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*Full Length Research Paper*

# Effects of different irrigation intervals and fertilizer applications on certain chemical contents of 'Braeburn' apple cultivar

**Ebru Kafkas[1]*, Adem Atasay[2], Ferhan K. Sabir[3], Hüseyin Akgül[2] and Kadir Uçgun[2]**

[1]University of Çukurova, Subtropical Fruits Research and Experimental Center, 01330, Balcalı, Adana, Turkey, [2]Eğirdir Fruits Research Experimental Center, 32500, Isparta, Turkey, [3]University of Selçuk, Faculty of Agriculture, Department of Horticulture, Konya, Turkey,

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**The aim of present study was to investigate effects of different irrigation intervals and fertilizers on total lipid, fatty acid and sugar accumulation of Braeburn apple cultivar under Mediterranean climatic conditions. Irrigation program was performed for two consecutive years with three different intervals (1, 3 and 7 days). In fertilization, 40 g N, 32 g $P_2O_5$ and 80 g $K_2O$ per tree (with four replications) applied in the same doses for both years, except for the increased N (50 g per tree) for second year, considering the vegetative growth of the trees. The fruits were commercially harvested in 2006 season. The lipid content ranged from 0.22% (daily irrigation without fertilizer) to 0.70% (irrigation in 7 days intervals with fertilizer). The highest fatty acid was obtained from treatment 3 (irrigation in 3 days intervals without fertilizer) (83.95%), while the treatment 6 provided the least value with 64.08% (irrigation in 7 days intervals with fertilizer). Although certain changes on fructose, glucose, sucrose and total sugars content were detected, the differences were not statistically significantly among the treatments.**

**Key words:** Apple, Braeburn, irrigation, lipids, sugars.

## INTRODUCTION

Placed within the temperate climate region on the world, Turkey has a significant potential in pome fruit production. Among pome fruits, apples are the most outstanding species of this group with annual production of 2 266 437 ton in 2007 (Anonymous, 2007). Therefore, apples and their derivatives considerably contribute to national income in terms of economy.

From biochemical perspective, apples constitute an important part of the human diet, since they are important source of monosaccharides, dietary fibre, minerals and various biologically active compounds, such as vitamin C, and certain phenolic compounds which are known to act as natural antioxidants. On the other hand, free amino acids and fatty acids as nutritive components of apple fruit, play important roles in human health and marinating fruit quality (Wu et al., 2007). Lipids and fatty acid are significant structural and metabolic constituents of plant/fruit cells. They are also essential components of membranes with their important roles for the compartmental and orderly function of most physical and chemical reactions occurring in a functional fruit cell (Song and Bangerth, 2003). Fatty acids are one of major precursors representing volatile formation during fruit maturation. Specifically, palmitic acid, stearic acid, oleic acid, linoleic acid and triacontane are the main lipids detected in the apple peel at harvest and they are believed to serve as precursors of important regulatory and volatile aroma substance (Lo Bianco et al., 2008). Recent studies indicate that reducing the dietary ratio of n-6 to n-3 fatty acids might play a significant role in decreasing the risk of heart diseases and cancer (Iso et al., 2002). Dietary recommendations for healthy eating

*Corresponding author. Email: ebru@cu.edu.tr

include the consumption of fruit juices (Williams, 1999) whose beneficial effects are ascribed to ascorbic acid partly, a natural antioxidant which might restrain the development of major clinical conditions including heart disease and certain cancers (Diplock, 1994; Drogoudi et al., 2008).

The sugar and acid content have a marked influence on the sensory quality of the apple fruit. The concen-tration of organic acids and sugars in apple juice differ due to genotipic differences as well as changes in weather and soil conditions. The main sugars present in apples are fructose, sucrose and glucose. Sorbitol, which is found in lower quantities in apple fruit but in larger amounts in the leaves, plays an important role in the metabolism of sugar accumulation during development (Ackermann et al., 1992; Hecke et al., 2006).

Genetic characteristics, geographical location, climate and soil conditions, environment, harvesting time and postharvest treatments influence the average contents of constituents synthesized in apple fruits (Ackermann et al., 1992; Wu et al., 2007). Especially, genetic structure controls enzyme systems and their activity in flavor formation (Reineccius, 2006). Therefore, each variety has specific biochemical activity under different environmental conditions depending on genotype and environment interaction. Somogyi et al. (1964) found that McIntosh apples grown with nitrogen fertilizers produce increased quantities of volatiles. It was hypothesized that increased nitrogen availability would likely result in higher levels of free amino acids during ripening.

In the present study, the effects of different irrigation intervals and fertilizers on total lipid, fatty acid and sugar accumulation of Braeburn apple cultivar grafted on M9 rootstock were investigated.

## MATERIALS AND METHODS

### Plant materials

The Braeburn apple cultivar grafted on M9 rootstock was grown in the experimental field of Egirdir Horticultural Research Institute (Egirdir/Isparta). The apple trees were planted with the configure-tion of 3.5 x 1.5 m distances in 2002. The plantation was carried out by using randomized split block design with four replication comprising four trees.

Irrigation program was performed for two consecutive years (2005 and 2006) with three different intervals (1, 3 and 7 days). For fertilization, 40 g N, 32 g $P_2O_5$ and 80 g $K_2O$ per a tree (with four replications; in April, May, June and October) were applied in the same doses for both years, except for the increased N (50 g per tree) for second year, considering the vegetative growth of the trees. The treatments were as follows; (1) daily irrigation without fertilizer, (2) daily irrigation with fertilizer, (3) irrigation for 3 days intervals without fertilizer, (4) irrigation for 3 days intervals with fertilizer, (5) irrigation for 7 days intervals without fertilizer, and (6) irrigation for 7 days intervals with fertilizer. The fruits of representative treatments were harvested for analysis at the commercial harvest seasons in 2006.

### FAME (fatty acid methyl ester) analyses

Homogenized fruit flesh samples were used for FAME analysis. Extraction of lipids was carried out following the method of Bligh and Dyer (1959). Boron trifluoride/methanol was used for prepara-tion of fatty acid methyl esters (AOAC, 1990).

### Gas chromatographic conditions

The fatty acid composition was analyzed by GC Clarus 500 with auto-sampler (Perkin Elmer, USA) equipped with a flame ionization detector and a fused silica capillary SGE column (30 m X 0.32 mm, ID x 0.25 µm, BP20 0.25 UM, USA). The oven temperature was 140°C, held 5 min, raised to 200°C at a rate of 4°C/min and to 220°C at a rate of 1°C/min, while the injector and the detector tem-perature were set at 220 and 280°C, respectively. The sample volume was 1 µl and the carrier gas was controlled at 16 psi. The split ratio was 1:100. Fatty acids were identified by comparing the retention times of FAME with a standard 37 component FAME mixture (Supelco). Triplicate GC analyses were performed and the results were expressed in GC area % as a mean value and ± standard deviation.

### Extraction of sugars

Approximately 100 g of each frozen sample was used and each replicate was used separately then, from this homogenized material 1 g of sample weighted and powdered with liquid nitrogen in a mortar. 20 ml of aqueous ethanol (80%, v/v) was placed in to a screw cap Falcon tube. Reaction mixture was placed in an ultrasonic bath, sonicated for 15 min at 80°C and then filtered, and the extraction procedure was repeated 3 more times. All the filtered extracts were combined and evaporated to dryness on the boiling water bath. The residue was dissolved with 2 ml of distilled water and filtered (Whatman nylon syringe filters, 0.45 µm, 13 mm, diam.) before HPLC analysis (Miron and Schaffer, 1991).

### HLPC conditions

The liquid chromatographic apparatus (Agilent) consisted of an in-line degasser, pump and controller coupled to a refractive index detector (Agilent) equipped with an automatic injector (20 ∝l injec-tion volume) interfaced to a PC running Class VP chromatography manager software (Shimadzu, Japan). Separations were performed on a 250 x 4.6 mm i.d., 5 ∝m, reverse-phase $NH_2$ analytical column (Beckman) operating at 40°C coloumn temperature with a flow rate of 1 ml $min^{-1}$. Elution was isocratic acetonitrile: water (3:1). Compo-nents were identified by comparison of their retention times to those of authentic standards under analysis conditions. A 20 min equilibrium time was allowed between injections.

### Quantitative and statistical analyses

All the samples were directly injected to the reverse phase chromatography column. For the stock solution of the sugar standards, glucose, fructose and sucrose were dissolved in water at a concentration of 30 mg $ml^{-1}$. All the samples and standards were injected three times each and mean values were used.

All the statistical analyses were carried out using SPSS 13.0 (SPSS Inc., Chicago, IL). Analysis of variance (ANOVA) was performed by ANOVA procedures.

**Table 1.** Total lipid (%) and relative fatty acid composition (%) of treatment in the apple cv. Braeburn.

| Lipid | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Lipid (%) | 0.70±0.09 | 0.46±0.17 | 0.29±0.02 | 0.43±0.04 | 0.41±0.11 | 0.22±0.00 |
| **Fatty acids (%)** | **Relative fatty acid content (%)** | | | | | |
| C8:0 | 15.13±1.19 | 13.95±0.47 | - | 14.28±1.58 | 11.95±3.16 | 1.54±0.13 |
| C10:0 | 14.28±0.16 | 13.53±0.62 | - | 13.20±1.29 | 11.99±2.72 | 12.80±0.63 |
| C11:0 | 6.12±0.03 | 5.51±0.05 | - | 5.57±0.48 | 4.83±0.83 | 4.67±1.08 |
| C12:0 | - | - | 0.52±0.01 | - | - | - |
| C13:0 | - | - | 0.02±0.01 | - | - | - |
| C14:0 | - | - | 4.30±0.04 | - | - | - |
| C15:0 | 0.09±0.05 | 0.09±0.05 | 0.40±0.01 | 0.28±0.23 | 0.10±0.06 | 0.13±0.01 |
| C16:0 | 7.20±1.10 | 9.54±0.52 | 26.70±0.21 | 8.31±0.66 | 11.69±3.22 | 11.47±0.76 |
| C17:0 | - | - | 0.47±0.01 | 1.02±0.03 | - | - |
| C18:0 | 0.93±0.12 | 1.18±0.00 | 5.39±0.06 | - | 1.79±0.79 | 1.14±0.07 |
| C20:0 | - | - | 0.15±0.02 | - | - | - |
| C21:0 | 0.03±0.04 | - | 0.04±0.01 | - | - | - |
| C22:0 | 0.12±0.11 | 0.23±0.09 | 0.05±0.03 | 0.29±0.02 | 0.26±0.03 | 0.38±0.00 |
| C23:0 | 0.44±0.02 | 0.38±0.03 | 1.29±0.01 | 0.38±0.02 | 0.30±0.06 | 0.27±0.04 |
| C24:0 | 0.36±0.02 | 0.27±0.03 | 0.11±0.02 | 0.29±0.04 | 0.23±0.04 | 0.22±0.00 |
| ∑ SFA | 44.7 | 44.68 | 39.44 | 43.62 | 43.14 | 32.62 |
| C14:1 | 0.44±0.02 | 0.22±0.30 | 0.04±0.00 | 0.45±0.00 | 0.47±0.03 | 0.52±0.04 |
| C15:1 | 0.02±0.03 | - | 0.06±0.00 | - | 0.03±0.04 | - |
| C16:1 | 0.30±0.16 | 0.30±0.38 | 5.61±0.03 | 0.23±0.30 | 0.16±0.15 | - |
| C17:1 | 0.17±0.01 | 0.19±0.01 | 0.09±0.00 | 0.23±0.02 | 0.25±0.01 | 0.22±0.03 |
| C18:1 | 3.39±0.66 | 3.04±0.29 | 9.86±0.45 | 3.69±1.28 | 6.99±5.91 | 2.67±0.63 |
| C20:1 | 0.12±0.07 | 0.26±0.25 | 0.28±0.01 | 0.26±0.13 | 0.11±0.03 | 0.30±0.03 |
| C22:1n9 | 0.06±0.01 | 0.05±0.00 | 0.04±0.01 | - | 0.03±0.04 | - |
| C24:1 | 0.19±0.06 | 0.09±0.04 | 0.05±0.07 | - | 0.03±0.04 | - |
| ∑ MUFA | 4.69 | 4.15 | 16.03 | 4.86 | 8.07 | 3.71 |
| C18:2 | - | - | 2.01±0.01 | - | - | - |
| C18:2cis | - | - | - | 20.48±3.80 | 22.48±0.31 | 22.68±2.40 |
| C18:2 n6 | 15.07±1.65 | 20.57±0.76 | | - | - | - |
| C18:3:n6 | - | - | 0.55±0.01 | - | - | - |
| C18:3 n3 | 2.42±3.17 | 5.04±0.13 | 0.89±0.01 | 4.40±1.99 | 4.16±0.41 | 4.84±0.30 |
| C20:2 cis | - | - | 0.12±0.03 | - | - | - |
| C20:3 n6 | - | - | 0.98±0.00 | - | - | - |
| C20:3 n3 | - | - | 7.68±0.11 | - | - | - |
| C20:4n6 | 0.23±0.02 | 0.19±0.01 | 0.18±0.04 | 0.18±0.01 | 0.14±0.04 | 0.16±0.03 |
| C20:5 n3 | 0.20±0.01 | 0.18±0.01 | 4.33±0.05 | 0.17±0.01 | 0.14±0.04 | - |
| C22:2 cis | 0.39±0.01 | 0.32±0.01 | 0.02±0.03 | 0.31±0.02 | 0.26±0.06 | 0.07±0.00 |
| C22:6 n3 | - | - | 11.68±0.15 | - | - | - |
| ∑ PUFA | 18.31 | 26.3 | 28.44 | 25.54 | 27.18 | 27.75 |
| ∑ | 67.7 | 75.13 | 83.91 | 74.02 | 78.39 | 64.08 |

**RESULTS AND DISCUSSION**

The effects of different treatments on total lipid and fatty acid composition of Braeburn apple cultivar are pre sented in Table 1. The highest lipid contents were detected in treatment 1, while the lowest value was obtained from treatment 6, ranging from 0.22 to 0.70%, respectively.

**Table 2.** Fructose, glucose, sucrose and total sugars content ($gL^{-1}$) of treatment in the apple cv. Braeburn.

| Treatment | Fructose | Glucose | Sucrose | Total sugars |
|---|---|---|---|---|
| 1 | 29.08±5.67 | 9.28±1.77 | 21.36±3.42 | 59.73±10.86 |
| 2 | 27.72±2.25 | 12.12±1.10 | 19.13±2.04 | 58.97±5.39 |
| 3 | 26.30±3.86 | 9.96±1.09 | 16.30±0.10 | 52.56±5.06 |
| 4 | 29.53±1.77 | 12.44±0.80 | 19.46±1.06 | 61.43±3.63 |
| 5 | 24.57±0.49 | 9.70±0.38 | 16.59±1.19 | 50.86±1.30 |
| 6 | 25.64±1.35 | 10.65±0.03 | 17.69±0.30 | 53.99±1.61 |

Results as mean ± SEM of measurements.

The fatty acid compositions among the treatments varied from 32.62 to 44.7% for saturated (SFAs), from 3.71 to 16.03% for monounsaturated (MUFAs) and from 18.31 to 28.44% for polyunsaturated acids (PUFAs). Among the fatty acids, palmitic (C16:0), oleic (18:1) and linoleic acid (C18:2 cis) were the most represented fatty acid in SFAs, MUFAs and PUFAs. Palmitic acid (C16:0) was found predominant for all treatment. This result is in agreement with Wu et al. (2007) who reported palmitic acid and linoleic acid as predominant in eight apple cultivars. Palmitic acids was followed by undecanoic acid (C10:0), linoleic acid (C18:2 cis), caprylic acid (C8:0) and linoleic acid methyl ester (C18:2n6), respectively. In a similar study, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid were detected in significant levels in apple *cv*. Golden Delicious (Song and Bangerth, 2003).

As for the total fatty acid amounts, the highest value was obtained from treatment 3 (83.95%), while the treatment 6 provided the least value with 64.08% (Table 1).

The treatment 1 had the highest amount of SFAs (44.7%), and the lowest PUFAs (18.31%). On the other hand, treatment 3 had the highest amount of PUFAs (28.44%) and MUFAs (16.03%), while caprylic acid (C8:0), linoleic acid methyl ester (C18:2n6) and palmitic acid (C16:0) were predominant in treatment 1 (15.13%), 2 (20.57%) and 3 (26.70%), respectively (Table 1). Linoleic acid (C18:2 cis) was predominant in treatment 4, 5, and 6 (20.48, 20.48 and 22.68%, respectively).

The amounts of fructose, glucose, sucrose and total sugars in the treatments are presented in Table 2. Fructose, glucose, sucrose and total sugars content were not significantly affected by the various treatments. According to the more recent studies, different irrigation methods (O'Connell and Goodwin, 2007; Lo Bianco et al., 2008) and fertilizer application (Sotiropoulos et al., 2008) and both applications (Pacholak et al., 2007) did not result in a significant difference in SSC of apples. Nonetheless, fructose followed by sucrose, was predominant among the sugars for all treatment. In a recent study carried out by Wu et al. (2007), fructose and glucose were found as predominant sugars in eight apple cultivars. Analyzing the apples grown by conventional and organic methods, Hecke et al. (2006) found fructose and sucrose as the main sugars.

The highest fructose level was observed in treatment 4 (29.08 $gL^{-1}$), followed by treatment 1 (29.08 $gL^{-1}$) and 2 (27.72 $gL^{-1}$). The least fructose was detected in treatment 5 (24.57 $gL^{-1}$). Sucrose level of treatments were ranged between 16.30 $gL^{-1}$ (treatment 3) and 21.36 $gL^{-1}$ (treatment 1). The highest glucose was detected in treatment 4 (12.44 $gL^{-1}$) in a similar manner observed in fructose level, whereas the lowest value was obtained from treatment 1 (9.28 $gL^{-1}$). Total sugar content of treatments were between 50.86 (treatment 5) and 61.43 $gL^{-1}$ (treatment 4). When the overall results were considered, slight increases in SSC were detected in fertilized apples in agreement with the results obtained by Raese and Drake (1997) who reported an increase of SSC and fructose content of Fuji apples fertilized with N.

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