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Influence of altitudinal variation on the physical and biochemical characteristics of apple (*Malus demostica*)

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ABSTRACT

This study was conducted to investigate the physical and biochemical attributes of three Indian apple (*Malus demostica* Borkh.) cultivars as affected by elevation and genotype. High performance liquid chromatography was used to analyse sugars, organic acids and phenolic composition. Fructose and glucose were the most dominant sugars irrespective of genotype and altitude, while malic acid was the dominant organic acid. Chlorogenic acid was the main phenolics and valeric acid the most abundant fatty acid in apples. The variation in the fruit weight amongst different apple genotypes was larger (133.40 to 186 g) than firmness (10.32 to 11.87 N). The genotype Golden Delicious grown at 1800 msl had the highest ascorbic acid (28.80 mg/100 g) and total antioxidant (9.98 µmol Trolox/g) content among the genotypes and elevations studied. The total phenol content was positively correlated with the elevation ranging from 82.5 mg/100 g (1400 msl) to 231 mg/100 g (1800 msl). A significant genotype × elevation interaction was found for sugars and organic acid content, minerals and physical attributes. This study confirms the role of elevation as effective factor on the physical and nutritional quality in apple fruits.

Key words: Apple genotypes, Elevation, Nutritional properties, Physical properties

Among fruit crops, apple (*Malus demostica* Borkh.) represents the third place worldwide with yearly production of 89.3 million tonnes (FAO 2016). China dominates in apple production, followed by the USA, while India stands fifth in the world. As per latest data, the apple production in India has reached 2.26 million tonnes/annum (Anonymous 2016-17), major part of which is used for fresh consumption. The Himalayan states, namely, Jammu and Kashmir, Himachal Pradesh and Uttarakhand are the major apple producing states of India. The apple cultivation in these areas is confined to the elevation, ranging from 1200 m to 3500 m above sea level (msl). Several varieties belonging to different groups such as Delicious (Royal Delicious, Red Delicious, Golden Delicious, Red Gold, Red Chief, Super Chief), spur type (Oregon spur, Silver spur), Gala (Gala Mischala, Galle Gala), Fuji (Auvil Early Fuji, Sun Fuji) etc., are grown in these areas. A few of them have become quite commercial while others still are in infancy. The nutritive value of apple fruit is very well known and constitutes variable amount of ascorbic acid, sugars, organic acids, phenolics and minerals. The therapeutic value of apple are widely known for curing several illnesses. Qualitative parameters such as skin colour, fruit firmness, sweetness and acidity and other organoleptic traits may influence the consumer acceptance for specific genotypes (Minas *et al.* 2018, Crisosto 2002).

Elevation of orchard has hardly been investigated in relation to its effect on physico-chemical composition and fruit quality. Various climate-related issues temperature, atmospheric pressure and like radiation (Korner 2007), could play a decisive role in the synthesis of colour and profile of phenolics. Similarly, some studies have revealed that the location of the orchard influences the chemical composition of fruits as in strawberry (Kallio *et al*. 2000), black mulberry (Koyuncu *et al*. 2004), raspberry (Anttonen *et al*. 2005) and quince fruits (Silva *et al*. 2002). It has been stressed that the changes in altitude always cause concurrent changes in light intensity, temperature and photoperiod. These elements establish the metabolism of plants and the content of several compounds in fruits

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which have an ultimate effect on the quality of fruits. Some investigations have shown that the elevation and genotypes may or may not influence the physico-chemical aspects of bluberry, peach and nectarine and guava (Correia *et al*. 2016, Frecon *et al.* 2002, Liverani *et al.* 2002, Solarte *et al*. 2014). All these studies necessitate appropriate selection of cultivation site (s) and varieties for good production, quality and profits. Thus, the aim of the present study was to compare the physical and nutritional composition of three commercially grown apple cultivars at two elevations.

MATERIALS AND METHODS

Three apple cultivars (Golden Delicious, Royal Delicious and Red Gold), were harvested from private orchards at Seobagh and Chatani, Kullu, Himachal Pradesh located at an elevation of 1400 msl and 1800 msl, respectively. The apples were harvested in 2015 at commercial maturity, having starch pattern index (SPI) of 2.5/4.0. After harvesting and sorting, the fruits were packed in cartons, transported to Delhi and stored at 2 °C and 80-90% relative humidity at the Division of Food Science and Postharvest Technology, ICAR-Indian Agricultural Research Institute, New Delhi, for further observations. The physical and nutritional attributes were determined in 20 randomly selected fruits of each cultivar.

Weight of apple fruit was recorded with the help of an electronic balance. Dimensions of fruit of different apple cultivars were measured using a vernier calliper. To determine the average size of the fruits, the geometric mean diameter ($D_g = \sqrt[3]{LWT}$) and arithmetic mean diameter $Da = (L + W + T)$ were calculated using average value of length and width of individual fruit. Firmness of apple fruit expressed in Newtons was determined using a texture analyzer and was defined as maximum force attained during puncture by a 2 mm probe. Peel colour of apple was determined using Hunter Lab System. The colour was expressed in L*, a* and b* coordinates where L* is a measure of lightness, positive values of a* indicate redness and negative values complement green. Positive values of b* are the vector for yellowness and negative for blueness.

The soluble solid content of apple was estimated using hand refractometer (0-50 ºB, ATAGO) and expressed as degree Brix (ºB) at 20ºC. Titratable acidity, ascorbic acid and total carotenoid content of the samples were determined as per the standard procedures and denoted as percentage (%), mg/100g pulp and mg/kg, respectively (Ranganna 1999). Total antioxidant activity in the fruits was determined by the cupric ion reducing antioxidant capacity method (Apak *et al*. 2004), and expressed as µmole Trolox/g. The total phenolic content was measured by the Folin-Ciocalteu method. The results were expressed as the mg gallic acid equivalents (GAE)/ 100 g of fresh weight (Singleton *et al*. 1999).

The sugars and organic acids were determined by high performance liquid chromatography (HPLC) (Kelebek *et al*. 2009). The sugars and organic acids standards were purchased. Waters high performance liquid chromatography comprising of binary pump model 515, 2414 refractive index (RI) and 2998 photodiode array (PDA) detector was used for all determinations. The sugars and organic acids in aqueous phase were quantified by using Aminex HPX-87H column operated with 5 mM H_2SO_4 as mobile phase at a flow rate of 0.5 ml min with the oven temperature at 50 °C using both detectors in series (PDA @ 210 nm). Sugars and organic acids concentration in the apples were expressed as g/l.

Phenolic acids extraction from apples was performed according to the method mentioned by Wu *et al*. (2007). A 20 µl volume of each sample was manually injected into the Water Alliance HPLC System. C18 column (5 µm, 4.6×250 mm) was used to determine the phenolics. The high performance liquid chromatography components include e2695 separation module and the 2996 photodiode array detector and the system was operated with Empower 2 Software (Waters Corporation). The mobile phase consisted of solvent A (water 0.1% formic acid), solvent B (acetonitrile 0.1% formic acid) with gradient programming of 100% A to 100% B and total run time of 55 min. and flow rate of 0.5 ml/min. The phenolics peaks were detected at 280 nm, and expressed as ml/l.

Trans-esterification method was used to establish fatty acid methyl esters (FAMEs). Fatty acids were methylated after dissolving the sample in methanol (2 ml) followed by the incorporation of few drops of concentrated sulfuric acid (H_2SO_4). The corresponding fatty acid methyl esters (FAMEs) were extracted with hexane by addition of salt solution (10 ml) for complete recovery. Gas chromatography–mass spectrometry (GC-MS) analysis of the prepared sample was carried out using 7890A GC equipped with a HP-5MS column (30 m × 0.25 mm, 0.25 µm, which was directly connected to a triple axis HED-EM 5975C mass spectrometer by the method of Wu *et al.* (2007). The injection volume was 1 µl with flow mode in split control. The carrier gas flow was set at 1 ml/min helium at 10 psi pressure. The oven temperature was initially held at 40 °C for 1 min, hereafter the temperature was raised with a gradient of 3 °C/min until the temperature reached to 60 °C and held for 10 min. Again the temperature was raised with a gradient of 2 °C/min up to 220 °C and held for 1 min. Finally, the temperature was raised up to 280 °C with increment of 5 °C/min. Total run-time was 111 min. Other settings included a 250 °C interface temperature, 200 °C ion source temperature and electron impact ionization (EI) at 70 eV. The fatty acid profile was represented as % relative area.

To determine macro and micro elements, fruit sample (one g) was digested in a microwave digestion system with concentrated nitric acid and diluted to 100 ml. The element concentrations were analyzed using ICP-MS with auto-sampling protocol and computed as mg/100g for macro elements and µg/g for micro elements.

The results were statistically analysed by two way analysis of variance on the data sets using SAS 9.3 software and significant effects (P< 0.05) were noted. Significant difference amongst the means was determined Tukey's HSD.

RESULTS AND DISCUSSION

Design of grading, conveying, processing and packaging systems is dependent on the physical attributes of horticultural produce. The dimensional characteristics of different cultivars can be used to characterize the cultivar and their shape. For the apple fruit cultivars grown in two different elevations, more than 186.0 g fruit weight was observed for cultivar Royal Delicious which also displayed maximum geometric mean (76.46 mm) and arithmetic mean diameter (76.50 mm), indicating the bigger size of the fruits. In contrast, the green/yellow cultivar Golden Delicious had the least fruit weight (133.40 g), geometric mean (66.17 mm) and arithmetic mean diameter (66.35 mm) (Table 1). The genotypes Red Gold and Royal Delicious presented the heaviest fruits (genotype × elevation interaction significant at P < 0.05). The fruits of Golden Delicious genotype had least fruit weight. Solarte *et al*. (2014) reported that the fruit weight of different guava genotypes was influenced by altitude. The fruits from the lower altitude of guava genotype Regional Roja and Guavatá Victoria had a higher fruit weight while the reverse trend was observed in fruits from the higher altitude of guava genotype Regional Blanca and Ráquira Blanca might be due to the variations between day and night temperatures and higher radiation at lower altitudes that displaces the balance between sugar accumulation and degradation resulting in increased fruit weight. Orazem *et al.* (2011) reported that the fruit size influences by genotype in peach fruit.

Peel colour coordinates (L*, a*, b*) exhibited significant interactions between genotype × elevation (P < 0.05). Regarding the colour, fruits of Golden Delicious and Red Gold grown at 1800 msl altitude showed the maximum L* values, whereas fruits of the Royal Delicious showed lower L* values may be due to genetic variability among genotype. The increase of L* values may be associated with degradation of chlorophyll with a differential susceptibility to degradation of chlorophylls among genotype (González *et al*. 2011). Genotypes Royal Delicious and Red Gold displayed higher a* values and with dark red coloured fruits. It is reported that the varied temperature range in a region of a temperate climate at an elevation highly influences the coloration of the fruits. The environmental factor that influenced the colour was temperature that increases the carotenoids in the skin of apple (González *et al*. 2011). Similarly, Gautier *et al*. (2008) and Medlicott *et al*. (1986) reported that the increase in the carotenoids content was associated with the increase of temperature. Environmental factors such as solar radiation wave lengths, day/night temperature or a combination of these conditions can regulate fruit ripening and the differences between these environmental factors increasing with altitude, determine the fruit quality at harvest. In peach the high altitude improved skin red blush intensity June Gold cultivar (Karagiannis *et al.* 2016). The maximum fruit firmness was exhibited by the Golden Delicious cultivar, while the lowest firmness was observed for Royal Delicious cultivar (Table 1). Fruits grown at 1800 m elevation recorded maximum fruit firmness as compared to those grown at lower elevation. Fruit firmness was influenced by cultivar, season, cultural practices and the harvest time (Beaudry 1992, Minas *et al.* 2018).

A variation in total soluble solids and titratable acidity was observed among the apple genotypes with respect to the altitude (Table 2). Soluble solid content ranged between 12.50 and 14.02 ºB. The Golden Delicious genotype displayed higher concentrations of soluble solid content followed by the Royal Delicious cultivar, whereas the Red Gold genotype reported the lowest content. Guerrero-Chavez *et al*. (2015) studied the effects of elevation on soluble solids content in strawberries. Further, titratable acidity (TA) was significantly (P < 0.05) influenced by the varied elevation of growing sites in the three apple genotypes. Royal Delicious and Red Gold apples grown in orchard situated at 1800 msl presented significantly higher titratable acidity (0.34%) as

Table 1 Effect of genotypes and elevations on physical attributes for apples in 2015

Genotype	Elevation (msl)	Fruit weight (g)	Fruit dimension (mm)				Firmness (N)	Colour		
			Width	Length	Geometric mean diameter	Arithmetic mean diameter		L*	a*	b*
Golden Delicious	1400	133.40^{d}	69.71^{b}	59.63	66.17^{d}	66.35^{d}	11.63^{a}	68.24^{a}	-8.76^{f}	40.06^{a}
	1800	152.00^{b}	70.63^{b}	63.70	68.24^{dc}	68.32^{dc}	11.87^{a}	69.29^{a}	10.37^{b}	42.00^{a}
Royal Delicious	1400	135.31^{d}	76.42^{a}	68.21	73.58^{ba}	73.68^{ba}	10.32^{b}	54.94^{d}	15.20^{a}	26.15^{d}
	1800	186.00^{a}	78.40^{a}	72.71	76.46^{a}	76.50^{a}	11.84^{a}	55.59^{d}	8.88^{c}	31.19^{c}
Red Gold	1400	142.00^{cd}	70.74^{b}	58.07	66.24^{d}	66.52^{d}	11.45^{a}	62.95^{b}	7.32^{d}	32.02^{c}
	1800	148.48^{cb}	75.56^{a}	64.00	71.49^{bc}	71.71^{bc}	11.83^{a}	60.04^{c}	1.34^{e}	36.28^{b}
Genotype (G)		<.0001	0.0003	<.0001	0.0001	<.0001	0.0001	<.0001	<.0001	<.0001
Elevation (E)		<.0001	0.0037	<.0001	0.0122	0.0061	<.0001	<.0001	0.0039	<.0001
G * E		<.0001	0.0003	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Values in rows with the same letters in superscript do not differ significantly (P< 0.05) according to Tukey's HSD.

compared to those grown at an altitude of 1400 msl (0.26%). The variation in the nutritional quality of the apple fruits may be observed as a result of various biotic and abiotic factors (Ali *et al*. 2011) but in the case of the current study the temperature and light intensity are possibly associated. Earlier, Liverani *et al.* (2002) and Frecon *et al.* (2002) has reported that the sweetness and acidity are significantly affected by genotype in peach and nectarine.

The vitamin C content varied from 14.40 (Red Gold) to 28.80 (Golden Delicious) (Table 2). Apples cultivated at 1800 m elevation exhibited the maximum vitamin C content. Our results are in accordance with the findings of Mphahlelea *et al*. (2014) and Crespo *et al*. (2010) who reported a significant effect of altitude on ascorbic acid concentration. According to Lee and Kader (2000), ascorbic acid accumulation in fruit is synthesised from sugars during photosynthesis under higher light intensity. This indicates that high ascorbic acid concentration in apple fruits harvested at higher elevation (1800 msl) could be associated with higher light intensity as compared to those harvested at lower (1400 msl) elevation which received lower light intensity during the growing season. The fruit cultivation under cool temperatures during day and night may influence the ascorbic acid content in fruit (Mercado-Silva *et al*. 1998, Nauer *et al*. 1974) probably due to a lower mean temperature which stimulates the synthesis of organic acids (Lee *et al*., 2000), lowers their utilization due to reduction in respiration rate (Bron *et al*. 2005) and enhances the translocation (Rathore *et al*. 1976).

Total phenolic content was higher in fruits grown at higher elevation (1800 msl) than those at lower elevation (1400 msl), averaging 147.75, 157.50 and 231 mg GAE/100 g in Golden Delicious, Royal Delicious and Red Delicious respectively could be related to variations of the climate conditions and/or depend on the genotype itself (Table 2). Intense solar radiation at higher elevation has often been implicated to having an impact on secondary metabolite profiles (Germ *et al*. 2009). Mphahlele *et al*. (2014) reported higher total phenolic content in pomegranate fruits harvested from plantations situated at higher (898 m) and lower elevations (222 m) than those harvested from middle (662 m) elevations. The accumulation of total phenolic concentration in harvested fruit at medium altitude may be due to high light intensity. The higher total phenolic content could be related to the less rainfall at this site. On the other hand, it could be suggested that fruit maturity among two locations varied due to climate effect thus resulting in variation in the phenolic content of the genotypes. Variations in the phenolic content of pomegranate juice have been reported to vary significantly due to geographical variation as well as exposure to extreme temperatures and maturity stage (Kondakova *et al*. 2009). Recently, D'Acunzo *et al*. (2017) reported that the gowning sites influence the total phenolic content of endives and chicory stems.

Antioxidant activity in the apple genotypes ranged from 2.77 (Red Gold) to 9.98 µmol Trolox/g (Golden Delicious). The antioxidant activity was the highest (9.98 µmol Trolox/g) in Golden Delicious fruits growing in 1800 m elevation. The genotype Red Gold showed the lowest antioxidant activity in both the elevations studied. Higher significant differences ($P < 0.05$) in antioxidant activity were reported in fruits of different genotypes and elevation (Table 2). In contrary to our findings, fruit harvested at lower altitude significantly showed higher antioxidant activity in pomegranate fruits than those harvested at higher and medium altitudes (Mphahlele *et al*. 2014). However, our findings are in agreement with those of Hakkinen *et al*. (2000) who reported higher AOX activity in strawberries grown at higher elevations. Higher antioxidant activity has been associated to higher total phenolic compound present in fruit (Tzulker *et al*. 2007). Apple cultivated at 1800 m elevation showed higher total carotenoid content compared with those from lower elevation. Carotenoids content varied between 46.44 and 104.50 mg/kg may be due to genetic variability among genotype and/or influence of elevation. Ouni *et al*. (2012) investigated that the oils obtained from olives cultivated at higher altitudes showed higher carotenoids content compared with those from lower

Table 2 Effect of genotypes and elevations on chemical composition for apples in 2015.

Genotype	Elevation (msl)	TSS (°B)	Titratable acidity (%)	Total phenol (mg/100 g)	Ascorbic acid (mg/100 g)	Total antioxidant activity (µmol Trolox/g)	Total carotenoids (mg/kg)
'Golden Delicious'	1400	13.49^{bac}	0.53^{b}	119.25^{d}	21.59^{b}	6.45^{b}	100.62^{b}
	1800	14.02^{a}	0.57^{a}	147.75^{c}	28.80^{a}	9.98^{a}	104.50^{a}
'Royal Delicious'	1400	13.19^{bdc}	0.26^{d}	82.50^{e}	21.18^{b}	5.55^{c}	46.44^{d}
	1800	13.80^{ba}	0.34^{c}	157.50^{b}	21.59^{b}	9.95^{a}	47.40^{dc}
'Red Gold'	1400	12.50^{d}	0.26^{d}	117.00^{d}	19.82^{c}	2.77^{d}	48.37^{dc}
	1800	13.01^{dc}	0.34^{c}	231.00^{a}	14.40^{d}	5.79^{c}	50.23^{c}
Genotype (G)		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Elevation (E)		0.0010	<.0001	<.0001	0.0023	<.0001	0.0038
G * E		0.9488	0.0037	<.0001	<.0001	<.0001	0.1919

Values in rows with the same letters in superscript do not differ significantly ($P< 0.05$) according to Tukey's HSD.

altitudes. Previously, Gil *et al.* (2002) and Giorgi *et al.* (2005) investigated that the antioxidant activity, phenols and ascorbic acid content were influenced by genotype.

The predominant free sugars for apple genotypes were fructose followed by glucose and sucrose. The low sucrose content in the apples is probably due to either the enzymatic hydrolysis or its conversion into other sugars during the ripening process. Significant differences ($P < 0.05$) in all sugars were found within the genotypes and elevation (Table 3). The Royal Delicious cultivar showed the highest glucose content (18.50 g/l) at 1800 m elevation, while Golden Delicious cultivar had the lowest value (9.50 g/l) at the lowest elevation. Fructose ranged from 15.55 g/l in Golden Delicious to 56.65 g/l in Royal Delicious and sucrose was the individual sugar present in lower amount in all cultivars, ranging from 9.85 g/l in Royal Delicious to 29.90 g/l in Red Gold. The contents of sugar compounds in this study compared to previous study of Zhang *et al*. (2010) and Wu *et al*. (2007). Correia *et al*. (2016) have previously reported that no significant differences in glucose, galactose and total free sugars contents were found among cultivars of blueberries grown at differing altitudes. Solarte *et al*. (2014) identified that the conditions of high altitudes improved the efficiency of fructose and glucose accumulation in the genotype Ráquira Blanca and Guavatá Victoria, but the effect was the opposite in the genotype Regional Roja and Regional Blanca. On the other hand, the lower altitudes increased sucrose content in the genotype Ráquira Blanca and Regional Roja possibly due to genetic variability among genotype or effect of altitude.

Apple fruits grown at a lower elevation were found to contain low levels of glucose, fructose and lower levels of sucrose in comparison to the apples grown at an 1800 m elevation. The higher elevation site is characterized by a lower temperature, whereas the lower elevation location is characterized by a higher temperature. Therefore, it was found that the cooler temperature at higher elevation most likely favours an increase in fructose and glucose as well as the accumulation of sucrose. Schwartz *et al*. (2009) have also reported a similar phenomena for strawberries. Fischer *et al.* (2007) also reported that the sugar concentration in gooseberry fruits was influenced by the altitude. Crespo *et al*. (2010) have also reported that the compositional variations in fruits in response to cultivation sites were cultivars specific.

Overall, the predominant organic acids in apple genotypes were malic acid and to a lower level succinic and citric acids (Table 3). Cultivars Golden Delicious and Royal Delicious exhibited the maximum amount of malic and citric acids while succinic acid concentration was highest in the Red Gold genotype. Fruits grown in 1800 m elevation developed maximum organic acids (Table 3). Crespo *et al*. (2010) reported that the organic acid content of the strawberry fruits was influenced by growing site. It was reported that the genotype and orchard practices affect the fruit quality by influencing physiological and metabolic processes (Berman and DeJong 1996).

Concentration of minerals of apple genotypes at different growing sites is presented in Table 4. Mineral content was significantly different between genotypes and growing sites. Fruits harvested at 1800 m elevations had higher mineral content than those harvested at lower elevations. Sodium (Na) content for the Royal Delicious genotype harvested at 1800 m altitude was 61.60 mg/100 g which was superior to that reported for the other genotypes. The fruits of Golden Delicious and Red Gold genotype from 1800 m altitude reported the maximum potassium (K) concentration and the least amount were reported in the Royal Delicious genotype harvested from lower altitude (Table 4). The maximum calcium (Ca) content was reported in Royal Delicious and Golden Delicious genotype harvested at 1800 m altitude with 39.15 and 35.06 mg/100 g, respectively. Variations were also reported among the genotypes and elevations with respect to the magnesium (Mg) content with a difference from 17.37 (Golden Delicious) (1400 msl) to 35.48 mg 100/g (Royal Delicious) (1800 msl). The Cu, Mn, Zn and Fe micronutrient varied among the apple genotypes and altitudes (Table 4). The highest Fe content was found in Red Gold genotype (4.70 µg/g) harvested at 1800 msl). Furthermore, fruits harvested at 1800 msl altitude had the

Table 3 Effect of genotypes and elevations on sugar content and organic acids for apples in 2015.

Genotype	Elevation (msl)	Sugars (g/l)			Organic acids (g/l)			
		Glucose	Fructose	Sucrose	Malic acid	Succinic acid	Citric acid	Acetic acid
Golden Delicious	1400	9.50^{f}	49.10^{c}	26.00^{c}	4.90^{d}	0.90^{d}	TR	TR
	1800	11.00^{e}	15.55^{e}	24.60^{d}	6.50^{b}	3.85^{b}	8.05^{a}	1.56^{a}
Royal Delicious	1400	17.00^{b}	48.00^{c}	9.85^{e}	5.50^{c}	0.15^{f}	TR	TR
	1800	18.50^{a}	56.65^{a}	13.40^{f}	5.80^{c}	1.10^{c}	TR	TR
Red Gold	1400	12.50^{d}	52.20^{c}	29.90^{a}	4.40^{e}	0.55^{e}	TR	TR
	1800	14.20^{c}	20.90^{e}	28.45^{b}	7.10^{a}	5.90^{a}	7.50^{b}	1.56^{b}
Genotype (G)		<.0001	<.0001	<.0001	0.4948	<.0001	<.0001	<.0001
Elevation (E)		<.0001	<.0001	0.2540	<.0001	<.0001	<.0001	<.0001
G * E		0.8919	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Values in rows with the same letters in superscript do not differ significantly ($p < 0.05$) according to Tukey's HSD. TR=Trace

Table 4 Effect of genotypes and elevations on minerals content for apples in 2015

Genotype	Elevation (msl)	Macro-elements (mg/100 g)				Micro-elements (µg/g)			
		Na	K	Ca	Mg	Fe	Zn	Mn	Cu
Golden Delicious	1400	54.50^{b}	936.00^{b}	15.74^{f}	17.37^{e}	1.27^{e}	0.51^{d}	0.13^{d}	0.07^{c}
	1800	60.20^{a}	994.00^{a}	35.06^{b}	24.52^{c}	2.10^{d}	0.40^{e}	0.12^{d}	0.08^{c}
Royal Delicious	1400	19.30^{c}	618.00^{f}	19.93^{e}	21.40^{d}	1.26^{e}	0.86^{b}	0.16^{c}	0.08^{c}
	1800	61.60^{a}	664.00^{e}	39.15^{a}	35.48^{a}	2.85^{b}	0.98^{a}	0.16^{c}	0.63^{a}
Red Gold	1400	19.80^{c}	782.00^{d}	28.95^{c}	26.42^{b}	2.53^{c}	0.51^{d}	0.23^{b}	0.12^{b}
	1800	20.00^{c}	827.00^{c}	26.24^{d}	22.65^{d}	4.70^{a}	0.68^{c}	0.42^{a}	0.11^{b}
Genotype (G)		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Elevation (E)		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
G * E		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Values in rows with the same letters in superscript do not differ significantly (P< 0.05) according to Tukey's HSD.

maximum Zn, Mn and Cu content in different genotypes. Our results corroborate with the findings by Horsley *et al*. (2014), Todea *et al*. (2014) and Manzoor *et al*. (2012) for apple genotype. The mineral contents in fruits are dependent on the cultivar, fertilization, soil and climatic conditions (Nour *et al*. 2011). As the management and manure application were the same for all of the cultivars, following the recommendations of Gonçalves *et al*. (2011), it is believed that such differences in the mineral contents are related to the characteristics that are intrinsic to each cultivar. These results are, therefore, important in the choice of a cultivar with superior properties for consumption, and even for facilitating genetic improvements to obtain superior cultivars.

Phenolic concentration varied significantly (P < 0.05) with genotypes and elevation. Individual phenolic compounds including the chlorogenic acid, coumaric acid, phloridzindihydrate, rutin, catechin and hydroxy cinnamic were identified by HPLC (Table 5). Among phenolics, the chlorogenic acid was identified in relatively high amounts, followed by the phloridzindihydrate and coumaric acid. The chlorogenic acid varied between trace in Red Gold (1400 msl) and 53.29 mg/l in Golden Delicious (1800 msl). Fruit from higher elevation (1800 msl) had significantly higher phloridzindihydrate concentration averaging 24.35 mg/l. Coumaric acid and hydroxy cinnamic acid did not vary much among all the elevations and genotypes. Rutin and catechin were identified in traces. Fruit harvested from higher altitude (1800 msl) had higher phenolics than those from lower elevation. As it was hypothesised, it is possible that the lower temperatures of the higher elevation sites trigger the mechanism for producing more phenolics. Similarly, cold stress induced the accumulation of phenolic compounds in apples (Thomai *et al*. 1998).

Table 5 Effect of genotypes and elevations on phenolic and fatty acids for apples in 2015

Genotype	Elevation (msl)	Phenolics (mg/L): Chlorogenic acid	Coumaric acid	Phloridzindihydrate	Rutin	Catechin	3-hydroxy cinnamic acid	Fatty acids (% relative area): Valeric acid (C5:0)	Levulinic acid (C5:0)	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)	13-Docosenoic acid (C22:0)
Golden Delicious	1400	17.74^{b}	3.58^{b}	4.72^{e}	TR	TR	0.53	TR	TR	TR	TR	TR	TR	TR
	1800	53.29^{a}	2.89^{c}	16.33^{d}	TR	TR	0.53	15.36	0.25	0.11	TR	TR	0.89	TR
Royal Delicious	1400	17.88^{b}	5.76^{a}	18.96^{c}	TR	TR	TR	TR	TR	TR	TR	TR	TR	TR
	1800	17.66^{b}	2.60^{d}	46.21^{a}	TR	TR	0.92	11.23	0.56	TR	TR	TR	0.26	TR
Red Gold	1400	TR	1.01^{f}	16.85^{d}	TR	TR	TR	TR	TR	TR	TR	TR	TR	TR
	1800	3.69^{c}	2.06^{e}	22.11^{b}	TR	TR	0.03	9.56	0.69	TR	TR	0.12	TR	TR

Values in rows with the same letters in superscript do not differ significantly (P< 0.05) according to Tukey's HSD. TR=Trace

In general, the total content of phenolics analysed were comparable to those reported by Wu *et al*. (2007) for apple. Hakkinen *et al*. (2000) reported significant differences in the total contents of phenolic compounds among the strawberry cultivars grown at two locations. Guerrero-Chavez *et al*. (2015) also reported that the phenolic acids concentrations in strawberry were influenced by growing site.

Among fatty acids, linoleic, linolenic, stearic, oleic, palmitic, palmitoleic and arachidic acids were identified through GC-MS (Table 5). Linoleic, oleic, palmitic and stearic acids were found as the predominant ones. In general, fatty acids contents in apple samples in this study were similar to those reported in previous study by Wu *et al*. (2007). Ouni *et al*. (2012) studied the composition of Chétoui olive oils of various altitude and found significant variations in the levels of the main fatty acids.

Overall, the findings of the present study showed that the growing apple at 1800 m elevation produce apples with superior quality. The fructose and the glucose were the predominant sugars and mallic acid was the major organic acid. The Royal Delicious cultivar had higher fruit weight and fruit dimension. The fructose sugar was present in greater amounts in Royal Delicious cultivar and the Red Gold cultivar features more sucrose. Titratable acidity, ascorbic acid, antioxidant, minerals and phenolics contents were also significantly influenced by the altitude and cultivars.

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