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Full Length Article

Comparative Antioxidant Potential and Bioactivity of Maize (*Zea mays*) Ear Tissues from Different Genotypes

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Abstract

The aim of this study was to determine the effects of maize (*Zea mays* L.) in diabetic patients *in vitro*. Three varieties (Golden, Sultan and Agatti: 85) were used to study the level of antioxidants and bio-molecules. Results showed that maize ears parts of all three varieties were more effective inhibitor in the oxidation process. Variety Golden silks was found to contain abundant activity of protease (22488 units/g f.wt.), α-amylase (390 units /g f.wt.), total sugar contents (307 mg/g f.wt.), and non-reducing sugar (288 mg/g f.wt.), and husks were rich in reducing sugar contents (16.9 mg/g f.wt.), and grains (kernels) in (15 mg/g f.wt.) protein content. Variety Sultan silks contained total phenolic content (38100 µM/g f.wt.), husk contained peroxidase activity (88655 U/g f.wt.), and grains manifested catalase (555 U/g f.wt.) activity. In varietyAgatti85 a highest activity of superoxide dismutase (292 U/g f.wt.) and malondialdehyde content (35 µM/g f.wt.) was recorded in silks. The findings of this research showed that maize probably inhibits the reaction of oxidation and decreases the complications occurring in diabetes mellitus. It was concluded that performed active parameters (total phenolics, SOD, peroxidase, catalase) may be responsible for higher antioxidant activity, which can be successfully used to reduce the extent of diabetes in the patients. © 2015 Friends Science Publishers

Keywords: Maize; Antioxidants; Ears parts; Diabetes; Oxidative stress

Introduction

World Population is reliant on medicinal plants for curing different diseases. Vascular impediments caused by diabetes are getting well-known considerations. Plants having recognized antioxidant and anti-glycation characteristics are getting much interest due to fewer side effects. Some plants have chemicals that can be utilized straight as drugs, which may have hypoglycemic properties and can be useful in the management of diabetic patients (Elosta *et al*., 2012).

Finding curing ability in plants is very old idea. For example, corn silk has been used for the prevention of diabetic problems in Chinese conventional medicine due to having excess of flavonoids (Farsi *et al*., 2008). “Metformin” is the only moral drug permitted for the treatment of type-II diabetes (Beisswenger *et al*., 1999) that is obtained from a medicinal plant (*Galega officialis*). It is traditionally utilized for curing diabetes (Oubre *et al*., 1970). Enhanced oxidative stress is a major cause for the growth of diabetes and problems related to it. Diabetes is generally an adjunct to the enhanced formation of free radicals or damaged antioxidant barricades. There are some known means e.g., protein kinase C, which link enhanced oxidative stress with diabetic problems (Maritim *et al*., 2003).

Information about medicinal plants has been systematically arranged in this area for further use in medicine has no side effects (Satyavati *et al*., 1987). Antioxidants protect in opposition to, glycation created liberate radicals and can have therapeutic latent (Ceriello *et al*., 1991). Furthermore, a study has reported that composites with both antiglycation and antioxidant characteristics have great effect in the treatment of diabetes mellitus (Duraisamy *et al*., 2003). A large number of medicinal, aromatic and other plants having chemical composites show antioxidant characteristics. Oxidation process is one of the most vital means for the formation of free radicals in drugs, foods and even in metabolites (Halliwell, 1994). Antioxidative defense is the most appropriate and effective means to eliminate the act of free radicals, which are the reason of oxidative stress. Antioxidants have the characteristics to distort chain reactions of free radical, which cause oxidative stress. In recent times attention has been focused in the therapeutic latent medicinal plants as antioxidants (Pourmorad *et al*., 2006).

In modern time, there has been significant progress in the recognition and consumption of plants with antioxidant activity. Diuretic, antilithiasic, uricosuric and antiseptic

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properties are usually accredited to corn silk, style/stigma of maize (*Zea mays* L.). In many parts of the world, corn silk has been utilized for the treatment of gout, edema, cystitis as well as for kidney stones, gout, nephritis and prostatitis (Ali *et al*., 2008).

Maize is a widely cultivated grain crop with a world production of 332 million metric tons. Around 40% of the yield (130 million tons) is used for maize. It is the 3rd most important cereal crop after wheat and rice in Pakistan and generally utilized as animal feed, human food and as a raw material for food industry and used in many medicines (Muhammad, 1979; Tahir *et al*., 2011). The objective of this study was to make comparison of active ingredients as antioxidants and other chemical constituents in the ear parts extracts of three maize varieties.

Materials and Methods

Sample Extraction and Analysis

For extraction and estimation of enzymes and other biochemical parameters all parts of maize ears (silk, grains, husk, rachis) were grounded in extraction buffer specific for different enzymes/biomolecules and centrifuged at 15,000×*g* for 20 min at 4°C. The supernatant was separated and used for assays of different enzymes and other biochemical changes. Experiments were laid out in completely randomized design (CRD) with three replicates.

Biochemical Analysis

Total soluble proteins: For protein estimation in different parts of maize, 5 µL of supernatant and 95 µL 150 mM NaCl were mixed with 1.0 mL of dye reagent (100 mg). Coomassie Brilliant Blue G-250 dye was dissolved in 50 mL of 95% ethanol and 100 mL 58% (w/v) phosphoric acid and dilute to one litter and the mixture was left for 5 min to form a protein dye complex. Then, the absorbance was measured at 595 nm (Bradford, 1976).

Superoxide dismutase (SOD): Ears samples (0.5 g) were homogenized in extraction buffer consisting of 50 mM potassium phosphate, pH 7.8, 0.1% (w/v) BSA, 0.1% (w/v) ascorbate, 0.05% (w/v) β-mercaptoethanol as described by Dixit *et al*. (2001). The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitrobluetetrazolium (NBT) following the method of Giannopolitis and Ries (1977).

Catalase (CAT) and peroxidase (POD): For the estimation of CAT and peroxidase, different ears parts (0.5 g) were homogenized in medium composed of 50 mM potassium phosphate buffer, pH 7.0 and 1 mM dithiothreitol (DTT). Activities of POD and CAT were measured using the method of Chance and Maehly (1995) with some modifications.

Malondialdehyde (MDA) content: The level of lipid peroxidation in different ears part was measured as MDA (a product of lipid peroxidation) by thiobarbituric acid (TBA) reaction using method of Heath and Packer (1968) with minor modifications (Zhang and Kirkham, 1994).

Protease activity: For the estimation of protease activity, samples were extracted in 50 mM potassium phosphate buffer pH 7.8. Protease activity was determined by the casein digestion assay described by Drapeau *et al*. (1974).

Amylase activity: The amylase activity was determined by the modified method Varavinit *et al*. (2002). One mL extracts of all parts was added to 1 mL of 1% starch solution. Then this mixture was incubated for 3 min. After incubation 1 mL DNS reagent (prepared by adding 1 g of 3, 5-dinitrosalicylic acid and 30 g of Na-K-tartrate in 50 mL distilled water followed by addition of 20 mL of 2 N NaOH and finally diluted to 100 mL) was added and placed in boiling water bath for 15 min. The reaction mixture was then cooled to room temperature and 9 mL distilled water added to each tube. After mixing, absorbance of solution was measured at 540 nm.

Reducing sugars: They were determined by dinitrosalicylic acid method (Miller, 1972). A 0.2 mL of extract were added to 1.8 mL distilled water and 1 mL DNS reagent and placed in boiling water bath for 15 min. The mixture was then cooled at room temperature and 9 mL distilled water was added to each tube, mixed well and absorbance of solution was measured at 540 nm.

Total soluble sugars: The amount of total soluble sugars in all parts of maize ear samples was estimated by phenol sulphuric acid reagent method described by Dubois *el al*. (1951). A 0.5 mL of 5% phenol solution was mixed with 2.5 mL of 96% H_2SO_4. Each tube was gently agitated during addition of acid and then allowed to stand in a water bath at 26–30°C for 20 min, and absorbance of colored solution was measured at 490 nm.

Total phenolic contents: A micro colorimetric method as described by Ainsworth and Gillespie (2007) used for total phenolics assay, which utilizes Folin-Ciocalteau (F-C) reagent. For this purpose, 0.2 g of sample was homogenized in 0.8 mL ice cold 95% methanol in an ice cold mortar and pestle. The samples were then incubated at room temperature for 48 h in the dark. The samples were centrifuged at 10,000×*g* for 5 min. The supernatant was removed and used for TPC measurement. A 100 µL of supernatant was mixed with l00 µL of 10% (v/v) F-C reagent, vortex thoroughly and then 800 µL of 700 mM Na_2CO_3 was added. Samples were incubated at room temperature for 2 h. Blank corrected absorbance of samples was measured at 765 nm.

Statistical Analysis

All experiments were conducted in triplicates using completely randomized design (CRD). The significance was ascertained by analysis of variance and Tukey (HSD) test at $p<0.05$ and where applicable at $P<0.01$ using XL-STAT software. Values presented in graphs are mean ± SD.

Results

Antioxidants Activities

Data for activity of CAT in various parts of edible ears of maize are presented in (Fig. 1). The activity of CAT was high (555 U/g f.wt.) in grains of maize variety Sultan as compared to other parts i.e. silk, husks and rachis. In variety Agatti 85 the activity of CAT was high in silk or grains than other parts while silks of variety Golden were superior in CAT activity as compared to other parts of ear (Fig. 1). Variety Sultan was high in CAT activity with respect to grain and rachises while the Golden in corn silk.

Highest SOD activity was noted in silks of variety Agatti 85 (292 U/g f.wt.) followed by Golden (255 U/g f.wt.) and (95 U/g f.wt.) in rachis of Sultan (Fig. 2). While comparing husk, it was observed that maximum SOD activity (237 U/g f.wt.) was observed in variety Agatti 85 while minimum in Sultan. Variety Agatti 85 was high in activity of SOD as compared to the others maize varieties. Similarly, grains of Agatti 85 showed maximum SOD activity (138 U/g f.wt.) than those of others.

A highest POD activity was found in the husk of variety Sultan (88655 U/g f.wt.) followed by silks of variety Agatti 85 with (52833 U/g f.wt.), while a minimum POD activity was found in rachis of variety Golden (3100 U/g f.wt.) (Fig. 3). Silks of variety Agatti 85 showed higher activity (52833 U/g f.wt.) than Sultan and Golden varieties. Husk of variety Sultan showed maximum activity of POD (88655 U/g f.wt.) followed by varieties Golden and Agatti 85. As for POD activity of rachis in all three varieties, Agatti 85 showed (19033 U/g f.wt.) higher activity than Sultan and Golden. Similarly grains of Sultan had higher activity (27266 U/g f.wt.) than Agatti 85 and Golden.

As shown in Fig. 4, maize varieties showed non-significant difference in silk protease activity, while it was comparatively lower in the rachis of Sultan (3880 U/g f.wt.). Protease activity in husks of ear was high in variety Golden (11810 U/g f.wt.) and low in Agatti 85 (4135 U/g f.wt). Rachis of Agatti 85 and Golden showed non-significant difference in protease activity while it was low in variety Sultan (3883 U/g f.wt). Similarly among grains, variety Sultan was at the top among three maize varieties and Agatti 85 was at the lowest level.

Biochemical Attributes

Total phenolic contents were high in silks of variety Sultan (38100 µM/g f.wt.) followed by Agatti 85 whereas lowest total phenolic contents (28121 µM/g f.wt.) was found in rachis of Golden (Fig. 5). The phenolic contents in silks were higher in variety Sultan as compared to Agatti and Golden. Variety Sultan showed slightly better phenolics contents in the husk than other varieties. Similarly total phenolic in rachis of variety Agatti 85 were greater than Sultan and Golden. Grains of Agatti 85 had superior phenolics contents (33484 µM/g f.wt.) than others.

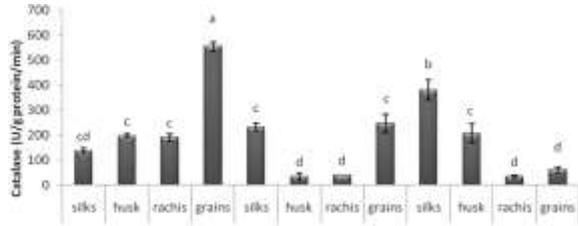

Fig. 1: Activity of catalase in various ears parts of maize varieties grown in field under natural conditions (Mean ± S.E.; *n* =3)

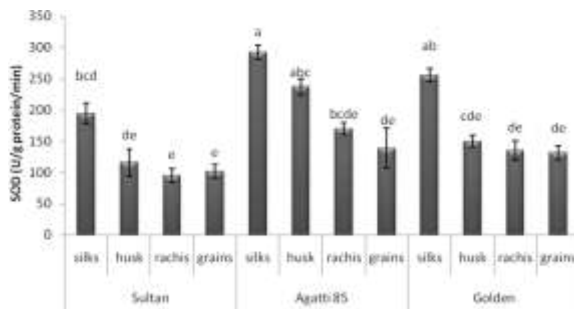

Fig. 2: Activity of superoxide dismutase in various ears parts of three maize varieties grown in field under natural conditions (Mean ± S.E.; n =3)

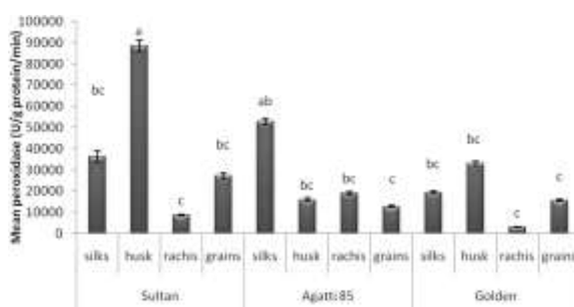

Fig. 3: Activity of peroxidase in various ears parts of maize varieties grown in field under natural conditions (Mean ± S.E.; n =3)

Highest total proteins contents were found in grains of variety Golden (15 mg/g f.wt.). Minimum soluble proteins (1.98 mg/g f.wt.) was present in rachis of this variety (Fig. 6). Silks of variety Golden had higher proteins (14.5 mg/g f.wt.) than those of Sultan and Agatti 85. Husk of variety Sultan showed higher soluble proteins contents (11.1 mg/g f.wt.) than the varieties Agatti 85 and Golden. Similarly rachis of variety Sultan showed larger proteins (11.211 mg/g f.wt.) than that of variety Agatti 85 and Golden (4.4 mg/g f.wt.), (2.1 mg/g f.wt.) respectively.

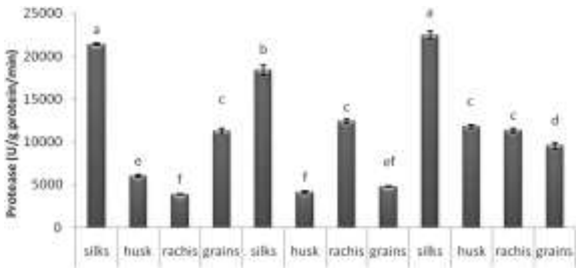

Fig.4: Activity of protease in various ears parts of three maize varieties grown in field under natural conditions (Mean ± S.E.; n =3)

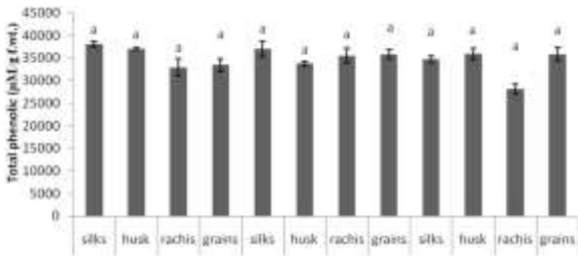

Fig.5: Total phenolic contents in various ears parts of three maize varieties grown in field under natural conditions (Mean ± S.E.; n =3)

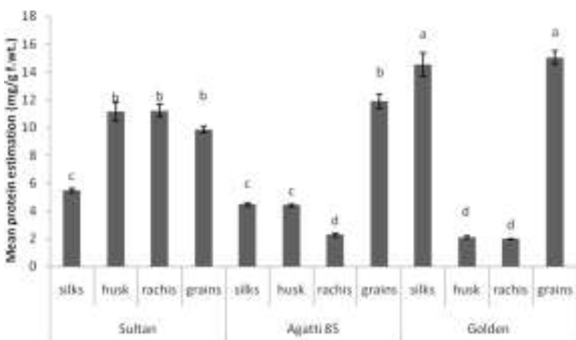

Fig.6: Total soluble proteins content in various ears parts of maize varieties grown in field under natural conditions (Mean ± S.E.; n =3)

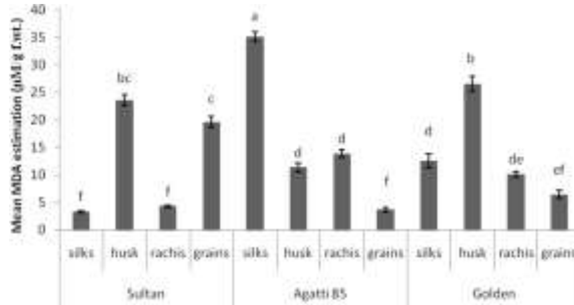

Fig.7: MDA contents in various ears parts of maize varieties grown in field under natural conditions (Mean ± S.E.; n =3)

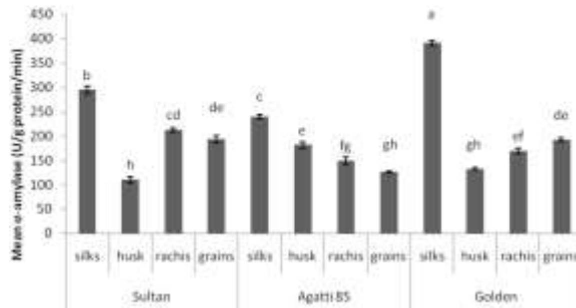

Fig. 8: Activity of α-amylase in various ears parts of maize varieties grown in field under natural conditions (Mean ± S.E.; n =3)

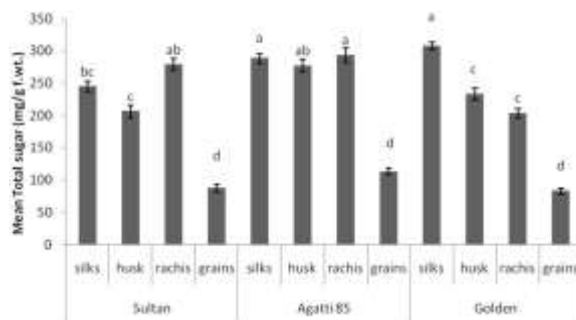

Fig. 9: Total sugar contents in various ears parts of maize varieties grown in field under natural conditions (Mean ± S.E.; n =3)

Maximum (MDA) contents were found in silks of variety Agatti 85 with 35 (µM/g f.wt.) while it was minimum in silks of variety Sultan and grains of variety Agatti 85 (Fig. 7). Level of MDA in husk part of variety Golden was found higher than other varieties. In rachis part, variety Agatti 85 contained higher contents than others. Similarly, grains of variety Sultan had MDA level of 19.6 (µM/g f.wt.) followed by variety Golden and variety Agatti 85.

The activity of α-amylase in the silks of maize variety Golden was the highest (390 U/g f.wt.) followed by in silks of variety Sultan (294 U/g f.wt.) (Fig. 8). A lowest α-amylase activity was present in husk of variety Sultan. While comparing the same parts, silks of variety Golden contained maximum α-amylase. Among the silks of all varieties the lowest value was (240 U/g f.wt.) showed by variety Agatti 85. While comparing husk, variety Agatti showed higher activity (181 U/g f.wt.) than that of variety Golden (132 U/g f.wt.), while variety Sultan displayed a lowest activity (110 U/g f.wt.). Similarly, a higher α-amylase activity (212 U/g f.wt.) was recorded for rachis of varieties Sultan and Golden, while a lower one in variety

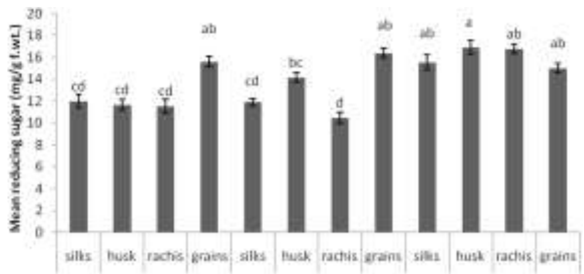

Fig. 10: Reducing sugar contents in various ears parts of maize varieties grown in field under natural conditions (Mean ± S.E.; n =3)

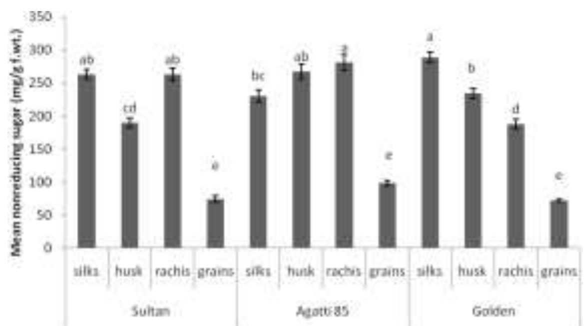

Fig. 1: Non-reducing sugar contents in various ears parts of maize varieties grown in field under natural conditions (Mean ± S.E.; n =3)

Agatti 85 (148 U/g f.wt.).

Silks of variety Golden showed the total sugar contents (307 mg/g f.wt.) which were non-significantly different from variety Agatti 85 and rachis of variety Sultan (Fig. 9). The lowest level was recorded in grains of variety Golden (82 mg/g f.wt.). The total sugar contents in same part of different varieties were found maximum in silks of variety Golden (307 mg/g f.wt.), which is slightly higher than of variety Agatti 85 (288 mg/g f.wt.) and variety Sultan showed less total sugar contents (245 mg/g f.wt.). While comparing husk, higher total sugar contents were recorded in variety Agatti 85 than variety Golden and variety Sultan. But difference between varieties Sultan Golden was less. Contents of total sugar in husk were (277, 233, 206 mg/g f.wt.) respectively. Similarly, rachis of variety Agatti 85 had greater total sugar contents (293 mg/g f.wt.) than that of variety Sultan and Golden. Minimum content was recorded in variety Golden (204 mg/g f.wt.).

The reducing sugar contents were in husk of variety Golden (16.9 mg/g f.wt.) and rachis of variety Golden were non-significantly different from grains and silks of variety Golden and grains of variety Sultan and variety Agatti 85 (Fig. 10). Overall results showed that in rachis, utmost contents were observed in variety Golden (16.8 mg/g f.wt.) followed by variety Sultan (11.5 mg/g f.wt.) and variety Agatti 85 (10.5 mg/g f.wt.).

Silks of variety Golden showed maximum non reducing sugar contents (288 mg/g f.wt.) followed by the 2nd highest value in rachis of variety Agatti 85 among all three varieties (Fig. 11). The minimum non reducing sugar contents were determined in grains of variety Golden (72.2 mg/g f.wt.). Whereas, the contents in variety Sultan were in-between of variety Golden and variety Agatti 85. While comparing husks, highest contents (267 mg/g f.wt.) were recorded in variety Agatti 85 and at 2nd it was (mg/g f.wt.) recorded in variety Golden and minimum contents were (mg/g f.wt.) in variety Sultan. Non-reducing sugar contents in the grains of all samples were slightly different from each other. The contents from highest to lowest were (98, 74 and 72 mg/g f.wt.), respectively.

Discussion

In various maize varieties, variety Sultan was high in CAT activity with respect to grain and rachis while the Golden in corn silks. In our study highest activity of CAT was observed in grains which are in agreement with previous finding on *Nigella sativa* in which Kaleem *et al.* (2006) observed high activity in grains. An increased CAT activity in sunflower (*Helianthus annuus*) grains was associated with a decrease in hydrogen peroxide level and in lipid peroxidation (Bailly *et al*., 2004). Activity of CAT is altered due to metabolic changes during various growth stages. In addition many environmental factors such as light (Feierabend *et al*., 1992) temperature (Omran, 1980; Nir *et al*., 1986), O_2 and CO_2 concentration (Fair *et al*., 1973; Monk *et al*., 1987) have been shown to influence catalase activity in mature plants. In fact this high level of CAT activity induced by grains drying was associated with a decrease in hydrogen peroxide level and in lipid peroxidation studied by Bailly *et al*. (2004).

Overall results showed that highest activity of SOD was observed in silks part of ear as compared to other parts. Silk is an important food part (El-Ghorab *et al*., 2007) which are used to produce novel natural antioxidants as well as a flavoring agent in various food products. Al-Rawi (2011) demonstrated that rise in the level of superoxide dismutase in serum and saliva of diabetic patients might be high due to the existence of elevated level of free radicals production. The SOD activity seemed to increase more in response to the high temperature than water deficiency (Wahid *et al.*, 2007; Farooq *et al*., 2009). Shahab *et al*. (2011) reported an increase in SOD activity of with increasing concentration of ions Na^+ and Cl^- in *Cassia angustifolia* grains and same behavior was also observed in tolerant cultivars of tomato, wheat, beet and cotton than those of sensitive.

Highest POD activity was found in the husk of variety Sultan followed by silks of variety Agatti 85, while the minimum in rachis of variety Golden. Overall, from these findings it is clear that the husk of ears part were rich in peroxidase activity and our results were in contrary to El-Ghorab *et al*. (2007) where, the extracts from Caper husk exhibited higher antioxidant activity of peroxidase then those of their essential oils. Hameed *et al*. (2008) suggested that POD activity (two folds) high in kabuli chickpea inheritably as compared to desi chickpea.

A highest protease activity was shown by the silks in all three varieties, while it was lower in other parts of maize. Study performed by Grudkowska and Zagdanska (2004) showed that decrease in protein contents might be related to high protease activity in plants which might be due to the reason that proteases are involved in the removal of abnormal, misfolded proteins, and protein rebuild in response to various environmental changes.

Total proteins contents were the highest in grains of variety Golden. This was slightly higher than silks of this variety. While comparing grains, variety Golden was at highest position among all varieties. In a study performed by Kravic *et al*. (2009) on maize (grains), high proline was one of the most plentiful amino acids, predominantly found in its bound form as a constituent of the storage protein zein. It accounts for a considerable protein of free amino acid pool in mature endosperm of maize (Hadzi-Taskovic, 1983).

Maximum MDA contents were found in silks of variety Agatti 85 while it was the minimum in silks of variety Sultan and grains of variety Agatti 85. In mango fruits cv. Nam Dok Mai highest MDA contents was observed in storage condition at low temperature and also electrolyte leakage are used to signify the lipid peroxidation of membrane lipids and permeability (Zhao *et al*., 2006; Junmatong *et al*., 2012). Guo *et al*. (2010) found that in the cotton leaf at flowering and boll-forming stage the MDA contents and endogenous hormones increased under normal watering applications.

Similarly, higher α-amylase activity was recorded for rachis of variety Sultan and Golden. The lowest activity showed by variety Agatti 85. While comparing the grains, variety Sultan showed higher activity than the variety Golden and variety Agatti 85. Fuchs *et al*. (1980) observed that in developing phase of mango fruits parts increased amylase activity corresponding to the increase in fruit part weight which suggested that changes taking place during ripening and development time as well as in the nature of the amylolytic enzyme activity during these development phases. Moreover, Lima *et al*. (2001) also found increased activity of α -amylase in mango fruits (*Mangifera indica* L.) and decreased starch content which was an important role of carbohydrate metabolism during ripening of mango.

Minimum soluble sugars content was recorded in variety Golden. While comparing grains of all three varieties, it was observed that they statistically classified in the same group due to non-significant difference between contents. Giorgini and Campos (1992) demonstrated that *Coffea arabica* grains were germinated and seedlings total soluble sugars content increased in the axis and starch contents decreased it was during germination endosperm degradation started due to metabolic changes. Farooq *et al*. (2006) found that increased in the level of soluble sugar direct relationship with α-amylase in rice grains. Present and previous reports thus support the view that increased de novo synthesis and activities of existing enzymes in seeds (Sung and Chang, 1993; Lee and Kim, 2000).

Grains of all three varieties showed statistically same reducing sugar contents. Guimaraes (2008) provided strong evidence to our results that reducing sugars and phenolics were the main antioxidant components found in all extracts of *Zea mays* parts. Overall these results suggested that rachis was used as a flavoring ingredient source and may act as a natural antioxidant supplement in various food goods (El-Ghorab *et al*., 2007). Fuchs *et al*. (1980) studied that in developing mango fruits parts during ripening there was a decrease in starch content and an increase in the reducing sugars contents.

Overall findings indicated that highest non-reducing sugar contents in silks were in accordance with Solihah *et al*. (2012), which showed increased in non-reducing sugar in silks linked that it had a variety of nutritional compounds including minerals, vitamins, salts, proteins, natural sugars, fibers and carbohydrate. Lima *et al*. (2001) described an increase in non-reducing sugars contents in fruit parts of mango and decrease in the starch content was linked to carbohydrate metabolism is an important feature during ripening of mangos.

High total phenolic contents in silk as observed in our study are in accordance with previous reports by Nurhanan and Wan (2012) on maize in which they observed high phenolic contents in corn silk. Corn silk contains phytochemicals such as steroids, anthocyanins, alkaloids, flavanoids, phenolics and terpenoid group of chemicals like citronellol and α-terpineol (Hasanudin *et al*., 2012).

Conclusion

SA higher phenolic content of maize was promising candidate for the improvement of natural glycation end products inhibitors for the treatment and prevention of diabetic difficulties and the degenerative belongings of aging. Great differences exist in the antioxidative enzyme activities and other biochemical constituents of maize. Thus these parts of maize may be used as hypoglycemic agents in the prevention of diabetes in the patients.

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