PHYSIO-CHEMICAL CHARACTERIZATION OF MANGO GERMPLASMS FOUND IN FARWEST PROVINCE OF NEPAL

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ABSTRACT

Mango fruits (Mangifera indica L.) are good source of antioxidants including vitamin C, phenolic compounds, carotenoids and other dietary compounds. This study evaluated the physical (weight, length, diameter and firmness) and chemical (total soluble solids, titratable acidity, pH, vitamin C, total phenol, flavonol and carotenoids) attributes of ten local mango germplasm from far western Nepal and the results were compared with commercial cultivar Maldha. Fruit attributes were analyzed at full ripening stage (6 days after harvesting). Physical traits showed significant variation, with local germplasms exhibiting smaller fruits weight (20.48–53.17 g), length (40.57–56.71 mm), and diameter (31.76–43.25 mm) than Maldha (160.44 g, 82.66 mm and 60.86 mm respectively), although firmness did not differ significantly. Maldha recorded the highest pH and total soluble solids (TSS), whereas, Kakune showed the lowest titratable acidity. Sanney exhibited the highest vitamin C (1.12 mg/ml) and total phenols (0.0095 mg/g GAE), while Moto Surukulle had the highest carotenoid content. During ripening, TSS, phenols, flavonols, and carotenoids increased while acidity declined. This study shows the significant variation among local germplasms and commercial cultivars with respect to physical and chemical attributes of fruits. Local germplasms showed greater antioxidant potential despite Maldah's superior fruit size and sweetness. Further multi-year evaluation, yield assessment and consumer preference studies are recommended to identify superior cultivars for cultivation and breeding programs in far-western Nepal.

Keywords*: *Antioxidants, Carotenoids, Germplasm, Flavonols, Phenol

INTRODUCTION

Mango (*Mangifera indica* L.) is the most popular tropical fruit in Nepal with highest acerage and production. It is grown on about 44,449 ha and produces around 517,552 MT, with an average yield of 11.64 MT ha^{-1} (MoALD, 2082). Improved mango varieties commonly grown in Nepal include Amrapali, Maldha, Dashari, and Jarda. In addition, NARC (2003) has documented several local germplasms, such as Sindhure, Kali, Supare, and Lohare from the lower hills, as well as Chinia, Sipiya, Chausa, and Safeda from the Terai and adjoining hill regions. Subedi et al. (2008) reported 132 different local and commercial mango cultivars from 11 districts of Nepal based on eco-geographical survey. Mango is not only valued for its taste and aroma but also for their high nutritional and biological value. It is a rich source of bioactive compounds such as vitamin C, phenolic acids, carotenoids, flavonoids, and antioxidants (Ramirez et al., 2014; Maldonado-Celis et al., 2019). Previous studies have shown that mango pulp has a high level of antioxidant activity, which includes scavenging free radicals and preventing lipid peroxidation. Additionally, it has been shown to boost the antioxidant enzyme system under isoproterenol-induced oxidative stress conditions (Bafna & Balaraman, 2005). Polyphenols and flavonoids are known to be a a major contributors to the total antioxidant activity of mango, which counteracts reactive oxygen species and oxidative damage (Ma et al., 2011; Rumainum et al., 2018). Mango carotenoids have also been shown to have a variety of biological activities. According to Venkateswarlu and Reddy (2014), these include serving as precursors of vitamin A, antioxidants, immune system boosters, cellular communication facilitators, UV radiation protectors, light-harvesting agents, and protectors against photooxidative damage.

Different local germplasms exist in different parts of the country. However, many of them are in threats due to introduction of commercial mango cultivars and high rate of logging of old mango trees (Subedi, 2008; Budathoki, et. al, 2004) Local germplasms are considered to possess various characteristics which are economically and culturally valuable than modern varieties. Although some study on physical attributes of fruit of local mango germplasms was made by Subedi et al. (2008), no publication on the characterization of phenolic compounds, to our knowledge, has been conducted in Nepal till date. The present study demonstrated the variation in physical and chemical attributes of local germplasms over commercial variety. This can be valuable in accessing the importance of these indigenous natural resources, their cultivation and further use in breeding program.

MATERIALS AND METHODS

Sample collection

Ten local mango germplasms (Dhole, Ratirumal, Mude, Sanney, Sinure, Kale, Hady, Rulli, Moto Surukulle, and Kakune) along with one commercial variety (Maldha) were selected from Shikhar Municipality, Doti, Far-west Province of Nepal for the study. The mangoes were harvested from 708 to 1165 masl where the maximum and minimum temperature lied between 27°C to 40°C and 7°C to 17°C, respectively and relative humidity ranged between 58% and 69%. Nine mature, regular-sized fruits representing all sides of the tree were collected for each germplasm. Analysis was carried out at three stages of maturity i.e. immediately after harvest (Maturity stage), 3 days after harvest (Partial ripening stage) and 6 days after harvest (Full ripening stage). The fruits were cleaned, air-dried, and immediately assessed for physical parameters. Samples were stored at room temperature until they reached full ripening. Laboratory analyses were carried out at the Central Laboratory of the Institute of Agriculture and Animal Science, Pakhlihawa Campus.

Chemicals and reagents

Sodium hydroxide (NaOH), Phenolphthalein, Oxalic acid, Ascorbic acid, 2,6 Dichloroindophenol dye (DCIP), Folin-Ciocalteu's reagent, Ethanol, Hydrogen Chloride (HCl) and Acetone were procured from Fisher Scientific. Sodium bicarbonate, Gallic acid, Beta-carotene and Quercetin were obtained form HiMedia Laboratories Pvt. Ltd. and n- Hexane was purchased from Mark Life Science Private Limited.

Physical parameter measurement

Fruit weight was measured using a digital balance (Phoenix instruments, Germany). Fruit length and diameter were measured using digital caliper (Model DC-515 Lutron instruments, Taiwan). Digital penetrometer (Model Fr-5120, Lutron instruments, Taiwan) was used for firmness measurement.

Sample preparation for chemical analysis

Selected fruits were peeled off and then pulp was chopped into little pieces. Then 10 gm of pulp was blended with 10 ml of distilled water and pounded coarsely in a mortar and pestle. Then it was squeezed through muslin cloth in a beaker. The filtrate was centrifuged (Laboratory Centrifuge) at 5000 rpm for 5 minutes. The supernatant was gathered and refrigerated until the analysis was completed.

TSS, pH, TA and Vitamin C measurement

Total soluble solids (TSS) were measured by placing 300 µL of the extracted juice on the prism of a digital refractometer (Milwaukee MA871). The method follows standard refractometry procedures described for fruit juice analysis (AOAC International, 2023). Sample pH was determined using a calibrated digital pH meter (BPH 231, Lutron Instruments) according to established guidelines for fruit samples (AOAC International, 2023).

Titratable acidity (TA) was analyzed by diluting 5 mL of the extracted juice with 45 mL of distilled water and titrating the mixture against 0.1 N NaOH using a single drop of phenolphthalein as the indicator until the light pink color appear. Total NaOH consumed for each titration was recorded and TA was calculated from the given formula for each sample of juice (AOAC International, 2023):

$$\%Acidity(w/w) = \frac{N \times V \times Eq.wt}{weight\ of\ sample(g) \times (1000)} \times 100$$

where N is the normality of NaOH (mEq/ml), V is the volume of NaOH consumed (mL), Eq. wt is the equivalent weight of the predominant organic acid

For the measurement of Vitamin C content, the widely used method, dye titration was used using 2,6-dichlorophenol indophenol (DCIP) (AOAC International, 2023). A dye solution was prepared by dissolving 50 mg of DCIP in 50 mL of distilled water with mild heating and making the volume up to 100 mL. For the sample titration, 1 mL of centrifuged juice was mixed with 25 mL of 0.5% oxalic acid and titrated with the dye until a stable color change was observed. Similarly, 1 mL of 0.1% standard ascorbic acid in 25 mL of 0.5% oxalic acid was titrated to determine the dye factor. Vitamin C content was then calculated using the standard formula (AOAC International, 2023):

$$Vitamin\ C\ (\%\ \frac{w}{v}) = \frac{\text{Volume of dye used for sample (mL)}}{\text{Volume of dye used for standard (ml)} \times \text{Concentration of standard ascorbic acid (\%)}}$$

Total phenol content

The Folin-Ciocalteu colorimetric test was used to calculate the total phenolic content using the Singleton and Rossi (1965) technique. A 100 µL aliquot of the produced extract was combined with 1 mL of Folin–Ciocalteu reagent, 1 mL of 10% sodium bicarbonate, and 4 mL of distilled water. After fully vortexing the mixture, it was left in the dark for an hour. Spectrophotometer was used at 760 nm to measure absorbance. Gallic acid served as the standard, and a calibration curve ($r^2 = 0.998$) was constructed by plotting absorbance at 760 nm against gallic acid standards from 0.0625 to 1.0 mg mL^{-1}. The total phenolic content was expressed as gallic acid equivalents (GAE) on a fresh-weight basis (mg g^{-1} FW)

Total flavonol content

The total flavonols was calculated following the method of Mazza et al. (1999) and expressed as Quercetin Equivalents (QE) on a fresh-weight basis (mg g−1 FW). An aliquot of 0.25 mL of prepared extract was transferred to a test tube, to which 0.25 mL of 0.1% HCl (made up in 10% ethanol) and 4.55 mL of 2% HCl were added. After thorough vortexing and standing for about 15 min, the absorbance was read at 360 nm using a spectrophotometer. The total flavonol content was calculated using a calibration curve (r2 = 0.990) with quercetin standards (0.0625–1.0 mg mL−1) prepared in 95% ethanol.

Carotenoid content

The carotenoid content was determined using the procedure given by Rumainum et al. (2018). One gram of freeze-dried pulp was meshed in a mortar and pestle and placed into a volumetric flask that was then wrapped with aluminum foil to protect from light. To this, 25 mL extraction solvent was added, which was prepared by mixing hexane, acetone, and ethanol in a 2:1:1 v/v ratio, and the mixture was then vigorously vortexed for ten minutes. Subsequently, 5 mL of distilled water was added and stirred for an additional 5 minutes. Absorbance of the hexane phase was measured using a spectrophotometer at 450 nm. Absorbance at 450 nm was plotted against known β-carotene concentrations for the measurement of carotenoid concentration using a calibration curve (r2 = 0.983) derived from β-carotene standards (0.03125–0.5 mg mL−1). Results are expressed as β-carotene equivalents on a fresh-weight basis (mg g^{-1}FW).

Statistical analysis

The experiment was conducted in Completely Randomized Design (CRD). Ten local germplasms and one commercial cultivar (test variety) constituted eleven treatments. Three extract for each germplasm was taken from three different fruits, so three tests

for each germplasm constituted three replications. The recorded data were originally compiled in Microsoft Excel. Analysis of Variance (ANOVA) was performed, and mean comparisons were made using Tukey's test at a 99% confidence level, applying GenStat Teaching & Learning Edition (18th version). Tables and figures were generated using Microsoft Excel 2010 and Microsoft Word 2010. The analysis of hierarchical clustering was performed with SPSS program (version 16).

RESULTS AND DISCUSSION

Physical attributes

The findings suggested a significant difference in fruit weight across germplasms while there was no significant variation in fruit weight at different maturity stages. The fruit length and diameter also varied significantly (Figure 1, Table 1). Commercial cultivar Maldha showed the highest fruit weight (160.44 g), fruit length (82.66 mm) and diameter (60.86 mm) followed by Mude (53.17 g, 56.71 mm length and 43.25 mm diameter) while Moto Surukulle showed the lowest (20.48 g) fruit weight and fruit diameter (31.76 mm) and Dhole showed the lowest fruit length (40.57 mm).

Budhathoki et al. (2004) also found out that the average fruit weight among 23 elite mango genotypes differed significantly and ranged from 21 g to 2500 g. During ripening, fruits gradually lose weight due to respiration, transpiration, and other biochemical changes (Gill et al., 2017; Thinh et al., 2013). In an experiment on fourteen hybrids and five superior mango selections, fruit length varied from 7.21 cm to 12.55 cm and width from 5.07 cm to 8.85 cm (Bora et al., 2017). Fruit size is influenced by both genetic factors and the environment.

Fruit firmness ranged from 0.4767 kg/cm² to 1.1433 kg/cm²; however, no significant differences were observed among the local germplasms and the commercial cultivar (Figure 2). Firmness value was significantly lowered as the fruit ripened for each germplasm. Fruit pulp softens during ripening regardless of temperature, due to enzyme-driven changes in cell wall structure, solubilization of pectin and cellulose, and hydrolysis of starch and other polysaccharides (Gill et al., 2017).

Table 1: Variation in mango fruit length and diameter of different mango germplasms

Mango germplasm	Fruit length (mm)	Fruit diameter (mm)
Dhole	40.57±2.19 ^{e}	32.19±1.85 ^{d}
Ratirumal	53.75±0.34 ^{bc}	40.97±0.82 ^{bc}
Mude	56.71±0.41 ^{b}	43.25±0.90 ^{b}
Sanney	42.79±2.11 ^{de}	35.23±1.04 ^{cd}
Sinure	52.72±1.65 ^{bcd}	38.37±0.44 ^{bcd}
Kale	47.34±1.14 ^{bcde}	34.01±0.52 ^{cd}
Hady	48.27±2.80 ^{bcde}	34.02±2.24 ^{cd}
Rulli	42.62±0.51 ^{de}	37.38±1.22 ^{bcd}
Moto surukulle	43.93±2.73 ^{cde}	31.76±1.60 ^{d}
Kakune	44.34±1.66 ^{cde}	34.78±0.43 ^{cd}
Maldha	82.66±1.92 ^{a}	60.86±0.28 ^{a}
Mean	50.52	38.44
Standard error	3.114	2.075
LSD (1%)	7.166	4.776
CV%	6.2	5.4

Values with the same letter(s) within a column are not significantly different at $p < 0.01$ by Tukey's test.

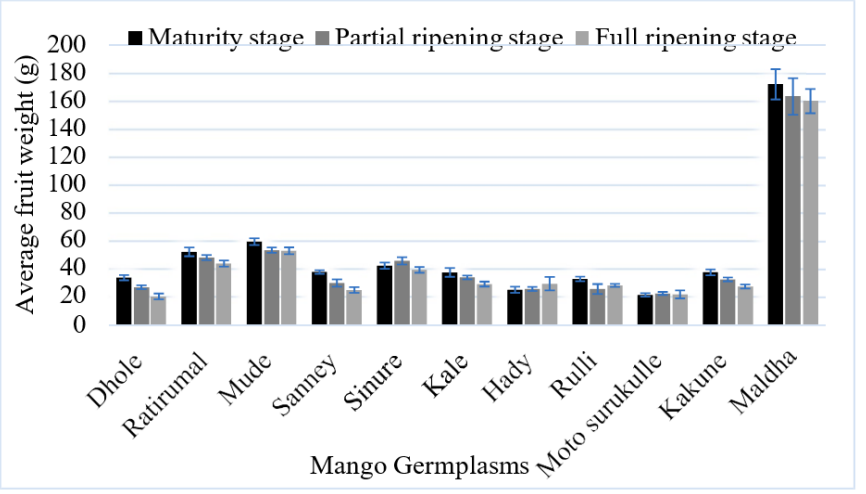

Figure 1: Variation in average fruit weight in different maturity stage of different mango germplasms

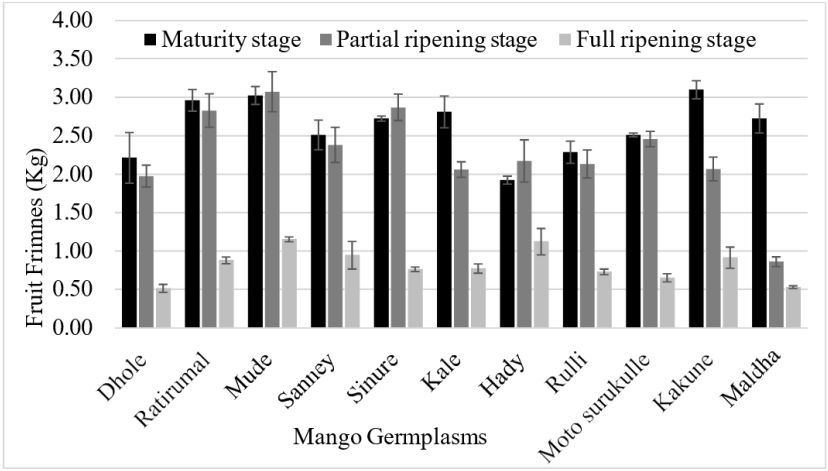

Figure 2: Variation in fruit firmness in different maturity stage of different mango germplasm

Chemical attributes

Maldha showed the highest TSS at partial ripening (8.3 °Brix), while Hady recorded the highest value at full maturity (10.4 °Brix). Mude recorded the lowest TSS at maturity while Sanney recorded it in full maturity stage. TSS increased markedly from maturity to full ripening in all germplasms except Dhole and Sanney. Significant variation in TSS content was observed among the germplasms at each ripening stage (Figure 3). The titratable acidity of germplasms at various stages of maturity varied considerably (Figure 4). Highest TA value was obtained in Sanney at maturity stage (3.08%) and partial ripening stage (2.56%) and in Kakune (2.79%) at full maturity stage. Lowest TA value was obtained in Maldha in each stages of ripening. Variation in Vitamin C content was seen among germplasms with highest value in Sanney in each stages of maturity (Table 2). Vitamin C content of local germplasm had varied significantly ranging between 0.19 mg/ml in Kale and 1.12 mg/ml in Sanney among germplasms at full maturity stage. Figure 6 displays the total phenol content at various stages of maturity of different mango germplasms. Sanney showed the highest total phenol content (0.0095 mg/g GAE) compared to the commercial cultivar Maldha (0.0014 mg/g GAE). Lowest value of total phenol was obtained in Rulli in maturity stage, Ratirumal at partial ripening stage, Mude at full ripeing stage. Similarly, significant increase in value of phenol content from maturity to ripening was observed in all germplasms except Ratirumal, Mude, Moto surukulle and Maldha. Significantly higher flavonol content was observed in Moto Surukulle at maturity

and full ripening stage and in Hady at partial ripening stage (Figure 5). Similarly, significant increase in flavonol content was observed in Ratirumal, Sanney, Kale, Hady, Rulli, Kakune and Maldha form maturity to full ripening stage. Significant variation was observed for carotenoid content among mango germplasms (Figure 7). Moto Surukulle showed significantly higher carotenoids value at all stages of maturity followed by Kale. Also, levels of carotenoids showed an increase from the mature to full ripening stages in all the germplasms.

Budhathoki et al. (2004) reported variation in TSS range (8–26 °Brix) among 23 local genotypes in Nepal. Starch hydrolysis and increased sugar accumulation are the primary causes of the rise in TSS during ripening (Iwanami et al., 2024), which improves sweetness and overall fruit palatability (Shafique et al., 2006). The titratable acidity (TA) content of local germplasms in Nepal varied from 0.2% to 0.6% (Budhathoki et al., 2004). Shafique et al. (2006) also noted a decrease in acidity with maturity. The breakdown of starch into more sugars and organic acid metabolism as the fruit ripens may be the cause of this decrease in acidity with maturity (Hussain et al., 2024). Manthey and Perkins Veazie (2009) reported a high variation of 5 cultivars of mango pulp in vitamin C content from 11 to 134 mg/100 g coming from different countries. Similarly, Kamruzzaman et al. (2014) documented high variability in pH, TSS, TA, and vitamin C content among five mango varieties in Bangladesh. Generally local or traditional varieties of mangos have higher levels of phytochemicals than commercial cultivars, which is consistent with previous research showing strong genetic control over phenolic accumulation in mangoes (Ajila et al., 2010; Abbasi et al., 2015). While Maldha's lower values correspond with trends in improved cultivars chosen primarily for yield and consumer traits rather than nutraceutical quality, Sanney's elevated phenolic content may reflect its intrinsic metabolic capacity for secondary compound synthesis (Shahidi & Naczk, 2004).

The higher flavonol content in Moto Surukulle at maturity and full ripening, and in Hady at partial ripening, indicates strong genotype-dependent variation in phenolic accumulation. The significant increase in flavonol levels from maturity to full ripening in several germplasms agrees with earlier studies showing that ripening enhances phenolic synthesis and extractability due to activation of the phenylpropanoid pathway (Masibo & He, 2008). Similar increases in flavonols during ripening have been reported in other mango cultivars, reflecting improved antioxidant potential at later stages (Abbasi et al., 2015). The rise in flavonol content from maturity to ripening is in accordance with Masibo and He (2008) showing that ripening enhances phenolic synthesis and extractability due to activation of the phenylpropanoid pathway.

Genetic influence might be the cause for significant variation for carotenoid content among mango germplasms (Boon et al., 2010). The increment in carotenoids from the mature to full ripening stages the germplasms corresponds with the degradation of chlorophyll and increased synthesis of pigments during the ripening process (Masibo & He, 2008). These results highlight the nutritional potential of fully ripe fruits for provitamin A.

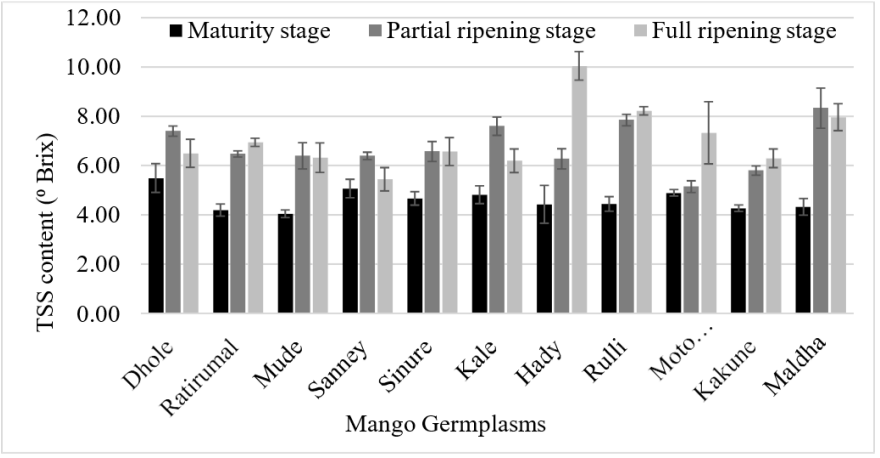

Figure 3: Variation in TSS in different maturity stages of different mango germplasms

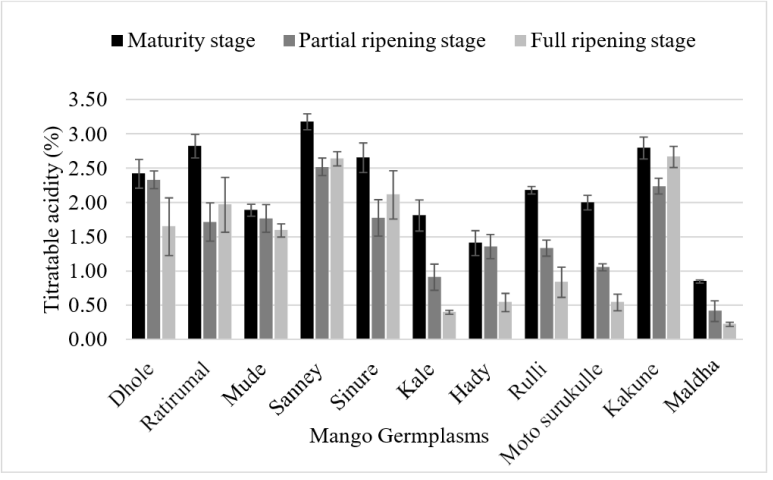

Figure 4: Variation in TA in different maturity stages of different mango germplasms

Table 2: Variation in Vitamin C content at different maturity of different mango germplasms

Mango germplasm	Maturity stage	Partial ripening stage	Full ripening stage
Dhole	0.3464±0.113 ^{bcd}	0.5772±0.049 ^{abc}	0.3481±0.082 ^{b}
Ratirumal	0.3790±0.057 ^{bcd}	0.3658±0.014 ^{bc}	0.3712±0.159 ^{b}
Mude	0.1372±0.011 ^{d}	0.2032±0.029 ^{c}	0.4848±0.097 ^{ab}
Sanney	1.0645±0.194 ^{a}	0.9368±0.121 ^{a}	1.1211±0.228 ^{a}
Sinure	0.6021±0.142 ^{abcd}	0.3170±0.070 ^{bc}	0.3333±0.033 ^{b}
Kale	0.3921±0.033 ^{bcd}	0.4040±0.131 ^{abc}	0.1970±0.027 ^{b}
Hady	0.4183±0.035 ^{bcd}	0.6966±0.195 ^{abc}	0.7424±0.236 ^{ab}
Rulli	0.2744±0.081 ^{cd}	0.2520±0.021 ^{c}	0.2348±0.030 ^{b}
Moto surukulle	0.1503±0.007 ^{d}	0.3232±0.020 ^{bc}	0.2815±0.032 ^{b}
Kakune	0.7056±0.019 ^{abc}	0.8129±0.029 ^{ab}	0.7651±0.008 ^{ab}
Maldha	0.8741±0.039 ^{ab}	0.6814±0.078 ^{abc}	0.5482±0.007 ^{ab}
Mean	0.486	0.506	0.493
Standard error	0.1525	0.1534	0.2044
LSD (1%)	0.3509	0.3530	0.4704
CV%	31.4	30.3	41.4

Values with the same letter(s) within a column are not significantly different at $p < 0.01$ by Tukey's test.

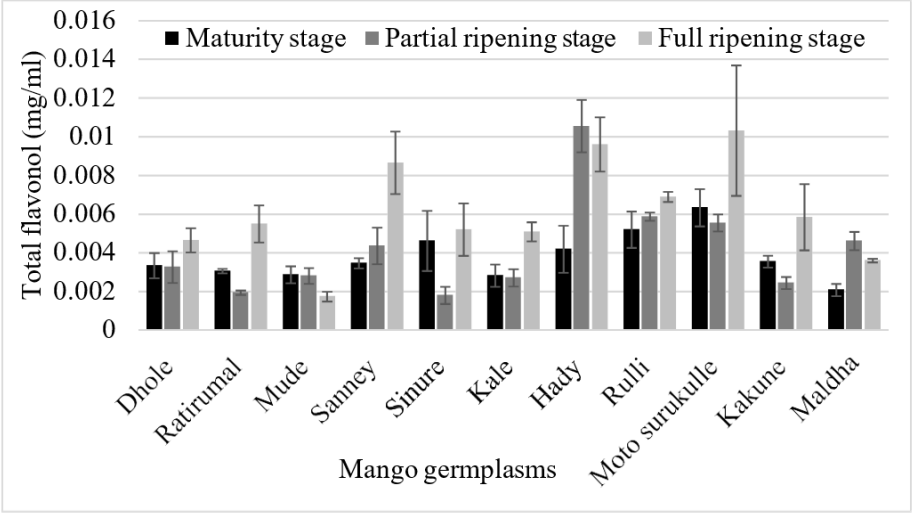

Figure 5: Variation in Total falvonol (mg/ml) in different maturity stages of different mango germplasms

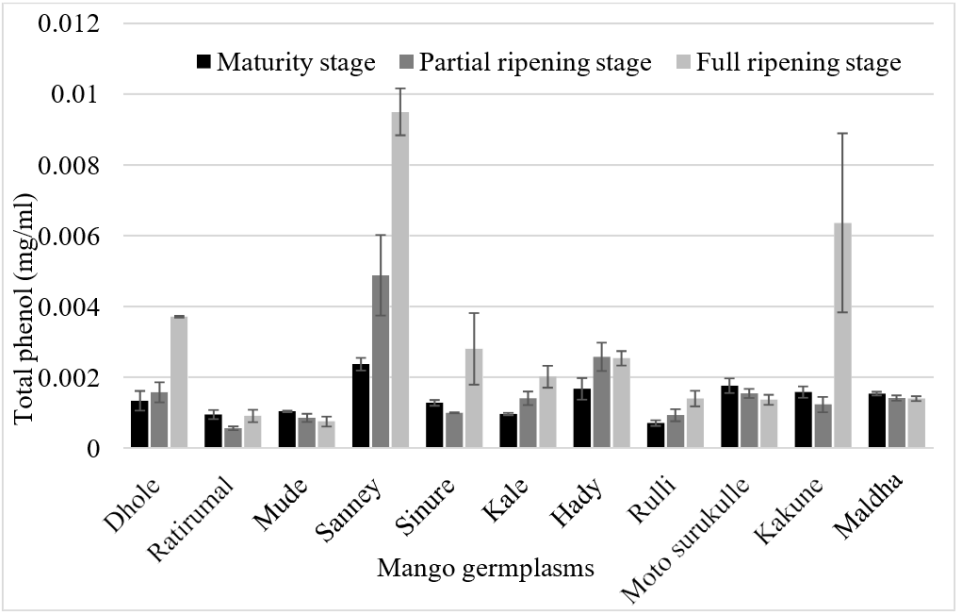

Figure 6: Variation in total phenol (mg/ml) in different maturity stages of different mango germplasm

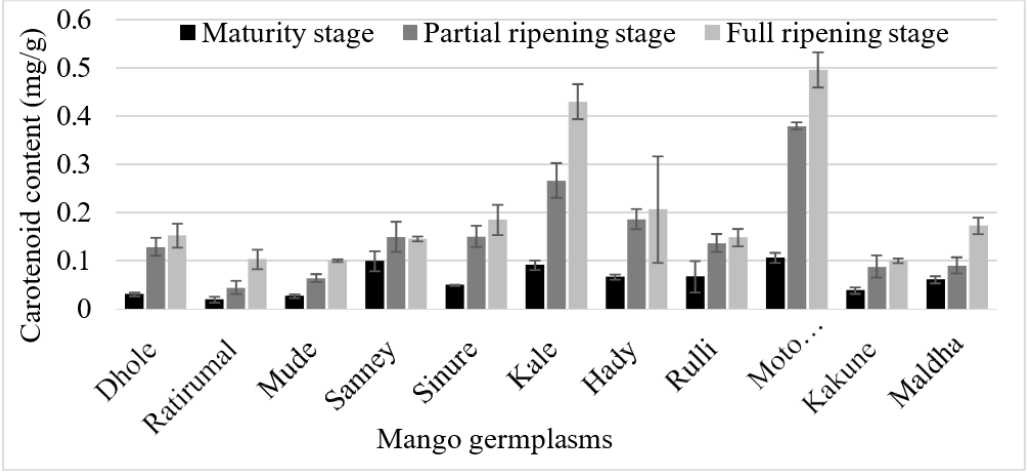

Figure 7: Variation in total carotenoid(mg/g) in different maturity stages of different mango germplasms

CONCLUSION

Local mango germplasms from Far-West Nepal displayed substantial variation in fruit physical and chemical attributes compared to commercial Maldha, with smaller sizes but often superior bioactive compounds like vitamin C and phenols in Sanney, and carotenoids in Moto Surukulle. Ripening enhanced TSS, pH, phenols, flavonols, and carotenoids across germplasms while reducing titratable acidity and firmness, highlighting their nutritional potential. These findings underscore the value of conserving and evaluating local germplasms for breeding superior cultivars adapted to the region, supporting mango genetic resource enhancement in Nepal.

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