

Research Article

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Assessment of Physical and Chemical Properties of Different Cultivars of Apple (*Malus spp*) in Mustang, Nepal

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Abstract This study was conducted at Temperate Horticulture Development Center, Marpha, Mustang to evaluate the physical and chemical properties of six different cultivars of apple (Tsukura, Saune, Red Delicious, Rich a Red Delicious, Royal Delicious and Golden Delicious) from January to September 2023. For the experiment, four trees of each cultivar were selected as replication and cultivar as treatment which was laid out in Randomized Complete Block Design (RCBD). Physical and chemical properties were studied by harvesting. Physical characteristics included individual fruit weight, fruit volume, fruit length, fruit width, individual seed weight, seed length and seed width and mesocarp thickness. Chemical characteristics included pH, TSS, TA and TSS/TA. Data analysis was done through R Studio. Physical and chemical characteristics varied considerably among cultivars. Physical characteristics revealed that the highest individual fruit weight (210.35 g) and size (Length 7.22 cm and Width 7.70 cm) was seen in Rich a Red Delicious and lowest in Tsukura whose weight was 22.25 g and size (Length 3.38 cm and Width 3.42 cm). Also, chemical analysis revealed that pH (4.57) was highest in Saune. TSS/TA was found maximum in Rich a Red Delicious (53.12) and minimum in Saune (17.30). From the study, it was found that Tsukura and Saune are suitable for juice purposes whereas the other four cultivars are suitable for fresh table consumption.

Keywords Apple (*Malus spp*); Cultivar; Physical characteristics; Chemical characteristics

1 Introduction

Apple is one of the edible fruits belonging to the genus *Malus* and family Rosaceae. Apple is a widely grown fruit tree especially grown in temperate regions of the world. Apple, with more than 7,500 (wild and commercial) cultivar types, a few are only famous (Kassebi and Korzenszky, 2022). Apples are bred for various tastes and uses, including cooking, and eating raw, dried apple, jam and cider production. The apple production, production area, and yield in Nepal are 52,753 Mt, 6,245 ha, and 8.45 Mt/ha, whereas in Mustang are 7,234 Mt, 565 ha, and 12.80 Mt/ha respectively (MoALD, 2023). Jumla district is the largest producer (13,958 Mt) of apples, while Mustang district has the highest productivity (12.80 Mt/ha) of apples. The export trend of Nepal at present scenario is null but the export was about 46297051 kg in the FY 2022/23. In FY 2017/18, Nepal used to export 3,075 kg of apples in the form of dried apples (MOICS, 2023).

Differentiating apple cultivars across regions is still lacking (Weihle et al., 2021). Some cultivars (local and novel) are not valued due to a lack of information regarding their features (Szot et al., 2022). Commercial use of different cultivars of apples is not properly known. Therefore, it is pivotal to determine the pomological and chemical characteristics of different apple cultivars to make their correct use. Physical characteristics refer to the fruit characteristics including fruit color, average fruit size, fruit firmness, number of fruits/kg, and average fruit weight. Chemical characteristics of apples include TSS, TA, pH, TSS/TA, etc. Physico-chemical properties (Fruit firmness, pH, TSS, and TA) of apples depend upon the cultivar, maturity stages, and temperature (Teferra et al., 2021).

This study was carried out to determine the physical and chemical characteristics of six different cultivars of apples found at Temperate Horticulture Development Center. Physical characteristics included individual fruit weight, fruit volume, fruit length, fruit width, individual seed weight, seed length and seed width and mesocarp thickness. Chemical characteristics included pH, TSS, TA and TSS/TA. They are crucial to determining the commercial value and consumer preference for the sweetness of different apple cultivars. It is very common to use the TSS/TA ratio to obtain better predictions of sweetness (Harkar et al., 2002). Apple fruit with TSS/TA ratios over 20 is considered to be sweet, while values under 20 are considered to be sour (Monteiro et al., 2018).

2 Materials and Methods

2.1 Research site

The research was carried out at Temperate Horticulture Development Center (THDC), located at Gharapjhong Rural Municipality-2, Marpha, Mustang district. The center is coordinated at 28°20' to 29°05' N and 83°30' to 84°15' E with an altitude of 2,650 masl. Among four blocks of THDC (A, B, C and D), the experiment was conducted in block A from January to September 2023.

2.2 Climatic observation

The meteorological data (temperature, relative humidity and precipitation) were estimated from the automatic weather station (Meteorological Station, Thakmarpha (Index No. 0604), Mustang) present in 'Block C' of THDC, Marpha. It is under the supervision of the Government of Nepal (Ministry of Energy, Water Resources and Irrigation, Department of Hydrology and Meteorology). Climatic variables during the study period were observed (Figure 1).

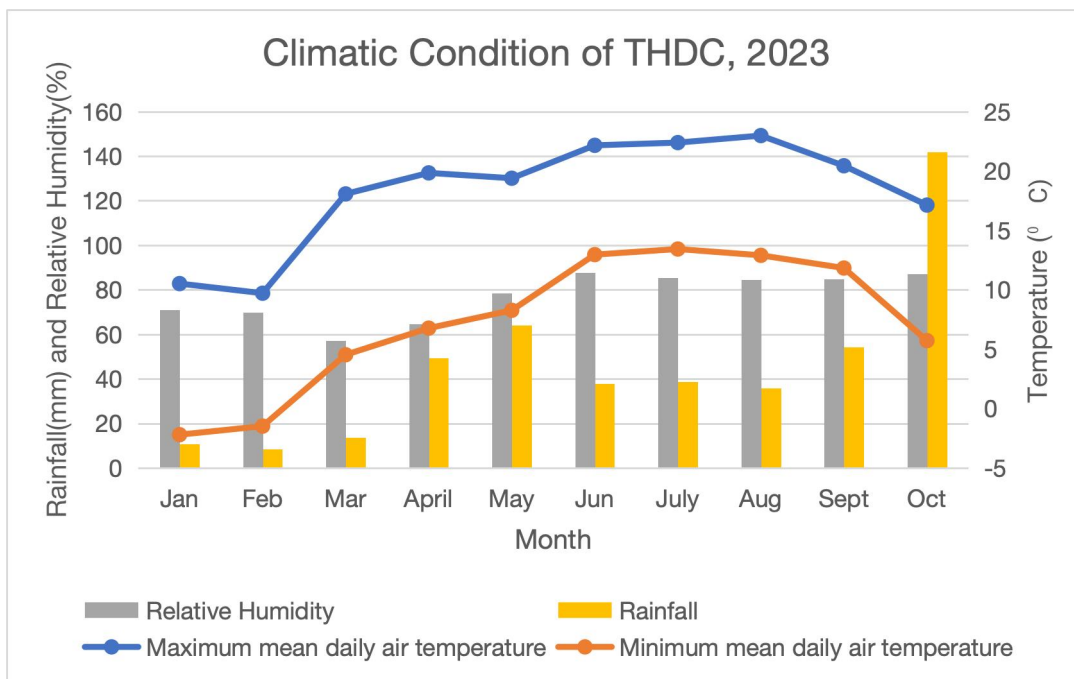


Figure 1 Mean daily minimum and maximum temperature, relative humidity and rainfall recorded at the experimental site

2.3 Plant materials and design

The experiment was carried out in Randomized Complete Block Design (RCBD) with 6 treatments and 4 replications in a single flowering season. Six cultivars of apple (Tsukura, Saune, Red Delicious, Royal Delicious, Rich a Red Delicious and Golden Delicious) were selected as treatment which was replicated four times (four trees per treatment) (Table 1). Training of all the trees aged 12-13 years was done on the Open Centre System. The rootstock used was the crab apple. They were planted at 5 m × 5 m distance.

Table 1 Plant materials used in research

S.N.	Cultivars	Origin
1.	Tsukura	Japan
2.	Saune (Local)	Nepal
3.	Red Delicious	United States
4.	Royal Delicious	n.d.
5.	Rich a Red Delicious	United States
6.	Golden Delicious	United States

2.4 Harvesting

Fruits were manually harvested on a random basis from each tree when they were fully ripened. They were harvested on different dates (Table 2) and then packaged in plastic bags and sent to the pomology lab of THDC for analysis. Poor quality and damaged fruits were eliminated and they were kept in a refrigerator chamber at 0 °C until use.

Table 2 Harvesting time of different apple cultivars at THDC, Marpha, Mustang, 2023

S.N.	Cultivars	Harvesting date
1.	Tsukura	2 nd July, 2023
2.	Saune	14 August, 2023
3.	Red Delicious	27 th September, 2023
4.	Golden Delicious	27 th September, 2023
5.	Rich a Red Delicious	27 th September, 2023
6.	Royal Delicious	27 th September, 2023

2.5 Determination of physical and chemical characteristics

2.5.1 Linear dimension

Thirty samples of fully ripened fruits were taken randomly for the study. Fruit and seed linear dimension (Mesocarp thickness, Individual fruit length, Individual fruit width, Individual seed length, and Individual seed width) was measured by using a vernier caliper with a level of accuracy of 0.01 mm (Jakobek et al., 2020; Korkmaz and Okatan, 2021).

2.5.2 Weight and volume

Thirty samples of fully ripened fruits were taken randomly for the study. The fruit and seed weight were measured using a digital weighing machine (Dolkar et al., 2021). The xylometric/water displacement method was applied to measure the fruit volume of the apple.

Water cannot be compressed, thus as long as the fruit absorbs a tiny amount of water, the change in water height should provide a close approximation of the fruit volume (Moreda et al., 2009). To minimize the error, a measuring cylinder of a small volume (300 mL) was used. Distilled water was half-filled in the cylinder and a single fruit was dipped in the cylinder at a time. The fruit was allowed to dip fully in water with the help of a metal sponge sinker and the rise in height was recorded. The difference between the initial and final reading of water in the measuring cylinder gives the fruit volume. The volume thus obtained is expressed in terms of mL or cm³ as 1 mL of water is equal to 1 cm³.

2.5.3 pH

The pH was measured using a digital pH meter containing electrodes for precision (Mehta et al., 2019). Before measurement, calibration of the pH meter was done. For calibration, the pH meter was put in the buffer solution of pH 4 and pH 7. Then, the pH meter was washed using distilled water and was made ready for measurement. After measurement, it was again washed with distilled water and calibrated for taking the next measurement.

2.5.4 Total soluble solids

Total Soluble Solids (TSS) of the fruit was measured by using an ATC-1E automatic hand-held refractometer (Atago, Tokyo, Japan) with a 0-32 °Brix scale at 20 °C (Rydzak et al., 2021; Muharfizah et al., 2023). The refractometer was calibrated to zero using distilled water before using it. The fruit juice was drawn into a vessel and one to two drops of fruit juice was poured into the prismatic surface. After covering the prismatic surface with daylight diffusion cover, the TSS was observed from the eyepiece.

2.5.5 Titratable acidity

10 mL of juice was obtained by crushing apples. The titratable acidity (TA) of the fruit was determined as per the general procedures prescribed by (Paul et al., 2010; Teferra et al., 2021). Firstly, the juice from the fruit was extracted. 10 mL of extracted juice was taken in a conical container and an equal volume of distilled water was added. Further, two drops of phenolphthalein indicator were added to the container to determine the endpoint of the reaction. The burette was filled with 0.1N NaOH and the initial reading was taken. The diluted fruit juice was then titrated with 0.1 N NaOH until the color changed to pink. The color change represents the end point of the reaction and the final reading of the burette was taken. The difference between the initial and final reading of the burette gives the volume of NaOH consumed by fruit juice for neutralization. The TA thus obtained was expressed as the TA% or percentage of dominant organic acid or gram of dominant organic acid per 100 mL of fruit juice. The dominant organic acid found in the plum is malic acid and hence the TA% was expressed as % malic acid (gm malic acid/100 mL of juice). The TA of the fruit can be calculated as suggested by Paul et al. (2010):

$$TA \% = \frac{V_b * N * M_{eq}}{V_a} * 100$$

Where, TA %: titratable acidity % or gm malic acid/100 mL of juice; V_b : total volume of NaOH consumed in the reaction, mL; N: normality of NaOH used (0.1N); M_{eq} : milliequivalent of malic acid (0.067 for malic acid); V_a : total volume of fruit juice used, ml (generally 10 mL is taken)

2.6 Statistical analysis

Data entry was done with the help of MS Excel. All the data were subjected to one-way ANOVA (Analysis of Variance), with cultivars as the treatment. The differences between mean values were determined using Duncan's multiple range test (DMRT) at a 5% significance level. DMRT is a simple and commonly used statistical tool for comparing the treatment means. All the statistical analyses were performed by using R packages.

3 Results and Analysis

3.1 Physical characteristics

For individual fruit weight, the maximum was found in Rich a Red Delicious (210.35 g) and least was found in Tsukura (22.25 g). Golden Delicious (160.40 g) was statistically at par with Red Delicious (174.65 g) for fruit weight. Similarly, the highest and least value of fruit volume (200 cm³ and 20 cm³), fruit length (7.22 cm and 3.38 cm) and fruit width (7.70 cm and 3.42 cm) were observed in Rich a Red Delicious and Tsukura respectively. Royal Delicious (6.95 cm) showed statistically similar figure with Rich a Red Delicious and Saune (6.07 cm) was statistically at par with Golden Delicious (6.32 cm) for fruit length. For fruit width, Royal Delicious (7.17 cm), Golden Delicious (6.89 cm) and Red Delicious (7.70 cm) had similar figures. The highest value for individual seed weight was observed in Golden Delicious (0.40 g), seed length was observed in Rich a Red Delicious (0.80 cm), seed width was observed in Saune (0.40 cm), and the mesocarp thickness was seen in Royal Delicious and Rich a Red Delicious (2.72 cm). The lowest figures for individual seed weight (0.10 g), seed length (0.64 cm), seed width (0.27 cm) and mesocarp thickness (1.52 cm) was seen in Tsukura. For individual seed weight, Saune (0.32 g), Red Delicious (0.32 g), Rich a Red Delicious (0.39 g), Royal delicious (0.34 g) and Golden delicious were statistically similar. Red Delicious (0.75 cm), Golden Delicious (0.75 cm) and Royal Delicious (0.77 cm) were statistically at par with Rich a Red Delicious whereas Saune (0.70 cm) was statistically at par with Tsukura

for seed length. Red Delicious (0.37 cm), Rich a Red Delicious (0.37 cm), and Golden Delicious (0.34 cm) were statistically at par with Saune whereas Royal Delicious (0.33 cm) was statistically par with Tsukura for seed width. Golden Delicious (2.45 cm) was statistically par with Rich a Red Delicious and Royal Delicious for mesocarp and Saune (1.72 cm) was statistically par with Tsukura for mesocarp thickness (Table 3; Table 4).

Table 3 Physical characteristics of apple fruits from different cultivars at THDC, Marpha, Mustang, 2023

Cultivars	Individual fruit weight (g)	Fruit volume (cm ³)	Fruit length (cm)	Fruit width (cm)
Tsukura	22.25 ^e	20.00 ^e	3.38 ^e	3.42 ^d
Saune	98.20 ^d	93.75 ^d	6.07 ^d	6.38 ^e
Red Delicious	174.65 ^c	173.75 ^b	6.55 ^{bc}	7.21 ^b
Golden Delicious	160.40 ^c	157.5 ^c	6.32 ^{cd}	6.89 ^b
Rich a Red Delicious	210.35 ^a	200.00 ^a	7.22 ^a	7.70 ^a
Royal Delicious	193.00 ^b	190.15 ^a	6.95 ^{ab}	7.17 ^b
LSD (0.05)	16.77	15.34	0.43	0.49
SE _m (±)	2.27	2.08	0.06	0.07
F probability	<0.001	<0.001	<0.001	<0.001
CV (%)	7.77	7.32	4.68	5.00
Grand mean	143.14	139.19	6.08	6.46

Note: LSD= Least Significant Difference, SE_m = Standard Error of Mean, CV: Coefficient of Variance; Means in the same column followed by the same letter(s) are not significantly different by DMRT at a 5% significance level

Table 4 Physical characteristics of apple seeds from different cultivars at THDC, Marpha, Mustang, 2023

Cultivars	Individual seed weight (g)	Seed length (cm)	Seed width (cm)	Mesocarp thickness (cm)
Tsukura	0.10 ^b	0.64 ^c	0.27 ^c	1.52 ^c
Saune	0.32 ^a	0.70 ^{bc}	0.40 ^a	1.72 ^c
Red Delicious	0.32 ^a	0.75 ^{ab}	0.37 ^{ab}	2.30 ^b
Golden Delicious	0.40 ^a	0.75 ^{ab}	0.34 ^{ab}	2.45 ^{ab}
Rich a Red Delicious	0.39 ^a	0.80 ^a	0.37 ^{ab}	2.72 ^a
Royal Delicious	0.34 ^a	0.77 ^{ab}	0.33 ^{bc}	2.72 ^a
LSD (0.05)	0.14	0.08	0.06	0.33
SE _m (±)	0.02	0.01	0.01	0.04
F probability	<0.01	<0.01	<0.01	<0.001
CV (%)	30.73	7.35	11.80	9.72
Grand mean	0.31	0.73	0.35	2.24

Note: LSD= Least Significant Difference, SE_m = Standard Error of Mean, CV: Coefficient of Variance; Means in the same column followed by the same letter(s) are not significantly different by DMRT at a 5% significance level

3.2 Chemical characteristics

The highest value of pH was found in Saune (4.30), TSS was found in Golden Delicious (11.87 °Brix), TA was found in Saune (0.60%) and TSS/TA was found in Rich a Red Delicious (53.12). The lowest value of pH was found in Golden Delicious (3.22), TSS was found in Tsukura (8.52 °Brix), TA was found in Rich a Red Delicious (0.20 %) and TSS/TA was found in Saune (17.30). Rich a Red Delicious (3.77) was statistically at par with Red Delicious and Royal Delicious having a pH of 3.87. Red Delicious (9.90 °Brix), Saune (10.27 °Brix) and Rich a Red Delicious (10.62 °Brix) were statistically at par with Royal Delicious (10.65 °Brix) for TSS. Red Delicious (0.23 %) and Golden Delicious (0.24 %) were statistically par with Rich a Red Delicious for TA. Tsukura (19.37) was statistically par with Saune for TSS/TA (Table 5).

Table 5 Chemical characteristics of different apple cultivars at THDC, Marpha, Mustang, 2023

Cultivars	pH	TSS (°Brix)	TA (%)	TSS/TA
Tsukura	4.30 ^b	8.52 ^c	0.44 ^b	19.37 ^e
Saune	4.57 ^a	10.27 ^b	0.60 ^a	17.30 ^e
Red Delicious	3.87 ^c	9.90 ^b	0.23 ^d	43.04 ^c
Golden Delicious	3.22 ^d	11.87 ^a	0.24 ^d	48.52 ^b
Rich a Red Delicious	3.77 ^c	10.62 ^b	0.20 ^d	53.12 ^a
Royal Delicious	3.87 ^c	10.65 ^b	0.30 ^c	34.95 ^d
LSD (0.05)	0.14	0.95	0.04	3.1
SE _m (±)	0.02	0.13	0.01	0.42
F probability	<0.001	<0.001	<0.001	<0.001
CV (%)	2.36	6.10	8.60	5.71
Grand mean	3.93	10.31	0.34	36.05

Note: LSD: Least Significance Difference, SE_m: Standard Error of mean, CV: Coefficient of Variance; Means in the same column followed by the same letter(s) are not significantly different by DMRT at a 5% significance level

4 Discussion

Poor yielding due to unfavorable climatic conditions (heavy rainfall during flowering season) could be the reason for significant variation in size among different cultivars (Yoon et al., 2020). Genetics study suggests that genes involved in cell division and cell expansion is potentially responsible for regulating fruit size (Devoghlaere et al., 2012). Furthermore, the differential genes related to auxin signaling, including the auxin synthetic genes *MdTAR1* and *MdYUCCA6* results in greater fruit weight in various cultivars (Bu et al., 2020). Thus, the higher fruit weight in Rich a Red Delicious cultivar may be primarily due to underlying genetic and environmental factors.

Low fruit weight and firmness of native cultivars are due to adaptive mechanisms to environment and genetic factors (Teferra et al., 2021). The physicochemical properties of apples depend upon cultivars, maturity stages, and temperature. The difference in pomological characteristics may be a result of cultivar, rootstock, cultural practices as well as variations in fruit formation (Bogbuga and Pirlak, 2012). Dolkar et al. (2021) observed the fruit weight of Royal Delicious was significantly higher than all the native cultivars of the Ladakh region. Korkmaz and Okatan (2021) found that fruit widths were found to vary from 77.61 mm to 55.24 mm and fruit length values were found to be between 65.92 mm and 54.89 mm. The fruit weight of different genotypes was found between 81.3 and 125.4 g as observed by Gecer et al. (2020) similar to Saune. Individual fruit weight of Red Delicious was found higher than Golden Delicious as observed by Loncaric et al. (2019) and also observed highest fruit weight, height and width were measured in the conventional apple cultivar, 'Red Delicious', and the lowest in traditional apple cultivar which was similar to Tsukura and Saune. Loncaric et al. (2019) showed that the mass of average one healthy seed was higher for conventional cultivars (60±10 mg) than traditional cultivars (48±11 mg) and also Golden Delicious showed similar results as Red Delicious similar to my findings. Sedlackova et al. (2021) found that the average weight of 10 seeds was 0.38-0.77/0.29-0.98 (g), the height of seeds was 6.68-9.90/6.16-9.83 (mm), and the diameter of seeds was 3.73-5.71/3.51-5.27 (mm), respectively for seeds of repository and self-grown seedling.

Our measured value in chemical characteristics varied significantly probably due to inter annual climatic variability and accessions (Mignard et al., 2022). Sweetness and sourness are considered important drivers for consumer preference (Endrizzi et al., 2015). Increase in sorbitol content is positively correlated with increase in TSS. Thus, Highest TSS in Golden Delicious was due to highest level of sorbitol content. The increase in acidity in certain cultivars of apple is due to accumulation of malic acid and L-ascorbic acid (Yoon et al., 2020). Therefore, highest value of TA was detected in Saune due to presence of highest concentration of dominant organic acids. The TSS, TA and TSS/TA (ripening ratio) harvested in different dates varied from 8.52-13.2 °Brix, 0.21%-0.30% and 28.17-61.81 for Royal Delicious according to Chalise and Giri (2019). Bhusan and Thomas (1998) found that the pH of Red Delicious, Golden Delicious, Royal Delicious and Rich a Red Delicious to be 3.7, 3.5, 3.6 and 3.7 respectively and TA to be Golden Delicious, Royal Delicious, Red Delicious and Rich a Red Delicious were (0.24±0.02)%, (0.24±0.01)%, (0.23±0.02)% and (0.21±0.01)%, respectively, which showed

correspondence with our research results. Dolkar et al. (2021) found that some native cultivars have the highest TA ($0.71 \pm 0.02\%$) among other cultivars as Saune (0.60 %). Korkmaz and Okatan (2021) showed that the TA of all 6 apple genotypes was $>0.64\%$. Kalkisim et al. (2015) found the average pH value of the native cultivar to be 3.8 and TA to be 0.69 %. Molina-Corral et al. (2021) found TSS of Golden Delicious (12.7-13.1 °Brix) was higher than Red Delicious (11.3-11.7 °Brix) grown at both locations in Washington, USA, and Chihuahua, Mexico similar to our findings.

5 Conclusion

From the study, it can be inferred that Rich a Red Delicious was found best cultivar in terms of sweetness, size and weight among six cultivars of apple. It is suggested that Tsukura and Saune (TSS/TA <20) can be cultivated targeting for juice purposes whereas the other four cultivars (TSS/TA >20) targeting for fresh table consumption in the Mustang region. Further investigation on physical and chemical properties of different cultivars of apple in Mustang is required before recommending different apple cultivars in Mustang, Nepal.

Authors' contributions

SS and NB were involved in conceptualization, conducting the experiment, data curation, editing, data analysis, and writing the original draft. PNA and SD were involved in supervision, manuscript revision, and providing the final structure to the manuscript. AKB was involved in data curation, data analysis and reviewing the manuscript. All authors read and approved the final manuscript.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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