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Örtüaltında ve Organik Olarak Yetiştirilen Bazı Muz Çeşitlerinin Farklı Olgunluk Dönemlerindeki Fiziko-kimyasal Özellikleri ve Antioksidan İçerikleri

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Özet

Bu araştırmada, örtüaltı ve organik büyüme sisteminde yetiştirilen iki farklı muz çeşidinin (Dwarf Cavendish ve Gross Michel) yeşil (hasat dönemi) ve sarı (olgun dönem) olmak üzere iki farklı dönemde meyve ağırlığı, meyve çapı, meyve uzunluğu, meyve yüksekliği, boy/en oranı, kabuk kalınlığı, kabuk ağırlığı, toplam suda çözünebilir kuru madde (SÇKM), pH, titre edilebilir asitlilik, meyve sertliği, meyve rengi ve antioksidan içerikleri gibi bazı fiziko kimyasal özellikleri belirlenmiştir. Araştıma sonucunda, en ağır (170.297 g) ve en geniş (37.059 mm) meyveler Gross Michel (kabukla birlikte) çeşidinden elde edilmiştir. Meyve uzunluğu ve meyve ağırlığı değerleri bakımından hem kabuklu hem de kabuksuz olarak Gross Michel muz çeşidi en yüksek değerlerde bulunmuştur. Toplam suda çözünebilir kuru madde, pH ve titre edilebilir asitlilik değerleri Dwarf Cavendish muz çeşidi için %22.1, 4.1 ve %0.48; bu değerler Gross Michel muz çeşidi için ise sırasıyla %21.533, 4.6 ve %0.503 olarak elde edilmiştir. Diğer taraftan meyve sertlik değerleri Dwarf Cavendish çeşidi için 4.233 N/mm, Gross Michel çeşidi için 4.500 N/mm olmuştur. Bununla birlikte, denemede yer alan çeşitler arasında kabuk rengi bakımından istatistiksel olarak belirgin bir farklılık bulunmamıştır. Ek olarak, meyve eti ekstraktlarında antioksidan aktiviteleri -serbest radikal giderme aktivitesi, , hidrojen peroksit giderme (H_2O_2) ve -metal (Fe^{+2}) şelatlama aktivitesi metodları ile belirlenmiştir. Olgun dönemde, Dwarf Cavendish muz çeşidinin meyve eti, Gross Michel muz çeşidinden daha yüksek antioksidan aktivitesi göstermiştir.

Anahtar Kelimeler: *Dwarf Cavendish, Gross Michel, Organik büyüme tekniği, Fiziko kimyasal özellikler, Antioksidan aktiviteleri*

Physico-chemical Properties and Antioxidant Activities of Under Protected and Organically Cultivated Some Banana Cultivars at Different Ripening Stages

Abstract

In this research, several physico-chemical properties such as fruit weight, fruit width, fruit length, fruit height, fruit length/fruit width, peel thickness, peel weight, total soluble solids (TSS), pH, titratable acidity (TA), fruit firmness, fruit color and antioxidant activities of the fresh (harvest stage) green and yellow (ripe stage) fruit pulp of two different cultivar of banana plants (Dwarf Cavendish and Gross Michel) cultivated in under protection and organic growing system were determined. At the end of the research, the heaviest (170.297 g) and the largest (37.059 mm) fruit was obtained from Gross Michel (with peel) cultivar. Also, in terms of the highest fruit length and fruit height was found with peel and without peel forms of Gross Michel cultivar. In point of total soluble solid content, pH and titratable acidity values were obtained 22.1 %, 4.1 and 0.48 % for Dwarf Cavendish banana cultivar, these values were found 21.533 %, 4.6 and 0.503 % for Gross Michel banana cultivar, respectively. On the other hand, fruit firmness value was found to be 4.233 N/mm for Dwarf Cavendish cultivar and 4.500 N/mm for Gross Michel cultivar. However, no statistically significant differences were found in terms of fruit peel color among the varieties in the experiment. In addition, the antioxidant activities of the fruit pulp extracts were evaluated by using -the free radical scavenging, hydrogen peroxide (H_2O_2) scavenging and metal (Fe^{+2}) chelating activities methods. Dwarf Cavendish banana cultivar fruit pulp had higher antioxidant activity at the ripe stage than Gross Michel banana cultivar.

Key Words: *Dwarf Cavendish, Gross Michel, Organic growing technique, Physico-chemical properties, Antioxidant activities*

Introduction

Banana fruit, the production of which is restricted in some regions in both the world and in Turkey, is a rather demanded product as it is delicious, exotic and nutritious. Turkey is residing in the north end of the countries producing banana. The share of Turkey in banana plantation lands is 0.20%, in production 0.84% and in imports 0.25%. All banana production of Turkey is met by Antalya and Mersin provinces in Mediterranean Region, and banana is produced in Anamur, Bozyazi, Alanya and Gazipasa counties and their

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periphery that provide protected microclimates by Toros Mountains (Akcaoz et al. 2009).

Organic farming is defined as a 'production system' that avoids or largely excludes the use of synthetic fertilizers, pesticides, and growth regulators. Instead, it relies on crop rotations, crop residues, animal manure, green manure, off farm organic wastes, mechanical cultivation, mineral bearing rocks, and aspects of biological pest control to maintain soil productivity and tilth, to supply plant nutrients and to control insects, weeds and other pests (Srivastavaa et al. 2002). So, organic products are becoming increasingly popular because of the concerns over environmental contamination and health benefits (Bourn and Prescott 2002). Several studies have shown that consumers have positive attitude towards organic food (Loureiro et al. 2001; Magnusson et al. 2001). Organic foods are associated with no concern, no risks and are seen as healthy (Tõnutare et al. 2009). In the past 10 years, so many review studies of the scientific literature comparing the nutrition of organic and conventional foods have been published. Many of these review studies (Reganold et al. 2010; Balci and Demirsoy 2008; Polat and Celik 2008; Abu-Zahra et al. 2006; Brandt and Molgaard 2001; Worthington 2001; Williams 2002; Magkos et al. 2003; Rembialkowska 2007; Benbrook et al. 2009; Lairon 2010) found some evidence of organic food being more nutritious, whereas a few review articles (Dangour et al. 2009; Doran and Parkin 1994) concluded that there were no consistent nutritional differences between organic and conventional foods.

The objective of the current study was to elucidate the effect of organic cultivation technology on some physico-chemical properties and antioxidant activities of Dwarf Cavendish and Gross Michel banana fruit cultivars.

Materials and Methods

Materials

Dwarf Cavendish and Gross Michel banana fruit cultivars were obtained from Akcami village in Akcami-Bozyazi-Mersin-Turkey (NL 36° 10' 01.43"; EL 32° 55' 44.72", its elevation is 2174 feet) at green stage without any ethylene treatment (harvest stage) and stored at 20 °C for 24 hr before being extracted (ripe stage) in the mid-June 2010 season. The organically and under protected grown bananas were obtained from an orchard certified to be Organic Farm (Certificate No: TR-OT-006-GD-013; Grower No: 006-3303-01-1) by EKOTAR (Control and Certification Body, Origin: Turkey, Location: Mersin) in Turkey. Greenhouse conditions were favourable for banana growing. No synthetic herbicides or insecticides were used. 5 t da(-1) farm manure (goat), 100 kg da(-1) Ormin-K, 1kg da(-1) B5A (liquid organic fertilizer) were used in the organic orchard during the growing season. The water management was done by sprinkler and drip system. Weed control was performed by hand weeding. Fruit samples were placed in plastic trays and frozen immediately in liquid nitrogen, then stored at -80 °C until analysis.

Methods

Physico-chemical analysis

Sampling: Ten fruits of each treatment were used for all analysis.

Determination of fruit mass: Fruit weight was measured by an electronic balance with an accuracy of 0.01 g. Each measurement was replicated 10 times.

Determination of size: From the samples, 10 fruits were selected at random for determining the physical characteristics. For each fruit, length and width values were measured using a digital calliper.

Acidity: Titratable acidity, expressed as % of citric acid, was determined in 10 ml of juice plus 50 ml of distilled water by titration to pH 8.1 with 0.1 N NaOH.

pH: The pH value was measured using a digital pH meter.

Total soluble solids: The total soluble solids (TSS), expressed as %, was determined in the juice of each sample using a portable refractometer at 21 ºC.

Color: Fruit color was evaluated by measuring Hunter L (brightness, 100 = white, 0 = black), a (+, red; -, green) and b (+, yellow; -, blue) parameters by means of a reflectance colorimeter (CR 300, Chromometer, Minolta, Japan). A white tile (No: 21733001) was used to standardize the instrument.

Fruit firmness: To estimate fruit firmness, peel from one side of the banana finger was removed and measurement was carried out at three different places using Penetrometer and recorded as force in Newton (N/mm). Average of ten readings was taken as measure of firmness of individual fruit.

Preparation of extracts for antioxidant activities: About 2.5 g fresh fruit samples were extracted by homogeny in mixer (Ultra turrax) with 50 ml solvent (50% water-methanol). The extracts were centrifuged at 4.000 x g for 3 min at 4°C after draining on coarse filter paper. And then the filtrate was drained by blue band filter paper (no: 391).

Free radical scavenging effect: The radical scavenging activity against the diphenylpicrylhydrazyl (DPPH) radical was evaluated according to the method of Serteser et al. (2008), with some minor modifications. The assay mixture contained 1.5 ml of 0.09 mg ml(-1) DPPH (Sigma Chemical Co., St Louis, MO, USA) in methanol, 1 ml acetate buffer solution (100 mM, pH 5.5). The dilutions between 0.4 and 4 mg ml(-1) were prepared with methanol. Then 3.9 ml DPPH solution prepared with $6x10^{-5}$ M methanol was

added to each 0.1 ml dilution and shaken well. The mixture was prepared and incubated for 60 min at room temperature in the dark. The absorbance of the remaining DPPH was determined at 517 nm against a blank. The scavenging activity was expressed as the IC_{50} value (mg ml(-1)). All analyses were carried out duplicate.

Linear regression equations of absorbance against concentrations were determined by measuring the absorbances of seven different concentrations of DPPH ($6x10^{-5}$ M) stock solution: A (517 nm)=15,465 (C DPPH)-0:0187 (R^2=0,987)

The remaining DPPH concentrations against absorbance values of sample series of different concentrations were calculated and then the remaining DPPH percentage was calculated:

% Remaining DPPH=[DDPH] sample/[DPPH] control

Exponential regression equation was obtained between the rate of the remaining DPPH percentage and the DDPH amount of sample in vitro, and the sample concentrations of plants that decrease the initial DPPH concentrations by 50% (efficient concentration [EC_{50}]). The antiradical activity (AE) was calculated by dividing EC_{50} values into 1.

Fe^{2+} chelating activity: The modified methods of Lim and Murtijaya (2007) were used for determination of the Fe^{2+} chelating activities of samples. One milliliter of extracts with different concentrations between 6 and 45 mg ml(-1) and 3.7 ml deionizer water were mixed. 0.1 ml of 2 mol $FeCl_2$ solution was added and shaken and kept at dark and room temperature for 70 min. Then, 0.2 ml of 5 mM ferrozin was added and shaken again, and the absorbance of the obtained Fe^{2+}-ferrozin complex after 10 min was measured at 562 nm. One millilitre of water was used instead of sample for the control. The equation is as follows (Yen and Wu 1999).

Chelating activity (%)=[1-(absorbance of sample/absorbance of control)]x100

H_2O_2 inhibition effect: The H_2O_2 inhibition effect of spice and plant extracts was determined by spectrophotometer (Ruch et al. 1989). One millilitre (2.6 and 10 mg/ ml) of sample, 3.4 ml of 0.1 M phosphate buffer (pH 7.4) and 0.6 ml of 43 mM H_2O_2 were mixed and after 60 min the absorbance of mixture was measured at 230 nm. Control solutions without H_2O_2 were prepared for each sample concentration. To determine the H_2O_2 concentration that was not involved in the reaction, a linear repression equation was used. Phosphate buffer (3.4 ml) was added to 0.6 ml 10.15, 25.43 at 230 nm. Linear equation formulas were obtained by the graphic of Standard curve of absorbance vs. different concentrations of (+)- Catechin

A (230)=0.0125 x C (H_2O_2, mM)+0.0873 (R^2=0.9783)

(+)-Catechin was used as the reference antioxidant. The equation used is as follows:

H_2O_2 inhibition capacity (%)=[1-(H_2O_2 conc. of sample/H_2O_2 conc. of control)] x100

Statistical analyses: Statistical analysis was done using the JAMP. Differences between means were analysed by ANOVA test ($p<0.05$) (Puskulcu and Ikiz 1989). This research was performed by three duplicates with a replicate.

Results and Discussion

In this study, some physico-chemical properties in terms of fruit weight, fruit width, fruit length, fruit height, fruit length/fruit width, peel thickness, peel weight, total soluble solids (TSS), pH, titratable acidity (AT), fruit firmness, fruit color and antioxidant activities of organically grown Dwarf Cavendish and Gross Michel banana fruit cultivar at different ripening stages.

The heaviest (170,297 g) and the largest (37,059 mm) fruit was obtained from Gross Michel (with peel) cultivar in the experiment. Also, in terms of the highest fruit length and fruit height was found with peel and without peel forms of Gross Michel cultivar. Both cultivars had the highest value and these values were statistically in the same group in point of fruit length/fruit width. There were no statistically differences among the varieties in terms of average peel thickness, and average peel weight (Table 1). Kachru et al. (1995) researched on the physical and mechanical properties of two varieties of green banana fruit, namely, Dwarf Scavendish and Nendran. They found that the average pulp to peel ratios were 1.39 and 2.32, and peel thickness were 3.65 mm and 2.95 mm, respectively. The maximum diameter of fruit without peel was 23.34 mm and 37.08 mm, respectively for the two varieties. The maximum effective length and width of the banana pulp resting at its most stable position was observed to be 137.0 mm and 66.5 mm, respectively for Dwarf Scavendish and 194.5 mm and 50.0 mm, respectively for Nendran. Similar results were obtained in my study.

According to Table 1, TSS value of Dwarf Cavendish banana cultivar (22.100 %) was higher than TSS value of Gross Michel cultivar (21.533 %). In terms of pH, titratable acidity and fruit firmness values were found statistically high in Gross Michel cultivar These values were obtained to be 4.500, 0.503 %, 4.500 N/mm for Gross Michel cultivar, respectively. Sonmezdag (2009) found that total soluble solid, pH and titratable acidity was 19.3 %, 4.87 and 0.52 % in naturally ripened 5^{th} class (according to the commercial color chart) banana fruit samples, respectively. While total soluble solid content went down, pH value went up depending on ripening. Cano et al. (1997) reported that total soluble solid was ranging from 16.30 to 24.5 g / kg and pH was ranging from 4.74 to 4.91 and total

acidity was ranging from 3.5 to 5.0 g / kg in Spanish and Latin American (*Gran Enana*) banana types. Torjia-Isasa et al. (1998) determined total soluble solid value to be 12 Brix via refractometer. Dadzie (1998) determined that pulp pH levels of FHIA- 01 and FHIA-02 were similar to those of Grande Naine and Williams. Results in my study were in parallel with these researchers' results.

Table 1. Some physico-chemical properties of ripe banana fruit

	Organically Grown Banana Cultivars				
	Dwarf Cavendish		**Gross Michel**		**LSD Value**
	With peel	**Without peel**	**With peel**	**Without peel**	
Fruit weight (g)	121.355±11.481 b	73.621±7.869 c	170.297±9.618 a	116.171±3.440 b	16.253
Fruit width (mm)	34.139±1.653 b	26.974±1.384 d	37.059±0.600 a	31.308±0.611 c	2.180
Fruit length (cm)	19.133±1.125 b	17.683±1.007 b	23.108±0.989 a	21.493±0.435 a	1.748
Fruit height (cm)	13.883±1.020 b	13.233±0.575 b	16.125±0.482 a	15.492±0.330 a	1.232
Fruit length/fruit weight	0.561±0.025 c	0.660±0.013 a	0.624±0.022 b	0.687±0.009 a	0.035
Peel thickness (mm)	3.307±0.454 a		3.263±0.409 a		0.979
Peel weight (g)	48.733±2.525 a		52.939±6.627 a		11.368
TSS (%)	22,100± 0.153a		21,533±0.100 b		0.293
pH	4,100± 0.100 b		4,600± 0.100 a		0.227
Titratable acidity (TA) (%)	0.48± 0.015b		0,503±0.015 a		0.035
Fruit firmness (N/mm)	4,233± 0.035b		4,500±0.020 a		0.065
Fruit Peel Color					
L	69,993±3.173a		63,637± 2.487 a		6.462
a	0,133±1.073a		0,843± 0.805 a		2.150
b	54,090±4.252 a		44,270± 6.700 a		12.720

** Values in all the lines not connected by same letter are significantly different (P<0,05)*

Fruit firmness value was found to be 4.233 for Dwarf Cavendish cultivar and 4.52 for Gross Michel cultivar (Table 1). In the previous studies; Sonmezdag (2009) found that banana fruit firmness was 3.44-4.62 N/mm range. Also, Cano et al. (1997) determined fruit firmness of ripe banana fruit to be 5.53-6.68 N/mm range. Vermier et al. (2009) explained that ethylene applicated 7th class banana fruit firmness was 1.98 N mm (-1). Dadzie (1998) found that both triploid cultivars were firmer than the tetraploid hybrids. Pulp firmness was similar in Grande Naine and Williams, but significantly different from FHIA-01 or FHIA-02. Bagnato et al. (2003) researched on the Cavendish bananas cv. Williams at ripening stage 6 (full yellow). They found that pulp firmness was 116 kPa and total soluble solid was 23 %.

Table 2. DPPH radical scavenging effects, Fe^{2+} chelating activity (%) and H_2O_2 inhibition activity (%) of fruit extracts

	Organically Grown Banana Fruit Varieties				
	Dwarf Cavendish		**Gross Michel**		**LSD value**
	With peel	**Without peel**	**With peel**	**Without peel**	
EC_{50}	3.333 c	1.920 d	3.450 b	3.520 a	
AE	0.300 b	0.521 a	0.290 bc	0.284 c	**0.011**
Fe Chelating Activity	26.720 b	32.883 a	21.270 c	19.857 d	**1.049**
H_2O_2 Inhibition	30.117 b	37.923 a	24.690 c	23.577 d	**0.911**

[a]Efficiency coefficient (EC_{50}) (mg sample/ mg DPPH): sample amount needed to decrease the DPPH concentration at the beginning by 50 %, [b]Antiradical activity (AE): 1 / EC_{50}.

** Values in all the lines not connected by same letter are significantly different (P<0,05)*

No statistically significant differences were found in terms of fruit peel color among the varieties in the experiment. Cano et al. (1997) found that objective color parameters *L**, *a**, *b**, showed significant differences between cultivars Enana and Gran Enana. Dadzie (1998) compared the post-harvest characteristics of the promising tetraploid banana FHIA-01 and FHIA-02 and plantain FHIA-21 and FHIA-22 and FHIA-03 hybrids with the commercial cultivars, Grande Naine, Williams, and Cuerno (Horn Plantain) respectively. The peel "L*" values, obtained for FHIA- 01 and FHIA-02, were similar to those of Grande Naine and Williams. In contrast, the peel "a*" value of FHIA-01 was significantly different from that of Grande Naine and Williams. At the mature unripe stage, the peel color of FHIA-01 was greener than that of Grande Naine and Williams.

DPPH, as a partically organic radical, is used to determine antioxidant activities of many plant extracts

and compounds (Brand-Williams et al. 1995). This method is based on decrease in alcoholic DPPH solution in presence of H binding antioxidant (DPPH+AH-DPPH –H+A). DPPH solution is dark violet colored and has a strong absorption range at 517 nm. It looses its color when transforming to DPPH-H and the absorbtion level decreases. The decrease in the absorption shows the cytochiometric decrease in DPPH. The sample amount which lower DPPH concentration 50 % is used to measure EC_{50} antioxidant activity. The lower EC_{50} value, the higher antioxidant power. The opposite of this situation is acceptable for the antiradical activity (AA). Radical scavenging activity, expressed as EC_{50}, ranged from 1.920 mg g(-1) to 3.520 mg g(-1). The inverse relationship was found between Antiradical activity and EC_{50} values in organically grown banana fruit pulp at different ripening stages. Because of a lower EC_{50} value indicates greater antioxidant activity.

The DPPH radical scavenging effects of two banana cultivars at different ripening stages are given in Table 2. According to Table 2, the highest antiradical activity was found to be 0.521 in Dwarf Cavendish banana cultivar fruit pulp at the ripe stage. Generally, the antiradical activity of fruit extracts of Dwarf Cavendish cultivar was found higher than Gross Michell. This effect is probably due to the high phenolic compound contents of Dwarf Cavendish banana cultivar. Chelating agents may have a great importance in rancidity of the oily foods, even they are not antioxidant materials. Because iron catalyses this reaction during lipid peroxidation, ferrozin forms a complex with Fe^{2+}. The amount of complex and red color decrease in the presence of the other chelating agents. Absorption values decrease by the color. The decrease in absorption shows effectiveness of chelating agent added except ferrozin (Serteser et al. 2008). According to Table 2, the highest chelating activity was found to be 32.883 % in Dwarf Cavendish banana cultivar fruit pulp at the ripe stage. H_2O_2 inhibitation activity method is used to eleminate $O_2\bullet^-$, even though superoxide radical anion ($O_2\bullet^-$) does not initiate lipid oxidation, directly. Because in the presence of metal ions, super reactive hydroxyl radical (.OH) may be formed by Fenton reaction ($Fe^{2+} + H_2O_2$-Fe^{3+} + OH- + .OH). For this reason, H_2O_2 inhibition activity is an important method in determination of antioxidant characteristic. According to Table 2, the highest H_2O_2 inhibition activity was found to be 37.923 % in Dwarf Cavendish banana cultivar fruit pulp at the ripe stage [20]. Bennett et al. (2010) indicate that banana cell walls could be a suitable source of natural antioxidants and that they could be bioaccessible in the human gut. Pérez-Pérez et al. (2006) found that Manzano banana (0.35±0.02 mM) had a higher antioxidant capacity than Cavendish banana (0.30±0.02 mM). Also, Meechaona et al. (2007) studied on "Kluai Khai" (KK), "Kluai Namwa" (KN) and "Kluai Hom" (KH) bananas obtained from a local market in the provinces of Kampangpech (for KK), Chiang Mai (for KN) and Nakornpratom (for KH), Thailand, in May 2007. At the end of their results, the banana oils of KK (90 µg ml(-1)), KN (73 µg ml(-1)) and KH (81 µg ml(-1)) showed moderate antioxidant activities compared to vitamin E when assayed by DPPH. The differences could be probably at least partially due to the different methods used.

As a result, organically grown Dwarf Cavendish banana cultivar should be considered to be a good source of natural antioxidants.

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