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Assessment of physico-chemical characteristics of strawberry (*Fragaria x ananassa* Duch cv Camarosa) during fruit growth and development stages using principal component analysis

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ABSTRACT

Purpose: The optimum harvest maturity for growers is depended on both product and marketing conditions. Non-climacteric fruits, especially strawberries, are generally harvested in fully ripen stage. The appearance, firmness, and phytonutrient compositions consist the main quality parameters in this fruits. In the present investigation, the strawberry fruits (*Fragaria x ananassa* Duch cv Camarosa) were harvested at seven different growth stages (50% growth, green, white, turning, 50% turning, ripening, over ripen) and evaluated for their physical and chemical characteristics. **Research method:** Correlation of parameters and growth stages were investigated by the statistical approach the principal component analysis (PCA). **Findings:** Results revealed an increase in the total soluble solids (TSS: 11.5 Brix) and decrease in titratable acidity (TA: 0.85 mg citric acid/100 g FW) and fruit firmness (1.27 N) parameters. The phenolic (151.43 mg GAE/g FW) and flavonoid contents (48.92 mg Q/100g FW) were decreased until turning stage, whilst the trend was increased afterwards. The vitamin C (AsA) amount was increased during ripening period, whereas it reached up to 42 mg ascorbic acid/100 g fresh weight in ripen fruit. The PCA plot indicated that increasing of the fruit ripening, TSS, AsA, and TAC (total anthocyanin concentrations) have been enhanced, although TA and Cl (chlorophyll) were declined. **Research limitations**: No limitations were encountered. **Originality/Value:** Optimum harvesting period is a considerable factor for both consumers and food industries, growth stages that it can be achieved to target production of fruits with stable and predicatble physical, chemical and phytochemical parameters.

INTRODUCTION

Strawberry fruit is known as one of the richest natural sources of antioxidants due to be rich in vitamins, minerals, anthocyanins, flavonoids, and other phenolic compounds (Aaby et al., 2012). Since, strawberry is a non-climacteric fruit, thus the optimizing of fruit quality is a considerable approach (Kim et al., 2013). Non-climacteric fruits are generally harvested fully ripen especially in case of strawberries, in which ripening does not continue normally following the harvesting; nevertheless, it has been shown that even when harvested at early stages of color development, the strawberries' color can change during storage (Nunes et al., 2006). Ripening is a biochemical process in fruits, in which physical and chemical characteristics including dramatic bioactive compounds production, such as reducing sugars, organic acids, ascorbic acid, anthocyanin, and ellagic acid for each fruit stages at maturity. In general, strawberry fruits become softer, redder, and sweeter during the ripening process (de Jesús Ornelas-Paz et al., 2013). The color, firmness, and chemical compositions are the most fruit's pivotal quality parameters accepted by consumers (Nunes et al., 2006). The fruit taste is strongly influenced by the contents of organic acids and total soluble solids (TSS) and the TSS/TA ratio (Šamec et al., 2016). Organic acids are involved in the texture, pH and color of strawberries (de Jesús Ornelas-Paz et al., 2013), whereas, accumulation of anthocyanins induces the color formation (Šamec et al., 2016). Ascorbic acid (AsA) as one of the most important organic acids in strawberries possesses beneficial roles in human health (Ganhão et al., 2019). Berry fruits, including strawberries, are rich sources of phenolic compounds including anthocyanins (glycoside derivatives of cyanidin, dolphinenide, malvidin, and polargonidine) that are responsible for the fruit color, as well as flavonoids mainly epicatechin, quercetin, and rutin. Furthermore, hydroxy-benzoic and hydroxy-cinnamic acid derivatives such as alginic acid, gallic acid, picomaric acid, and ferulic acid have been reported as the dominant fruits constituents (Tarola et al., 2013). The quality attributes of strawberries as infertile fruits are strongly influenced by its ripening stage, whereas they should be removed from the herb at full maturity stage to avoid further ripening leading to spoiling (de Jesús Ornelas-Paz et al., 2013). Different growth patterns have previously been reported in different strawberry cultivars (Perkins-Veazie & Huber, 1988); moreover, various trends in sugar and organic acid contents have been studied on several strawberry cultivars at several growth stages (Aubert et al., 2021; Ganhão et al., 2019). Numerous studies have examined changes in the phytochemical and qualitative composition of strawberry fruit at different stages of growth and in different cultivars (Aaby et al., 2012; Aubert et al., 2021; de Jesús Ornelas-Paz et al., 2013; Ferreyra et al., 2007; Ganhão et al., 2019; Kim & Shin, 2015; Nunes et al., 2006; Šamec et al., 2016). Cordenunsi et al. (2003) described changes in stiffness, anthocyanins, phenolic compounds, citric acid, and sugars amounts in several selected cultivars. In this experiment, the cultivars' type analyzed as a crucial factor in determining the fruit quality after harvesting and increasing of shelf life, moreover it was indicated that the geographical growth environment and cultivation system affect the fruit quality. Physicochemical studies of several strawberry cultivars were formerly analyzed during ripening stage, and results showed significant enhancement of their soluble solids, fructose, total phenols, total anthocyanins, malic acid content, and total antioxidant activity (Kim & Shin, 2015).

Since the optimum harvesting period is a considerable factor for both consumers and food industries, the present study was conducted to investigate the physicochemical properties of strawberry fruit growing under hydroponic conditions at different growth stages.

MATERIALS AND METHODS

Sample collection

Strawberries (*Fragaria x ananassa* Duch cv Camarosa) were cultivated under hydroponic conditions in the greenhouse of Shahid Chamran University of Ahvaz. Figure 1 exhibits different growth and development stages of the strawberry fruits. Sampling was based on the method of Jia et al. (2011). In accordance with the day's temperature (heat unit) after fully flowering, fruits were harvested at different stages (at least 80 fruits in each stage), sepals were subsequently removed, fruits' fleshy parts sliced to smaller pieces, then transferred in aluminum foil to a freezer at –80 °C until experiments.

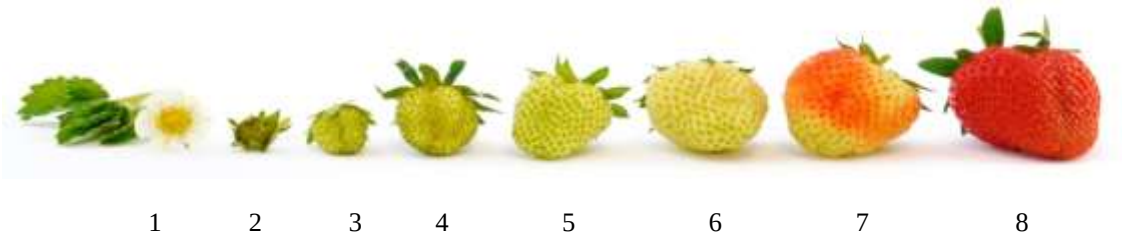

Fig. 1. Growth and development stages of strawberry (1: flowering, 2: fruit set, 3: 50% growth, 4: green, 5: white, 6: turning, 7: 50% turning, 8: ripening, respectively).

Quality assessment

In order to assess the fruit's firmness, a Lutron digital firmness tester (FG-5020, Taiwan) was made by penetrating its flesh (diameter 5 mm), where the results were expressed in newton (N), and four fruits were considered for each replication (Amiri et al., 2021). The total soluble solids (TSS) of the fruits' juice were measured with a hand-held refractometer (Atago, Japan, Model N-50E) (Amiri et al., 2020). Furthermore, the titratable acidity (TA) of the fruit extract was experimented *via* titrating 0.01 N NaOH to the endpoint of pH 8.1. The results were expressed in mg equivalents of citric acid per 100 g of fresh weigh (FW) (Amiri et al., 2021). The concentration of soluble sugars was analyzed by following method: 3 mL of the anthron reagent was added to 100 µL of the fruits' ethanolic extract, then placed in a boiling water bath for 10 min, after cooling the samples, the wavelength was read at 625 nm by means of UV spectrophotometer (UV-2100, New Jersey). The standard curve was prepared by utilization of a standard glucose solution in a concentration series 20 to 100 mg/L (Palma et al., 2014).

Assessment of bioactive parameters

The ascorbic acid (AsA) concentrations of the fruits' juice were evaluated using 2,6-dichlorophenol indophenol method (Hernández-Muñoz et al., 2008). Methanol (80%) was added as solvent to 1 g of fruit's flesh homogenate in a falcon tube, then shacked at 200 rpm for 3 h and centrifuged at 6,000 rpm (Centric 250IVD) for 15 min. The supernatant was utilized to evaluate antioxidant capacity (AC) and total phenolic contents (TPC) based on the methods described by Slinkard and Singleton, (1977). Briefly, to assess TPC, 200 µL of the sample was added to 1.5 mL of 10% Folin–Ciocalteu reagent. Thereafter, 1.5 mL of 6% sodium carbonate solution was added and vortexed after 5 min. A UV-2100 spectrophotometer (UV-Shimadzu, China) was operated to read absorbance after incubation in dark for 90 min, and the results were presented as mg GAE (Gallic acid equivalents). g-1 FW. Moreover, the pH differential method introduced by Lee et al. (2005) was applied to determine total anthocyanin concentration (TAC). The absorbance was recorded at 520 nm

and 700 nm in buffers at pH 1.0 and 4.5, while the findings are stated as mg of pelargonidin-3-glucoside (PG). g^{-1} FW.

AC was determined by FRAP (ferric reducing antioxidant power assay reported by Benzie and Strain (1996) with slight modifications. Furthermore, 80 µL of supernatant was combined with 3.6 mL of FRAP reagent (10 mM of 2,4,6-tripyridyltriazine solution in 40 mM of HCl, 20 mM of $FeCl_3.6\ H_2O$, and 0.3 M of sodium acetate buffer), then incubated at 37 °C for 50 min. The absorbance was read at 593 nm and compared to a FRAP blank. The data obtained are expressed as mmol Fe II. g-1 FW.

Total flavonoid contents were assessed by calorimetric $AlCl_3$ (Zhishen et al., 1999). 500 µL of methanolic extract was dissolved into 5 mL distilled water. After 5 min, 150 µL $NaNO_3$ (5% V/V), and 300 µL 10% $AlCl_3$, while after 6 min, 2 mL NaOH (1 M) and 2 mL of distilled water were added to the solution, and absorbance of the solution was read at 510 nm. The total curve was plotted using quercetin and the concentration of flavonoids was represented as mg of quercetin per 100 g fresh weight (mg Q/100g FW).

Statistical analysis

The treatment was examined through an entirely randomized design analysis (CRD) employing SAS (v. 9.1). The mean differences were determined using LSD (least significant difference) test at $p < 0.01$. Figures were prepared using XLSTAT (Addinsoft, New York, USA). Principal component analysis (PCA) was implemented for phytonutrient properties varied by growth stage to analyze possible correlations.

RESULTS

Quality assessment

As demonstrated in Table 1, the trend of decreased firmness was observed during fruit ripening stage. From 50% fruit growth to color change stages, a decrease in stiffness was detected with a steep slope, although in the last growth stages, the stiffness was declined with a lower slope.

Table 1. Soluble solids, titratable acidity, pH, TSS/TA, and firmness in strawberry's different growth and development stages

Stages	Firmness (N)	TSS/TA	TA (g citric acid/100 g)	pH	TSS (Brix)
50% growth	32.52 ^{a}	5.16^{e}	1.54 ^{a}	3.26^{c}	8^{d}
Green	24.90 ^{b}	6.42^{de}	1.32 ^{b}	3.24^{c}	8.50^{cd}
White	11.61 ^{c}	7.48 ^{d}	1.15 ^{bc}	3.25^{c}	8.67^{cd}
Turning	5.48 ^{d}	7.53^{d}	1.12 ^{bcd}	3.20^{c}	8.50^{cd}
50% turning	2.30 ^{e}	9.18^{c}	1.50^{cde}	3.41^{b}	9.67^{bc}
Ripening	1.64 ^{e}	11.26^{b}	0.94^{de}	3.35^{b}	10.67^{ab}
Over ripe	1.27 ^{e}	13.41^{e}	0.85^{e}	3.51 ^{a}	11.50 ^{a}

Means with the same letter in each column are not significantly different at the 5% probability level according to LSD

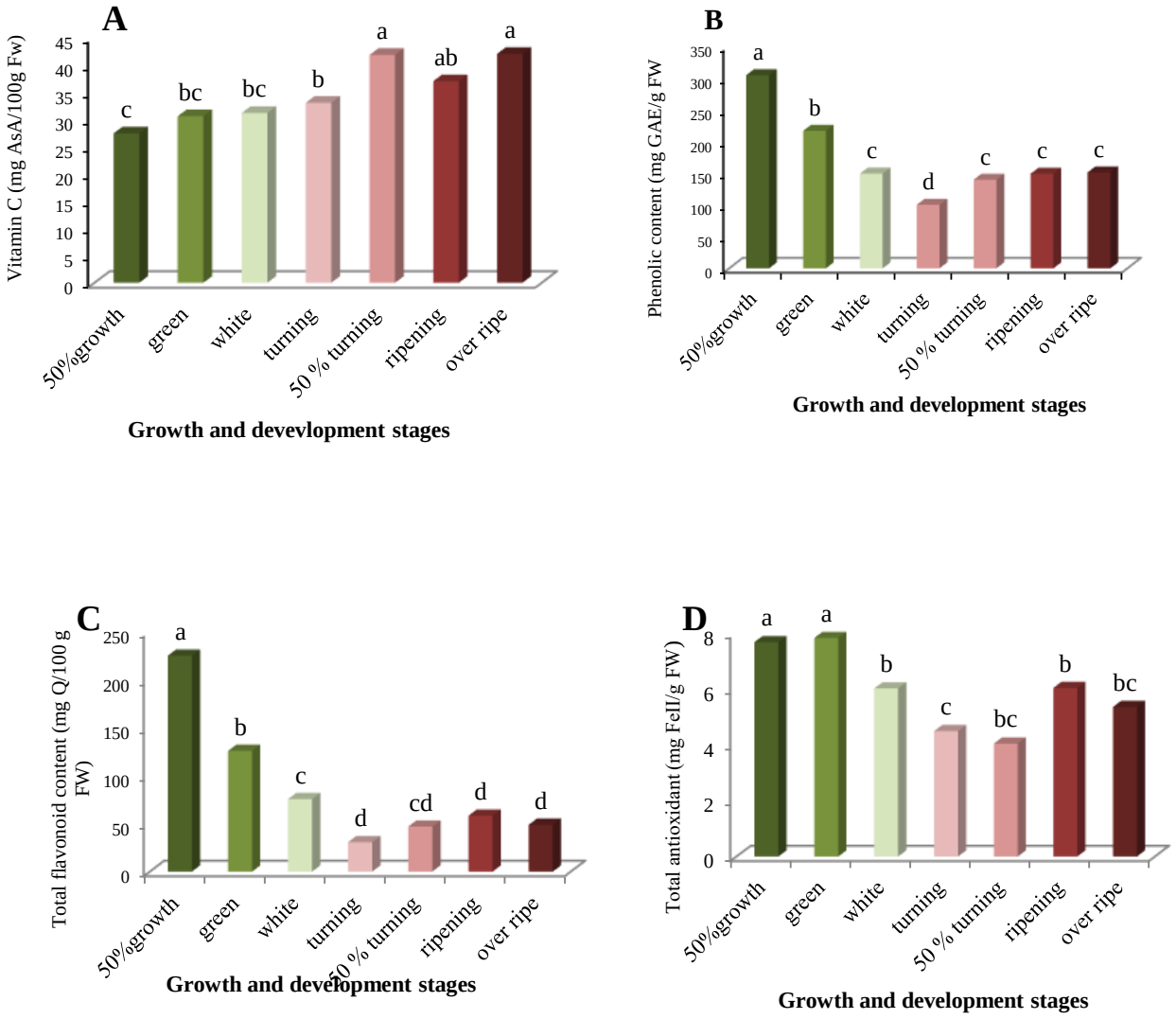

Fig. 2. Ascorbic acid (A) total phenol (B), total flavonoid contents (C), and antioxidant activity (D) in different growth and development stages (50% growth, green, white, turning, 50% turning, ripening, over ripe) of strawberry fruits.

Bioactive parameters

The vitamin C amount of the fruit tissue was increased as it was growing, whereas no significant difference of this content can be observed from the 50% discoloration to the over ripen stage (Fig. 2A). As exhibited in Figure 2B, the richest strawberry fruit in TPC was interestingly recorded in unripens samples, and by turning its color this parameter was gradually ascended.

As depicted in Figure 2C, total flavonoid contents of the fruit samples were highly correlated to their ripening stages. The fruits at starting growth phase possessed the highest total flavonoid contents (TFL), 225.35 mg Q/100 g FW). During the growth stages, a decreasing trend was recorded, although it was slightly increased from color turning (30.71 mg Q /100 g FW) to over ripen phases (49 mg Q /100 g FW). Antioxidant capacity of the fruits revealed a decreasing trend from the first growth stage to the stage of discoloration (Fig. 2D).

Afterwards, this potency has been increased through enhancement of vitamin C and phenolic contents.

Soluble carbohydrates

Soluble carbohydrates in the strawberry fruit are fundamental traits that influenced by several factors such as genetics and climatic conditions. As demonstrated in Figure 3, toward ripening stage soluble carbohydrate was increased, whereas no significant difference of this content can be observed from the ripening to the over ripen stage**.**

Chlorophyll and anthocyanins

The fruits' chlorophyll (Chl) amount was decreased until the discoloration stage (18.68 mg/100g FW), whilst from this stage onwards, chlorophyll was destroyed by the anthocyanins synthesis, as expected (Fig. 4, a and b). Decomposition of Cl in the last days of development and before fruit coloring turns the fruits color to white, in which usually coincides with the cessation of the fruit growth. Afterwards, strawberry fruit's staining is mainly occurred in the last few days of development and the fruits color changes from white to red or pink due to suddenly increasing of anthocyanin synthesis.

Principal Component Analysis

Multivariate exploratory methods are extensively utilized in order to statistically classify different samples based on a wide range of parameters. Principal component analysis (PCA) is known as one of the best techniques owing to its simplicity, interpretation quality, and effectiveness to explaining variations in data set, where it was conducted *via* XLSTAT statistical software. The PCA was implemented on evaluation of physicochemical attributes in different fruits' growth stages (Fig. 5). PCA1 and PCA2 describe 77.03 and 17.11% of total variations. TSS, TA, TPC, AC, and TAC were considered as the variables. In accordance with the PCA plots, several parameters were enhanced during the fruits' ripening periods. As shown in Figure 5, close relationships among C, AsA, TAC, as well as TSS with ripe and overripe stages, in addition significant correlation of C with white and turning fruit growth stages were detected. Consequently, by increasing of the fruit ripening, TSS, AsA, and TAC were enhanced, although TA and Cl were declined.

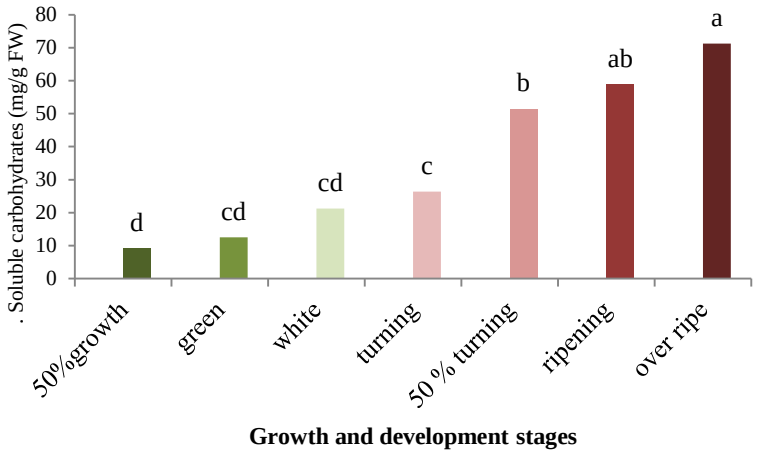

Fig. 3. Soluble carbohydrates in different growth stages (50% growth, green, white, turning, 50% turning, ripening, over ripe) of strawberry fruits.

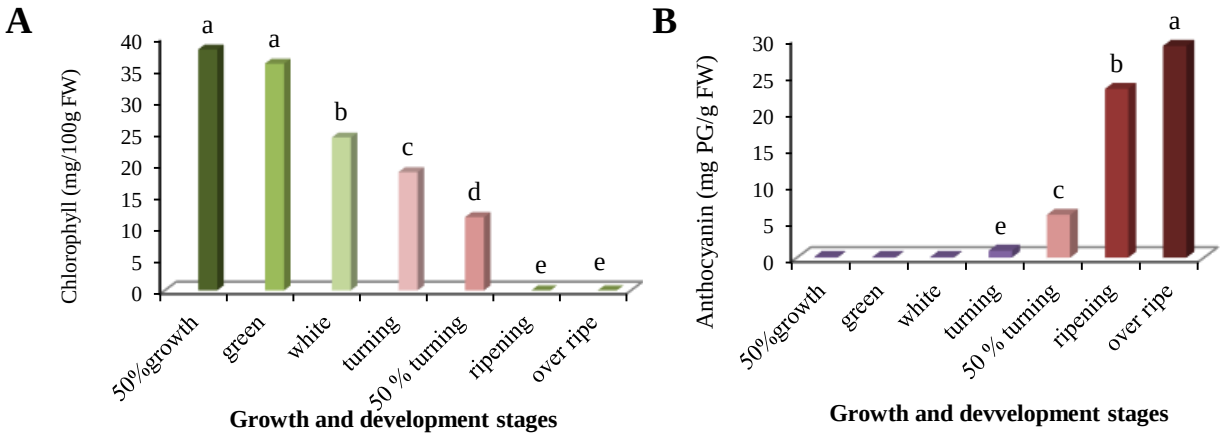

Fig. 4. Chlorophyll (A) and anthocyanin (B) contents in different growth and development stages (50% growth, green, white, turning, 50% turning, ripening, over ripe) of strawberry fruits.

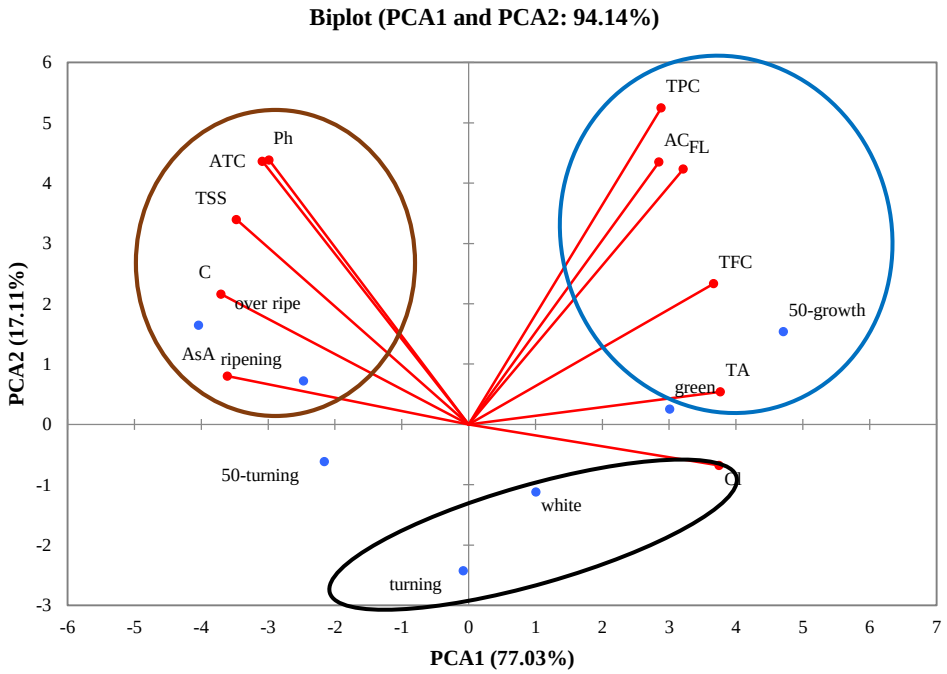

Fig. 5. Principal component analysis (PCA) biplot of the phytonutrient properties with stages of growth and development (50% growth, green, white, turning, 50% turning, ripening, over ripe); TSS: total soluble solids, TA: titratable acidity, TPC: total phenolic content, AsA: ascorbic acid content, AC: antioxidant capacity (AC), ATC: total anthocyanin content, TFC: (Total phenolic contents), C: carbohydrate content, Cl: chlorophyll, pH, FL (Total flavonoid content).

DISCUSSION

Parameters like firmness, titratable acids (TA), total soluble solids (TSS) and their ratio (TSS/TA) are indispensable factors in determining strawberry fruit quality (Šamec et al., 2016). Stiffness as one of the most important quality characteristics, is related to the texture of the fruit, and plays a key role in marketing and consumers' opinion. The firmness of the

strawberry fruit decreases rapidly during storage (Vandendriessche et al., 2013). A former study showed that the fruit tissue's firmness was decreased from white to semi-staining stage, and uniformly from this phase onwards ends of the fruit ripening (Ménager et al., 2004). In another report, the stiffness range of different strawberry cultivars in ripen fruit has been described from 1.6 to 3 N (Ménager et al., 2004).

Despite possessing several natural organic acids comprising malic acid, succinic acid, and oxalic acid, citric acid is identified as the most abundant one in strawberries, whilst its concentrations varied 4 to 12 mg/g throughout different ripening stages. Fully ripen strawberry fruit has a sour and sweet taste with a pleasant aroma. Similar tendencies have been reported for many strawberry cultivars during growth stages (Ménager et al., 2004; de Jesús Ornelas-Paz et al., 2013). Fruits accumulate mainly organic acids as the energy reserve during the first period of development. Organic acid and amino acid accumulation shifts toward sugar synthesis during the later stage of the fruit development (Perotti et al., 2014); whereas its content is decreased during ripening, thus the ratio of sugar to acidity (which determines the taste) reaches to the desired level (Perkins-Veazie and Huber, 1988). Based on our findings, the amount of soluble solids was increased in the final stages of fruit growth, in consistent with Sturm et al., (2003) and (Hwang et al., 2019) reports. The 7:1 ratio of sugar to acid was defined as sweetness and the 6:1 ratio was defined as the acidity of the fruit. According to the results of Chandler Montero et al., (1996) and Osogrand (Nunes et al., 2006), soluble solids in strawberry fruit were the most important characteristics of the quality, and as an acceptability factor by consumers. Soluble sugars are major sources of cellular metabolism, accounting for 99% of glucose, fructose, and sucrose (Kim & Shin, 2015).

The increased ascorbic acid content according to the ripening stage was similar to the previous studies (de Jesús Ornelas-Paz et al., 2013; Kim et al., 2013). Vitamin C (AsA) is the most important phytochemical in strawberry (Šamec et al., 2016). Previously, Chandler cultivar showed the amount of phenolics was decreased until the 21st day after fruit formation and increased from the 28th day due to anthocyanin accumulation (Moing et al., 2001). In the present study, the TPC in ripen fruit was analyzed 151.42 mg GAE/100 g FW, where it is higher than the amount reported in Chandler (120 mg GAE/100 g FW), and Osogrand cultivars (97 mg GAE/100 g FW) (de Jesús Ornelas-Paz et al., 2013). Kim and Shin, (2015) documented similar TFC range in diverse fruits cultivars. Another study reported higher TFC in the white stage (795 mg Q/kg) compared to the ripe stage (576 mg Q/kg), which is in consistent with the present findings (Shin et al., 2008).

Unripen Janghee fruits showed higher TPC and TFC than ripen fruits (Hwang et al., 2019). In a previously experiment performed on Selva cultivar, a direct correlation between radical scavenging potency, phenolic constituents, and AsA contents has been approved (Ferreyra et al., 2007; Hwang et al., 2019). Moreover, Šamec et al. (2016) determined phenolic substances and AsA as the antioxidants of strawberry fruit. According to study of Moing et al., (2001), the soluble sugars concentration exhibited an opposite trend with fruit ripening, then a low content was measured up to 10 days after fruit formation, nevertheless it was increased to more than 500 mg/g during fruit ripening. In agreement with our results, Cao et al., (2015) reported the soluble carbohydrates amount in ripen strawberry fruit between different cultivars (65 to 96 mg/g FW). Jia et al., (2011) further indicated that the Cl content was increased from the fruit formation period to 14 days later; however, after that until end of the development period, the Cl concentration has rapidly been decreased to nearly zero in the mature fruits. Total anthocyanin content has previously been reported higher in ripen than in unripen fruits (Aaby et al., 2012; de Jesús Ornelas-Paz et al., 2013; Hwang et al., 2019).

CONCLUSION

In the present, *via* utilization of PCA, interrelationships of the physical, quality characteristics, and biochemical parameters of seven different growth stages of strawberries, grown under hydroponic conditions were evaluated. The classification of variables and parameters in PCA biplots confirms the specific growth stages properties which are important for potential commercial or industrial applications. Our results point out that growth stages have an effect on phytonutrient content, along with physical and chemical properties of strawberry fruits. Accordingly, we have shown that proper growth stages can be achieved to target production of fruits with stable and predictable physical, chemical, and phytochemical parameters.

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Conflict of interest
The authors have no conflict of interest to report.

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