Journal of Food Technology 3 (3): 444-448, 2005

Nutraceutical Phenolics (Total Polyphenols, Chlorogenic [5-o-caffeoylquinic] Acid) in Tubers, Leaves, Stalks and Stems of New Developed Sweetpotato (*Ipomea batatas* L.): Alterations in Tubers During Short-term Storage

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Abstract: New genotype of sweetpotato (*Ipomea batatas* L.) was developed by agronomic micropropagation method in Aegean Area in Turkey. Total phenolic contents and chlorogenic acid (5-O-Caffeoylquinic acid) (CA) concentration of new genotype sweetpotato (Hatay Red) were determined by UV-visible spectrophotometer. Total phenolic content of tubers of that micropropogated crop was 184.47±0.06 mg 100 g^{-1} ($p<0.01$) whereas the total chlorogenic acid amount was 23.36±0.04 mg 100 g^{-1} ($p<0.01$). Total phenolic contents in leaves , stalks and stems of sweetpotato were also high and their amounts were as 333.52±0.13 mg 100 g^{-1}, 132.26±0.07 mg 100 g^{-1} and 180.95±0.09 mg 100 g^{-1}, respectively ($p<0.01$). Leaves of sweetpotato contained 38.36 mg chlorogenic acid 100 g^{-1}($p<0.01$) whereas stalks and stems had 25.84 mg 100 g^{-1} and 123.62 mg 100 g^{-1} of chlorogenic acid ($p<0.01$). With the exposure of tubers of that new cultivar to dark and normal light conditions storage for up to 15 days, influences upon total phenolics and chlorogenic acid contents were investigated. It was monitored that 31.8% of total polyphenols were decreased at the 12 days of storage at the normal light source ($p<0.01$). Increasing of chlorogenic acid was 25.3% of initial level at 6 days of storage whereas 75.8% of initial at 12 days of storage at the same light conditions ($p<0.01$). Whole parts of sweetpotato could be utilized valuable source of food and animal feed after harvesting due to high phenolic contents.

Key words: Total phenolics, sweetpotato (*Ipomea batatas* L.), chlorogenic acid , micropropagation

INTRODUCTION

Sweetpotato (*Ipomea batatas* L.) is one of the ten most important crops that feeds millions of people in the developing world and is grown in many tropical, subtropical, and temperate regions. More than 133 million tons are produced per year in more than 100 developing countries as valuable source of food, animal feed and industrial raw material [1,2]. Sweetpotatoes is not related to the potato (*Solanum tuberosum* L.) and belong to the morning-glory family (*Convolvulaceae*) which are cropped as annuals and unlike the is a tuber, or thickened stem-the sweetpotato is a storage root [1,3].

Polyphenolic compounds has a strong a antioxidative and anticancerogenic effects for human health. Essentially, plant polyphenols may reduce the risk for certain cancers[4,5] and coronary heart diseases [6,7].

Chlorogenic acid (3-O-caffeoylquinic acid) (Fig. 1.) constitutes about 90% of the total phenolic compounds of potato tubers [8].Chlorogenic acid may be involved in defenses against insects and phytopathogens in plants [9] and has a strong *in vitro* antioxidative effects [7]. There is a great interest to determine chlorogenic acid and related phenolic compounds in fruits and vegetables including potatoes [10-14].

Sweetpotato contain chlorogenic acid and three kinds of isochlorogenic acids as the main phenolic constituents. These four phenolic compounds has been suppressed the melanogenesis equally. Therefore the extract from sweetpotato was found to suppress the melanogenesis of mouse melanoma B16 cells [15]. It has been reported that sweetpotato (*Ipomea batatas* L.) is a functional alternative food and has high amount of micronutrients and polyphenolic compounds [10]. Leaves, stalks, stems and

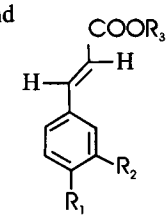

Fig. 1: The chemical structure of chlorogenic acid (R_1=R_2=OH, R_3=Quinic Asid)

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tubers of sweetpotato contain phenolics and chlorogenic acid is a major polyphenolic component [16]. The objectives of this research was to determine the total polyphenols and chlorogenic (5-O-Caffeilquinic) acid content of new developed sweetpotato (*Ipomea batatas* L.) genotype in Aegean Area in Turkey and to investigate the storage conditions for up to 15 days on total phenolics and chlorogenic acid contents of this new cultivar.

MATERIALS AND METHODS

Plant Material: A local sweetpotato (*Ipomea batatas* L.) genotype named Hatay Red with orange flesh color was used as genetic material in the study conducted in the Department of Field Crops of the Ege University during 2000-2002. Rootstocks of Hatay Red were planted in clay pots as starting material. The growing tips of the newly developed plants were then cultured *in vitro* in the modified Murashige-Skoog growth medium with NAA (Naphthaleneacetic acid) in mg L^{-1}. The cultures were kept in the growth cabinet at 24°C±2 under 16 h illumination. Nodes were taken from the developed seedling and they were recultured in order to increase the number of micropropagated plantlets. 7-8 cm of seedlings were transplanted to seed beds in 40cm × 40 cm spacings containing 1:1:1 (v/v/v) soil, sand, and manure mixture.

New developed plants (Hatay Red) were harvested by hand in November 2002 and tuber, leaf, stalk and stem samples were used in total polypenol and chlorogenic acid analysis.

Chemicals: Folin-Ciocalteu's phenol reagent were purchased from Sigma (St.Louis,MO). Chlorogenic acid (Kod: C-3878) and polyvinylpolypyrrolidone was obtained from Sigma Chem.Co.(St Louis,U.S.A). Metanol (analytical grade) was purchased from J.T.Baker. Sodium carbonate (Na_2CO_3) and all organic solvents were of analytical grade and obtained from Merck (Darmstadt,Germany). Bidistile water was purchased from LabScan (Turkey).

The Determination of Total Phenolic Compounds: Dry sweetpotato tuber material was grounded (0.5 g) was extracted with 20 mL of 80% aqueous methanol using Ultra blender for 1 min at 4°C and then sample was centrifuged (10 min,4000×*g*) and successive extracts were combined. Total phenolic amounts in this extract was determined according to Folin-Ciocalteu procedure (Singleton and Rossi,1965). 500 µL of sweetpotato metanol extract were transferred to 1 mL of bidistile water. 1 mL of Folin-Ciocalteu phenol reagent was added and combined solutions were mixed. After 5 min,1 mL of saturated sodium carbonate solution was added and the solution was also mixed with stirring. 45 min after adding the saturated sodium carbonate solution to the Folin-Ciocalteu mixture, the adsorbance at 765 nm. was measured (Jasco UV-vis 540 spectrophotometer). The total phenolic content was determined from a standard curve using chlorogenic acid and expressed as chlorogenic acid (CAE) equivalents. To determine the total phenolics in leaves, stalks and stems of sweetpotato, the same procedure was performed.

The Determination of Total Chlorogenic Acid Contents: Methanol extracts of tubers, leaves, stalks and stems parts of sweetpotato samples were obtained using above-mentioned procedure. The UV absorption method (328 nm) was modified from a published method concerning quantitative chlorogenic acid determination (Aoki,Yahagi and Tamura ,1984). After adequate diluting with methanol, sweet potato methanol extract were mixed with iso-propanol and benzene (4:4:2, v/v/v). The mixture were charged with polyvinylpolypyrrolidone column (ID 21×65 mm) and eluted with methanol after rinsing twice with benzene and methanol mixture (6:2,v/v). Chlorogenic acid standard solutions were used for plotting calibration curve (R^2 = 0.9999). The method used was validated by using the tuber part of sweetpotato (*Ipomea batatas* L.) sample (R^2 = 0.9999).

Statistical Analysis: Three different extraction of sweetpotato (Hatay Red) in triplicate (n=3) were analysed for each part. All determinations were in twice. Data were analyzed with Statistica for Windows ('98 edition ,Ver . 6.0, StatSoft Inc., Tulsa , OK) by one-way analysis of variance (Kruskal-Wallis ANOVA) with sweetpotato total polyphenols and chlorogenic acid contents as the source of variance.

RESULTS AND DISCUSSION

New genotype of sweetpotato (*Ipomea batatas* L.) was developed by agronomic micropropagation procedure in Aegean Area in Turkey. Total polyphenols and chlorogenic (5-O-Caffeilquinic) acid contents of new developed sweetpotato (*Ipomea batatas* L.) genotype were determined. For whole parts of new developed

Table.1: Dry matter, total phenolics and chlorogenic acid contents of new sweetpotato genotype ($p<0.01$)

Sweetpotato (Hatay Red)	Dry matter (g 100 g^{-1})	Total phenolics (mg 100 g^{-1})	Chlorogenic Acid (mg 100 g^{-1})
Tuber	30.94±0.02	184.47±0.06	23.36±0.04
Leaf	14.02±0.04	333.52±0.13	38.36±0.08
Stalk	7.14±0.01	132.26±0.07	25.84±0.02
Stem	16.83±0.02	180.95±0.09	123.62±0.10

Values are means of triplicate determinations. All analysis in duplicate.

sweetpotato, the total phenolic contents and chlorogenic acid amounts was provided in Table 1.

The dry mass data were analyzed in whole parts of sweetpotato genotype ($p<0.01$). The tubers of sweetpotato showed a high content of dry matter (30.94±0.02) (Table 1). The leaves, stalks and stems contained 14.02±0.04 ; 7.14±0.01 and 16.83±0.02 as g per 100 g, respectively (Table 1).

The total polyphenol concentration in 100 g was 184.47±0.06 ; 333.52±0.13 ; 132.26±0.07 and 180.95±0.09 mg, in tubers, leaves, stalks and stems of new sweetpotato genotype, respectively ($p<0.01$) (Table 1). The total chlorogenic acid content was dedected (in 100 g) as 23.36±0.04 ; 38.36±0.08 ; 25.84±0.02 and 123.62±0.10 mg, in tubers, leaves, stalks and stems of this crop (Table 1). Method validation was performed with recovery analysis of chlorogenic acid by using standard addition procedure ($R^2 = 0.9999$). It is estimated that the differences between the total polyphenol content and chlorogenic acid content may be due to the presence of other polyphenolic compounds except for chlorogenic acid.

Not only tubers but also leaves, stalks and stems of sweetpotato have high amount of polyphenols. It is reported that plant polyphenols having esterified carboxylic acid, such as caffeic acid ester with catechol structure and have an inhibitory influence on the cell damage caused by active oxygen species [18], and suppress harmful bacterial growth [6,23]. The tuber parts of sweetpotato was stored for up to 15 days at dark and normal light source. During short-term storage, the changes of total polyphenols and total chlorogenic acid (5-caffeoylquinic acid) contents of sweetpotato were investigated as shown in Table 2 ($p<0.01$).

After harvesting, total polyphenol contents of sweetpotato tubers were determined as 184.47±0.06 mg 100 g^{-1} and decreased during 3 days of storage at dark light (178.32±0.11 mg 100 g^{-1}) ($p<0.01$) (Table 2). At the 6 day of storage, 170.55±0.23 mg total polyphenol 100 g^{-1} was found in tubers and low amounts of changes were detected at the same light conditions. There were no significant differences regarding total polyphenol contents between 3, 6 and 6 days of storage ($p<0.01$) (Table 2). 9.49% of total polyphenols were decreased at the 15 days of storage at dark light as shown in Table 2. At the normal light source, initial content of polyphenols was decreased, continuously for up to 12 days and there were no significant differences concerning total polyphenol contents between 12 and 15 days of storage ($p<0.01$) (Table 2). During 3 days of storage at day light, 162. 56±0.07 mg total polyphenol 100

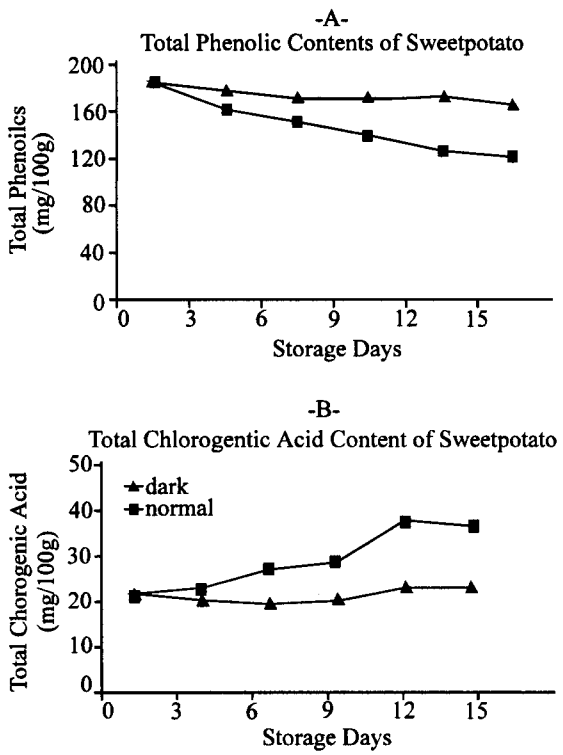

Fig. 1: The changes of A. total polyphenols and B. total chlorogenic acid (5-caffeoylquinic) contents of sweetpotato tubers at 2 different light sources ($p<0.01$)

g^{-1} was determined whereas at 9 days of storage 139.89±0.09 mg100 g^{-1} total polyphenols was dedected ($p<0.01$) (Table 2). 18.2% of total polyphenols were decreased at the 6 days of storage at normal light whereas 31.8% of that were decreased at the 12 days of storage at the same conditions ($p<0.01$)(Table 2).

Total chlorogenic acid (5-caffeillquinic acid) concentrations of tubers were monitored for up to 15 days. Initially, chlorogenic acid amount was 23.36±0.04 mg 100 g^{-1} in tubers at dark conditions and decreased, slightly. After 6 day of storage, chlorogenic acid amount of tubers increased to 24.92±0.08 mg 100 g^{-1} at the dark light conditions ($p<0.01$) (Table 2). There were no significant differences concerning total chlorogenic acid contents between 12 and 15 days of storage at dark ($p<0.01$) (Table 2).

Total chlorogenic acid of sweetpotato tubers were determined at normal light conditions and it is reported that the chlorogenic acid contents in sweetpotato tubers

Table 2: The changes of total polyphenols and total chlorogenic acid (5-caffeoylquinic acid) cont sweetpotato tubers with the exposure to dark and normal light ($p<0.01$) for up to 15 days (Values are means of triplicate determinations. All analysis in duplicate)

		Total polyphenols (mg 100 g^{-1})					
		Expose time					
	Light source	0 day	3 days	6 day	9 day	12 day	15 day
Sweetpotato	dark	184.47±0.06	178.32±0.11	170.55±0.23	169.72±0.15	171.38±0.20	166.97±0.34
(Hatay Red)	normal	184.47±0.06	162.56±0.07	150.78±0.10	139.89±0.09	125.78±0.13	122.35±0.06
	Total chlorogenic acid (5-caffeillquinic acid) (mg 100 g^{-1})						
		Total polyphenols (mg 100 g^{-1})					
		Expose time					
	Light source	0 day	3 days	6 day	9 day	12 day	15 day
Sweetpotato	dark	23.36±0.04	21.83±0.09	20.76±0.12	21.55±0.05	24.92±0.08	24.57±0.16
(Hatay Red)	normal	23.36±0.04	24.76±0.05	29.28±0.17	30.98±0.13	41.07±0.10	39.62±0.04

has been increased, continuously. Increasing of chlorogenic acid was 25.3% of initial level at 6 days of storage whereas 75.8% of initial at 12 days of storage. Chlorogenic acid concentration has been so increased due to sweetpotatoes exposed to normal light ($p<0.01$) (Table 2). Zucker[19] reported the accumulation of chlorogenic acid and other polyphenols at the potatoes expose the light, at the sites of infection of potatoes by certain microorganisms, at sites of mechanical damage and at bruised potatoes.

Chlorogenic acid in potato tubers is also associated with the phenomenon referred to as after-cooking blackening (ACB). In the flesh of susceptible cultivars a colorless iron-chlorogenic acid chelate is formed during the cooking process that, on exposure to air, is oxidized to a bluish-gray ferric (Fe^{+3}) compound [20]. Before not known the positive effects of phenolic compounds to human health, this discoloration were regarded as a severe quality defect, although it does not affect taste or nutritive value[21]. Before not known the healthy effects of phenolics, stimulation of chlorogenic acid by cultivar-dependent and environmental conditions such as soil, climatic conditions, storage period, and light resulted in tubers of a lower quality at the end point of sale [22]. Currently, consumer has preferred the colorness in potato products due to the antioxidant concept.

Whole parts of sweetpotato (*Ipomea batatas* L.) could be utilized valuable source of food and animal feed due to high phenolic contents. Our further study concerning detailed polyphenol determination of sweetpotato is in progress.

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