268.81 ± 08.15	3.62 ± 0.08	1827.83 ± 5.67	2100
5	YH-5482 (n = 4)	404.93 ± 14.05	3.30 ± 0.04	1562.52 ± 9.77	1971
6	YH-1899 (n = 5)	594.73 ± 14.13	1.70 ± 0.08	1561.25 ± 9.71	2158
7	30Y87 Pioneer (n =3)	1057.03 ± 06.50	4.55 ± 0.13	1861.55 ± 4.78	2923
8	NT-6621 Syngenta (n = 4)	1869.17 ± 09.06	4.72 ± 0.12	2036.41 ± 12.9	3910
9	FH-1046 (n = 4)	1123.05 ± 19.95	2.55 ± 0.10	1899.06 ± 7.19	3025
10	YH-5490 (n = 6)	985.19 ± 14.79	5.60 ± 0.11	1929.64 ± 6.78	2920
11	FH-1036 (n = 3)	742.14 ± 12.13	3.95 ± 0.17	1966.55 ± 11.6	2713
12	YH-5521 (n = 3)	25.03 ± 00.58	1.68 ± 0.08	9354.85 ± 3.59	9382

Data is mean of triplicate analysis ± standard deviation.

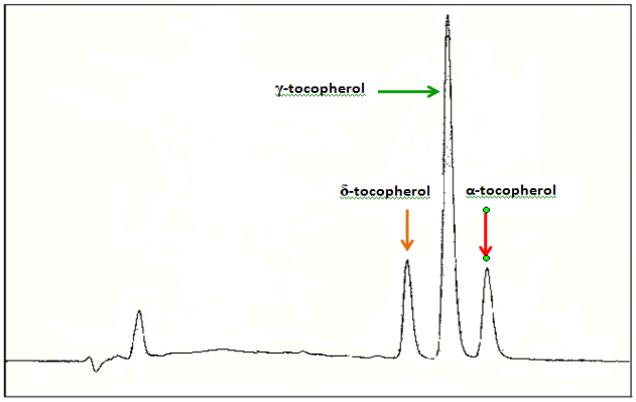

Figure 1. Chromatogram of certified reference standard of tocopherol (δ –tocopherol = 3 µg/mL, γ-tocopherol = 3.75 3 µg/mL, α-tocopherol = 4 µg/mL).

The study presents the correlation between high level of tocopherol (vitamin E) content and proximate chemical composition in various genotypes of maize. The results support the objective of studying various maize varieties to provide additional information regarding the parameters that help induce resistant in maize genotypes, especially the increase in tocopherol inhibit aflatoxigenic fungi which is beneficial for human health. The incidence of high levels of tocopherol (vitamin E) during genetic variation in maize genotypes may be suitable. Additionally, further studies are required to recognize the mechanistic reaction of tocopherol (vitamin E) and its effect on inhibition or control of fungus attack.

DISCUSSION

Tocopherol (Vitamin E) is the major lipophilic antioxidant having scavenging ability for alkoxyl and peroxyl radicals. It contributes physiological role to prevent lipid peroxidation during seed dormancy, germination and early seedling development (Sattler *et al.*, 2004). The interaction of proximate parameters with tocopherol plays an important role in food production and commercialization in preventing lipids and lipid-containing foodstuffs from oxidation during storage, thus extending their stability and shelf life. Maize is a good source of tocopherols and tocotrienols with variable concentration, and individual compound varies in their biological and antioxidative activity (Saldeen and Saldeen, 2005).

Recent studies in Pakistan conducted by Qamar *et al*. (2016) have reported the moisture content ranging from 8.98 to 10.45% in maize grain which agree with those obtained in the present study. The differences may be due to generic maize genotypes in the tested cultivars. Agronomic practices and environmental factors also contribute to moisture retention by the crops (Enyisi *et al.*, 2014). The results of crude protein showed that grains of maize contained protein content ranging from 1.75±0.02 to 5.25 ± 0.10% which are different to the results reported in the literature (Qamar *et al*., 2016; Feil *et al*., 2005) carried out in maize grains.

The proximate chemical composition of the studied maize genotypes showed the presence of crude fiber in the range of 2.12 ± 0.10 to 2.55 ± 0.05%. Our results are contradictory to the published results of some authors (Barikmo *et al*., 2004) in terms of finding high concentration of crude fiber and the differences may be due to varietal differences or environmental factors during growth period of the genotypes or other artifacts. The data regarding fat content are presented in Table 1. Fatty acid content obtained fat content (5.20 ± 0.08 - 2.83 ± 0.03%) are in accordance with Garcia-Lara et al., 2013 who reported the crude fat in resistant and susceptible maize genotypes grown in Mexico to be the range of 3.0 to 5.0%.

Data on tocopherol (vitamin E) level and its relationship with proximate chemical composition in cereal is scanty. However, antioxidants like tocopherol has been shown to control or hinder toxigenic fungi which produce mycotoxin (aflatoxin B_1 and fumonisins) during storage in maize and rice (Nesci *et al*., 2007; Samapundo *et al*., 2007; Iqbal *et al*., 2014). In this study, the average level of α-, γ- and δ-tocopherol in different genotypes of maize were significant in NT-6621 Syngenta, YH-5521 and YH-5490. The sum of tocopherol (α- + γ- + δ-TOC) was significant in YH-5521. The presence of antioxidant in maize genotypes is desirable to reduce the concentration of contaminant (mycotoxin) in studied crop as low as possible, particularly in view of the elevated production and export of maize and derived products from Pakistan. The high presence of tocopherol in maize provides protection against the formation of mycotoxin which may otherwise occur, as a result of sun-drying of grains before storage as well as large storage of grains. Therefore, fungal production and subsequently the formation of mycotoxin may arise owing to delay in drying under high humidity environment, with poor post-harvest management (Ruadrew *et al*., 2013). The routine human diet can be improved by adding maize genotypes in raw as well processed form as the maize is rich in fiber, protein, fat content and antioxidant (tocopherol).

***Conclusions*:** The finding of the study highlights the association between tocopherol (vitamin E) level and proximate chemical composition in different maize genotypes. The results have revealed a negative correlation between tocopherol content and proximate chemical composition in maize genotypes of diverse nature. Further studies are desirable at genetic and molecular levels to probe the mechanistic background. Tocopherols trait needs to be enhanced in crops to protect humans from the oxidative stress mediated by active oxygen and nitrogen species. The protective role of vitamin E against atherosclerosis,

cardiovascular diseases, cataracts, neural tube defects and cancer has been the subject of extensive studies.

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