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Comparative quality evaluation of mandarin (*Citrus reticulata* Blanco) fruits from the Darjeeling Hills

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ABSTRACT

The research was carried out to assess the quality of Darjeeling Mandarin fruits through various morphological, physicochemical and biochemical parameters in a comparative manner. Mandarin samples were collected from farmer's orchards located in places of Darjeeling and Kalimpong hills such as Mirik, Mungpoo, Sittong, and Samsing. Comparatively, samples from Silugaon, Mirik had the superior fruit weight, pulp weight, juice volume and vitamin C; Mungpoo samples showed higher peel weight, number of segments, number of seeds, total soluble solids (TSS) and total phenolic content; while lower values of such parameters were recorded for Sittong-1 samples. Peel thickness is an important character of citrus fruits that determines the mechanical protection and flavor. In this study, the peel thickness was measured to be in a range of 1.82 – 2.97 mm. TSS was found in a range of 9.61º-13.8ºBrix which is quite acceptable as per previous reported standard (>8ºBrix). The recorded pH values (3.95 – 4.14) were not satisfactory which revealed a higher acidic level in fruits as per consumer acceptability (>4.35 pH). Significantly higher phenolic content was recorded for samples collected from Mungpoo, Darjeeling. The location-based correlation analysis showed that all samples were positively correlated (>96% similarity) with each other. Principal component analysis revealed clear location-specific differentiation among the mandarin samples, identifying Labda for superior nutraceutical traits and Sittong-1 for yield-related attributes, thereby supporting targeted breeding and site-specific cultivation under the Himalayan conditions.

Key words: Darjeeling mandarin, peel thickness, bioactive compounds, correlation analysis, PCA.

INTRODUCTION

India is one of the world's top producers of fruits and vegetables due to its varied environment, which allows for the growth of a broad variety of crops. Approximately 12% of the world's fruit is produced in India, making it the second-largest producer of horticulture products (Pavithra, 14). Citrus group makes the 3rd highest production after banana and mango and the states like Madhya Pradesh, Tamil Nadu, Gujarat, West Bengal, Bihar are the major fruit producing states (Roy *et al.,* 17). Among the citrus group, Darjeeling mandarin (*Citrus reticulata*) is a distinctive variety of mandarin orange cultivated in the Darjeeling region of West Bengal, India. This fruit is renowned for its vibrant color, harmonious sweet-tart taste, and delightful fragrance, making it an integral part of the area's agricultural landscape (Gaikwad *et al.,* 6). Furthermore, the Darjeeling Mandarin is packed with vitamin C and antioxidants, making it not only a delicious addition to the diet but also a fruit that offers considerable health benefits (Martí *et al.,* 13). The superior quality and flavor of these mandarins can be attributed to the unique climatic conditions found in Darjeeling, characterized by cool temperatures, high altitudes, and well-drained soil.

Cultivating the Darjeeling mandarin plays a crucial role in the local economy, providing farmers with a reliable source of income while promoting sustainable horticultural practices. Despite its significance as a major cash crop supporting the livelihoods of hill communities, its production and orchard area have declined markedly in recent decades (Sarkar *et al.,* 18). The decline is largely attributed to increased incidence of diseases such as citrus greening, tristeza, dieback, and canker, along with ageing orchards, use of poor-quality planting material, rising temperatures, inadequate orchard management, labour shortages etc. Furthermore, intrusion of external mandarin ecotypes such as Nagpur, Khasi, Kinnow mandarin etc., have disturbed the genetic purity of the local Darjeeling mandarin. These constraints collectively threaten the sustainability of Darjeeling mandarin cultivation, underscoring the need for identifying and conserving the superior quality clone of Darjeeling Mandarin. The identification, conservation and multiplication of elite clone of healthy planting materials, followed by the production of grafted plants, towards reviving local mandarin cultivation. Therefore, it becomes critical to identify high-quality fruits and superior phenotypes for future crop improvement initiatives. This research investigates the characteristics of fruits collected

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from pure Darjeeling mandarin ecotype to support the restoration initiative of Darjeeling mandarin and enhance its production and productivity.

MATERIALS AND METHODS

Fruit samples were collected from the sixteen farmer's orange orchard of major mandarin growing region of Darjeeling hill. Ten fruits from each tree in an around Darjeeling were collected, among them, 7 samples were from Silugaon, Mirik (Darjeeling), 3 were from Mirik busty (Darjeeling), 5 were from Labda, Mungpoo (Darjeeling), 10 were from Sittong-1 (Darjeeling) and 1 sample was from Samsing (Kalimpong). Ten fresh mandarins that are free from insect infestation from each plant from sixteen farmers from five different places in and around Darjeeling were collected. The places are listed in Table 1.

The collected fruit samples were transported to the laboratory of ICAR–Indian Agricultural Research Institute (IARI), Regional Station, Kalimpong, for detailed physical and biochemical evaluation. All analyses were carried out using freshly harvested fruits under standardized laboratory conditions. The study was conducted to characterize fruit quality attributes through measurement of key morphological and biochemical parameters. Physical characterization included assessment of fruit weight (g), peel weight (g), peel thickness (mm), fruit volume (mL), pulp weight (g), number of segments per fruit, fruit diameter (mm), seed weight (g), number of seeds, and juice volume (mL). Fruit, peel, pulp, and seed weights were measured individually using an electronic weighing balance and expressed in grams. Fruit volume was determined using the water displacement method, where the volume of displaced water corresponded to fruit volume and was expressed in milliliters. Peel thickness was measured after carefully removing the peel and recording the values using a slide caliper. Fruit dimensions were recorded by measuring fruit length and breadth, and fruit diameter was subsequently estimated using standard dimensional calculations. The number of fruit segments and total seeds per fruit were determined manually. For estimation of juice volume, fruit segments were separated after peeling and deseeding, followed by homogenization using a mortar and pestle. The homogenized juice was filtered through filter paper and the final extract volume was measured using a graduated measuring cylinder. Biochemical characterization was performed to determine total soluble solids (TSS), pH, total titratable acidity (TA), ascorbic acid (vitamin C), and total phenolic content (TPC) following established analytical procedures (Ahamad *et al.,* 2). Total soluble solids were estimated from freshly extracted fruit juice using a digital refractometer (HI96801, Hanna Instruments) and values were expressed as °Brix. Prior to measurement, the refractometer was calibrated using distilled water to ensure accuracy. The pH of fruit juice was determined using a pre-calibrated digital pH meter by directly immersing the electrode into the juice sample. Total titratable acidity was determined following the method described by Gurung *et al*. (8). Approximately 2 mL of fruit juice was diluted with distilled water and titrated against standardized 0.1 N sodium hydroxide (NaOH) using phenolphthalein as an indicator until the appearance of a stable pink endpoint. The acidity values were expressed as percentage acidity. Ascorbic acid content was estimated by the 2,6-dichlorophenol indophenol dye titration method as described by Ahamad *et al.* (1). Fruit juice samples were diluted with 4% oxalic acid solution and titrated with the standardized dye until a persistent light pink color developed. The vitamin C content was calculated and expressed as mg per 100 g fresh weight. Total phenolic content was quantified using the Folin–Ciocalteu colorimetric method following Gurung *et al.* (8). Briefly, fruit juice samples were reacted with Folin–Ciocalteu reagent and sodium carbonate solution followed by incubation at room temperature for color development. Absorbance was measured spectrophotometrically at 765 nm. Gallic acid was used as the calibration standard and total phenolic content was expressed as micrograms of gallic acid equivalent per milliliter (µg GAE/mL) significance of treatments was tested through mean value.

Table 1: Collection sites and geographic details of Darjeeling mandarin accessions.

Sample code	Site of collection		Elevation (m)	Latitude	Longitude
MSG1-MSG7	Silugaon	Mirik, Darjeeling	1495 m	26.89°N	88.19°E
MB15-1- MB15-3	Mirik Busty	Mirik, Darjeeling	1495 m	26.89°N	88.19°E
LCP1-LCP5	Labda	Mungpoo, Darjeeling	300 to 1,880 m	26.96°N	88.37°E
S11-S24	Sittong-1	Sittong, Darjeeling	964.11m	26.55°N	88.22°E
SMSNG1	Samsing	Dooars, Kalimpong	914 m	26.99°N	88.81°E

RESULTS AND DISCUSSION

The collected fruit samples have a varied range of 70.1 g to 156 g in fruit weight. The highest average fruit weight was noticed in the sample MSG5 (156 g), followed by MSG2 with 153.8 g, LCP3 with 127.3 g and the lowest fruit weight was S13 (70.1 g). MSG2 sample was found to be superior in majority of the parameters including fruit volume, pulp weight, fruit diameter, peel weight, juice volume and seed weight. MSG2 has a fruit volume of 148.9 ml, peel weight of 39.7 g, pulp weight of 112.2 g, fruit diameter of 73.53 mm, seed weight of 3.188 g and juice volume of 69.3 ml. The lowest value in fruit volume was shown by LCP1 with 59.6 ml, in peel weight it was S11 (17.5 g). S11 had the lowest average fruit diameter as well as seed weight with 55.52 mm and 1.417 g. In case of juice volume and pulp weight, the minimum value was found in the sample of S13 with 46.9 g and 24.8 ml. Peel thickness is an important character of citrus fruit, as variation in peel thickness can alter the mechanical protection and flavor as by affecting density of vascular bundle and oil glands (Jentzsch *et al.,* 9). In present study, the peel thickness had a range of 1.818 mm to 2.97 mm, where the highest thickness was in MB-15-2 and lowest was in MB-15-3. The number and size of fruit segments influence juice amount and sugar-acid balance, which are key factors in citrus fruit quality (Jiao *et al.,* 10). The average number of segments has been found within 8.5 to 10.2, with maximum cases it was found near about 9 segments. The sample MB-15-2 has been found with highest number of segments and lowest was in S13 with a segment average number of 8.5. Seed number and weight can influence the fruit size and uniformity (García *et al.,* 7) and fewer seeds indirectly improve the nutrient distribution and taste profile (Khefifi *et al.,* 11). The minimum seed number was observed in MSG1 with an average number of 11.8; while the sample SMSNG1 had a significant average of seed number (22.19).

The biochemical characterization, TSS, vitamin C content, total acidity and total phenol content was analyzed. Sugar content is a typical measurement in a variety of crops. The sugar content is a standard metric in several crops. As the fruit's flesh matures, it accumulates nutrients in the form of starch, which subsequently transforms into sugars during ripening. The sugar content, expressed in degrees Brix (°Brix), quantifies the fruit's sweetness by assessing the soluble solids, which encompass sucrose, glucose, fructose, citric acid, and minerals present in the juice. In TSS, the highest amount was found in the sample MSG6 (13.8ºBrix) followed by S13 (12.29ºBrix), LCP5 (11.92ºBrix) and the lowest TSS was noticed in the sample MSG4 (9.61ºBrix). However, none of the sample had less than 9ºBrix as citrus species specially the sweet oranges must possess a TSS above 8ºBrix (Lado *et al.,* 12). The findings revealed that the pH of all the fruit juices was ranged between 3.954 to 4.145. The highest value has been found in MSG1 (4.145), followed by MB-15-3 (4.138), MSG2 and MB-15-1 with both having a pH value of 4.134. The lowest pH (3.945) was found in both MSG7 and LCP3. Less pH denotes the higher acidic level in fruits which may make it less palatable for consumers and can also dominate natural sugar content, however less pH value increase shelf life by providing microbial resistance (Dissanayake, 3).

The titratable acidity content in selected mandarin fruits varied between 1.96% and 0.52%, with the highest acidity observed in the sample of MSG4 (1.96%), followed by LCP5 (1.73%), S14 (1.41%) and lowest in the sample of MB-15-3 (0.52%), as seen in Table 1. Among the twenty-six samples, most of the samples (23) showed higher acidity percentage compared to the previously reported acidity percentage (0.48-0.82%) of Darjeeling mandarin by Gurung *et al.* (8). The acidity of these samples is due to the presence of organic acids like citric acids, malic acid and ascorbic acids and different acidity level might be due to varying concentration of sugars. Moreover, the variety and ripeness can directly influence the acidity level in mandarin fruits (https://virtualfair.sarsef.org). Different environmental factors like water availability, mineral nutrients and temperature have an impact on the accumulation of citric and malic acids in fruit cells (Etienne *et al.,* 4). Vitamin C or ascorbic acid content among different mandarin fruits was found between 48.96 and 32.4 mg/100 g. MSG3 exhibited the highest vitamin C concentration of 48.96 mg/100 g which suggests a higher antioxidative potential and MSG3 might holds a more efficient metabolic pathway for ascorbic acid synthesis or retention. Samples like S11, S13, S14, S21 and S23 all showed lowest vitamin C with a value of 32.4 mg/100 g. Interestingly, even the lowest concentration of vitamin C observed in this present study were notably higher than previously documented vitamin C content of Darjeeling mandarin (11.79 mg/ 100 g) reported by (Fahim *et al.*, 5). This difference of vitamin C content may be due to several factors like sample variations, harvesting period, methods of extraction, storage duration, genotype variation etc. In the context of total phenolic content, the highest and significant phenolic content was found in LCP4 and LCP5 with a value of 208.2 and 122.67 µg GAE/ml of juice respectively, followed by MB-15-3 (112.1 µg GAE/ml) and MSG5 (100.8 µg GAE/ml). The results are

Table 2: Physical and biochemical characterization of Darjeeling mandarin from each tree.

Plant number	Fruit weight (g)	Fruit volume (ml)	Peel weight (g)	Peel thickness (mm)	Pulp weight (g)	Fruit diameter (mm)	No, of segments	No. of seeds	Seed weight (g)	Juice volume (ml)	TSS	pH	Vit C (mg/ 100 g)	Total acidity (%)	Total phenol (µg GAE/ml)
MSG1	113 ± 13.58	92.7 ± 11.57	26.9 ± 5.13	2.449 ± 0.32	83.9 ± 11.29	64.97 ± 3.25	8.9 ± 0.88	11.8 ± 3.26	2.099 ± 0.55	49.3 ± 9.01	10.68 ± 0.33	4.145 ± 0.21	35.28 ± 3.01	0.68 ± 0.27	40.33 ± 1.12
MSG2	153.8 ± 25.33	148.9 ± 26.30	39.7 ± 10.51	2.635 ± 0.56	112.2 ± 17.73	73.53 ± 4.51	9.7 ± 0.67	17.5 ± 3.50	3.188 ± 0.99	69.3 ± 11.21	10.52 ± 0.42	4.134 ± 0.24	33.12 ± 9.31	0.92 ±0.20	55.7 ± 1.07
MSG3	124.3 ± 13.43	109.3 ± 27.38	33.7 ± 4.64	2.81 ± 0.29	89.5 ± 9.80	66.77 ± 3.35	9.7 ± 1.16	19.3 ± 5.68	3.125 ± 0.78	56.2 ± 6.51	11.2 ± 0.51	4.099 ± 0.18	48.96 ± 5.45	1.12 ± 0.00	59.93 ± 0.99
MSG4	104.1 ± 24.20	82.9 ± 13.83	25.3 ± 4.30	2.304 ± 0.48	82.4 ± 18.79	63.32 ± 4.58	9.5 ± 1.08	16 ± 3.92	2.344 ± 0.81	43.6 ± 9.78	9.61 ± 0.61	4.051 ± 0.14	44.64 ± 3.21	1.96 ± 0.13	74.77 ± 1.37
MSG5	156 ± 11.41	102.8 ± 20.65	30 ± 5.08	2.19 ± 0.40	95.9 ± 10.35	65.97 ± 3.03	9.5 ± 0.85	16.4 ± 1.65	2.611 ± 0.50	55.1 ± 6.66	9.81 ± 0.55	4.056 ± 0.11	46.8 ± 9.17	1.14 ± 0.16	100.8 ± 1.87
MSG6	73.8 ± 10.47	75.8 ± 10.23	17.4 ± 4.72	1.842 ± 0.29	54.8 ± 6.76	55.74 ± 3.76	8.8 ± 0.84	16.8 ± 6.87	1.928 ± 0.61	31.4 ± 4.22	13.8 ± 1.14	4 ± 0.12	39.2 ±	1.24 ±	90.34 ± 2.19
MSG7	117 ± 12.10	110.8 ± 13.48	37 ± 3.39	2.824 ± 0.53	80 ± 10.51	69.87 ± 3.12	9.4 ± 0.55	17.6 ± 2.97	2.924 ± 0.49	44.2 ± 7.16	10.44 ± 1.03	3.954 ± 0.17	43.2 ±	1.45 ±	67.71 ± 0.96
LCP1	112.3 ± 6.41	59.6 ± 6.77	26.6 ± 4.27	2.107 ± 0.47	83.1 ± 5.86	65.87 ± 1.88	9.6 ± 1.07	15.8 ± 4.39	2.056 ± 0.40	48 ± 4.92	11.67 ± 40.59	4.08 ± 0.08	37.44 ± 5.45	1.23 ± 0.43	53.77 ± 2.8
LCP2	100.7 ± 10.68	96.5 ± 14.81	28.5 ± 4.17	2.25 ± 0.28	71.1 ± 8.06	63.18 ± 2.66	9.9 ± 0.74	17.6 ± 3.13	1.929 ± 0.43	41.9 ± 5.65	10.24 ± 0.55	4.085 ± 0.14	36.72 ± 5.91	1.04 ± 0.26	36.2 ± 0.74
LCP3	127.3 ± 16.96	101.7 ± 26.79	33.6 ± 7.73	2.681 ± 0.54	92.9 ± 10.24	69.72 ± 3.47	9.7 ± 1.16	18.4 ± 4.67	2.088 ± 0.55	52.4 ± 6.83	11.15 ± 0.46	3.954 ± 0.13	41.76 ± 5.45	1.08 ± 0.22	71.7 ± 1.16
LCP4	96.8 ± 8.39	91.3 ± 7.26	24.6 ± 2.72	2.242 ± 0.24	70.5 ± 6.60	63.01 ± 2.37	9.1 ± 1.20	13.4 ± 2.80	1.727 ± 0.59	37.5 ± 6.90	10.67 ± 0.53	4.047 ± 0.11	38.72 ± 8.45	1.36 ± 0.29	208.2 ±1.29
LCP5	89.4 ± 11.05	84.7 ± 15.32	23 ± 5.03	2.157 ± 0.62	65.4 ± 8.07	61.35 ± 2.83	9.5 ± 0.97	15.8 ± 2.70	1.998 ± 0.51	34.7 ± 5.54	11.92 ± 0.59	4.047 ± 0.09	33.52 ± 5.24	1.73 ± 0.49	122.67 ± 0.85
S11	70.3 ± 9.12	70.8 ± 12.35	17.5 ± 2.72	1.967 ± 0.36	52.2 ± 7.25	55.52 ± 2.77	8.7 ± 1.06	14.8 ± 2.74	1.417 ± 0.21	30.3 ± 3.40	11.23 ± 0.41	4.041 ± 0.15	32.4 ±	1.28	79.87 ± 1.14
S12	74.6 ± 7.69	82.5 ± 14.84	20.1 ± 4.28	2.134 ± 0.24	51.9 ± 5.09	57.62 ± 2.75	8.9 ± 1.20	15.6 ± 3.34	1.514 ± 0.37	28 ± 4.83	10.38 ± 0.55	4.004 ± 0.11	39.6 ±	0.83	56 ± 1.00
S13	70.1 ± 8.21	72.8 ± 10.92	22.9 ± 4.01	2.362 ± 0.47	46.9 ± 5.00	57.04 ± 2.71	8.5 ± 0.85	15.8 ± 2.35	1.636 ± 0.37	24.8 ± 2.62	12.29 ± 0.62	4.052 ± 0.13	32.5 ±	0.64	85.03 ± 0.43
S14	84.9 ± 19.69	87 ± 22.40	27.1 ± 8.77	2.354 ± 0.37	57 ± 11.55	59.91 ± 4.92	8.7 ± 1.16	19.3 ± 4.67	2.339 ± 0.52	31.5 ± 7.60	11.46 ± 0.60	4.047 ± 0.13	32.4 ±	1.41	64.83 ± 1.04
S15	98 ± 12.86	101.1 ± 14.15	30.1 ± 4.84	2.216 ± 0.30	67.8 ± 10.87	61.86 ± 3.75	9.6 ± 0.97	16.5 ± 5.08	2.013 ± 0.62	39.6 ± 6.13	9.83 ± 0.93	4.023 ± 0.17	43.2	1.02	51.33 ± 1.82
S16	91 ± 9.96	97.7 ± 13.70	23.9 ± 5.53	1.999 ± 0.33	63.9 ± 7.95	60.42 ± 2.98	9.3 ± 0.95	19 ± 3.68	1.982 ± 0.46	36.3 ± 5.96	10.81 ± 0.69	4.047 ± 0.11	38.4	1.34	76.37 ± 1.5

Contd...

Table 2 contd...

Plant number	Fruit weight (g)	Fruit volume (ml)	Peel weight (g)	Peel thickness (mm)	Pulp weight (g)	Fruit diameter (mm)	No, of segments	No. of seeds	Seed weight (g)	Juice volume (ml)	TSS	pH	Vit C (mg/ 100 g)	Total acidity (%)	Total phenol (μg GAE/ml)
S21	82.9 ± 12.89	86.9 ± 14.45	22.4 ± 4.38	1.873 ± 0.36	62.3 ± 7.02	57.64 ± 4.21	9.2 ± 0.63	20.1 ± 2.73	2.055 ± 0.31	33.6 ± 4.53	11.91 ± 0.66	4.068 ± 0.17	32.4	0.96	61.06 ± 0.93
S22	86.3 ± 9.73	91.9 ± 11.59	24 ± 3.53	2.393 ± 0.37	63.6 ± 7.07	60.4 ± 3.01	9.1 ± 0.74	18.3 ± 2.41	2.191 ± 0.30	35.6 ± 4.55	11.83 ± 0.85	4.063 ± 0.15	36	1.22	10.47 ± 0.58
S23	85.2 ± 7.25	92.7 ± 11.17	21.7 ± 3.30	1.841 ± 0.36	63.4 ± 4.97	60.07 ± 1.89	8.7 ± 0.67	20.1 ± 2.51	2.201 ± 0.25	36.7 ± 3.06	11.54 ± 0.87	4.01 ± 0.11	32.4	1.02	98.6 ± 0.71
S24	81.7 ± 15.99	88 ± 16.15	21.7 ± 3.33	2.16 ± 0.36	58.1 ± 7.72	59.23 ± 5.41	9.6 ± 0.70	21.3 ± 2.11	1.945 ± 0.28	33.7 ± 5.52	11.11 ± 0.85	4.03 ± 0.18	46.8	1.15	78.45 ± 1.36
MB 15-1	118.8 ± 10.47	123.2 ± 7.60	36.8 ± 4.87	2.608 ± 0.32	81.6 ± 7.83	69.04 ± 3.55	9.2 ± 1.10	19 ± 3.54	2.848 ± 0.53	48 ± 3.94	11.76 ± 0.67	4.134 ± 0.13	43.2	1.28	66.25 ± 1.52
MB15-2	118.6 ± 14.66	116.4 ± 10.74	39.6 ± 4.39	2.97 ± 0.55	78.2 ± 11.21	69.18 ± 2.72	10.2 ± 0.45	19.2 ± 3.96	3.096 ± 0.81	43 ± 5.10	11.08 ± 0.48	3.972 ± 0.12	39.6 ± 2.93	0.9	89.17
MB 15-3	77.2 ± 9.44	79.8 ± 8.17	21.2 ± 3.70	1.818 ± 0.35	56.6 ± 7.13	59.95 ± 3.76	9.2 ± 0.45	20 ± 2.65	2.27 ± 0.36	30 ± 3.08	10.96 ± 0.62	4.138 ± 0.16	36 ± 5.09	0.52 ± 0.28	112.1 ± 2.12
SMSNG1	99.81 ± 10.91	102.72 ± 10.80	36.44 ± 2.50	2.91 ± 0.39	69.83 ± 8.29	61.81 ± 3.42	9.36 ± 0.53	22.19 ± 1.69	2.71 ± 0.17	39 ± 5.17	10.67 ± 0.25	4.09 ± 0.07	45.36 ± 3.21	1.1 ± 0.34	68.8 ± 5.47

provided in Table 2. This higher total phenolic content may be related to greater phenylalanine ammonia-lyase enzyme activities which is accountable for the synthesis of phenolic acids, flavonoids and related free radical scavenging molecules (Pineda-Hidalgo *et al.,* 16).

Correlation analysis between the different parameters of collected samples from different region showed variable results. Correlation analysis revealed distinct groupings of traits. Fruit weight was found to be positively correlated with pulp weight, juice volume and fruit diameter, while has negatively correlated with TSS. Fruit volume was positively correlated with seed weight and peel weight, and negatively correlated with TSS. Significantly strong positive correlation was observed between peel weight and peel thickness. TSS was also negatively correlated with pH and Vitamin C. A previous study also showed an inverse relation between TSS, vitamin C and titratable acidity. pH was strongly negatively correlated with total acidity which is expected as higher the acidity level lowers the pH value. Fruit weight, pulp weight, juice volume, and fruit diameter formed a tightly correlated cluster, representing size and yield attributes. Peel-related traits showed high positive associations with pH and vitamin C, suggesting their structural and biochemical link. In contrast, TSS was negatively correlated with most physical and biochemical traits, indicating an inverse relationship between sweetness and fruit size or antioxidant content. These findings support the trait segregation observed in PCA and highlight potential trade-offs in breeding for size versus quality. Thus, this correlation study found that sweeter fruits are smaller in size and have more TSS value. The overall correlation between sample traits is given in the Table 3.

The location based comparative analysis revealed that an average data was also analyzed to find out the difference between the physical and biochemical characterization of collected fruit samples based on their location. The average physical data of the different samples is given in Table 3 and the Figure 1 is also provided to show the graphical comparison between them. Physical parameter of fruits from different location had different values. Fruit weight and pulp weight were found to be varied between 82.5 g to 120.28 g and 58.71g to 85.52g respectively with Silugoan, Mirik being the highest. In case of peel weight and number of segments, the samples from Labda, Mungpoo showed highest average value. Sample from Mirik busty, Darjeeling had shown with highest fruit volume (106.46 ml) and fruit diameter (66.07 mm), and sample of Samsing, Kalimpong showed highest number of seeds (22.19), seed weight (2.71

Table 3: Correlation of different parameters of samples.

	Fruit weight	Fruit volume	Peel weight	Peel thickness	Pulp weight	Fruit diameter	No. of segments	No. of seeds	Seed weight	Juice volume	TSS	pH	Vit C	Total acidity	Total phenol
Fruit weight	1.000														
Fruit volume	0.657	1.000													
Peel weight	0.385	0.836	1.000												
Peel thickness	0.377	0.731	0.972	1.000											
Pulp weight	0.989	0.541	0.284	0.302	1.000										
Fruit diameter	0.901	0.649	0.352	0.268	0.868	1.000									
No. of segments	0.698	0.492	0.443	0.395	0.679	0.875	1.000								
No. of seeds	-0.202	0.497	0.826	0.799	-0.304	-0.186	0.018	1.000							
Seed weight	0.556	0.988	0.884	0.787	0.434	0.529	0.397	0.610	1.000						
Juice volume	0.983	0.531	0.249	0.270	0.996	0.837	0.610	-0.334	0.427	1.000					
TSS	-0.663	-0.632	-0.776	-0.871	-0.643	-0.426	-0.454	-0.419	-0.643	-0.626	1.000				
pH	0.243	0.861	0.953	0.872	0.116	0.254	0.278	0.869	0.921	0.094	-0.585	1.000			
Vit C	0.362	0.706	0.896	0.947	0.294	0.139	0.152	0.740	0.777	0.290	-0.878	0.825	1.000		
Total acidity	0.144	-0.563	-0.473	-0.268	0.284	-0.119	-0.097	-0.595	-0.597	0.302	-0.163	-0.688	-0.172	1.000	
Total phenol	0.080	-0.250	-0.281	-0.340	0.119	0.419	0.638	-0.382	-0.350	0.052	0.326	-0.370	-0.611	0.028	1.000

g) and peel thickness with 2.91 mm. However, number of seeds and seed weight was observed minimum in Labda, Mungpoo and Sittong-1, Darjeeling which showed it better quality fruits. All the data are given in Table 4.

Biochemical analysis of fruit samples from different location did not show any similar highest pattern in any particular location. TSS or total soluble solids is one of the criteria to identify any fruit's sugar content and quality. For instance, samples from Labda, Mungpoo was found to be superior in TSS and total phenolic content with 11.3 ºBrix and 98.5 µg GAE/ml. The second highest TSS was found in the samples collected from Mirik Busty (11.26ºBrix), all the other samples had more than 10.6ºBrix. So, all the samples from different location had excellent amount of sugar content. However, the highest TSS content (12.00ºBrix) of Darjeeling mandarin from Sikkim and Kalimpong was previously reported by

Fig. 1. Representation of sample collection sites.

Table 4: Average physical properties of mandarin samples from different location.

	Silugaon, Mirik	Labda, Mungpoo	Sittong-1, Darjeeling	Mirik Busty, Darjeeling	Samsing, Kalimpong
Fruit weight (g)	120.28	105.3	82.5	104.6	99.81
Fruit volume (ml)	103.31	86.76	87.14	106.46	102.72
Peel weight (g)	30	87.26	26.03	32.53	36.44
Peel thickness (mm)	2.43	2.28	2.12	2.46	2.91
Pulp weight (g)	85.52	76.6	58.71	72.13	69.83
Fruit diameter (mm)	65.75	64.64	59.24	66.07	61.82
No. of segments	9.35	9.56	9.03	9.53	9.36
No. of seeds	16.48	16.2	18.08	19.4	22.19
Seed weight (g)	2.6	1.95	1.92	2.73	2.71

Kishore et al. (2010) and Gurung *et al.* (8). Juice quantity or juice volume, another indication of good fruit was found highest in the samples collected from Silugaon, Mirik (49.87 ml), followed by Labda, Mungpoo (42.9ml) and Mirik Busty, Darjeeling (40.33 ml), whereas the lowest juice volume was recorded in the sample of Sittong-1, Darjeeling (33.01 ml). pH, which is a criterion to evaluate acidity level of a fruit has been found to be in range of 4.03 to 4.09, which make all juices to be acidic in nature. Vitamin C or ascorbic acid is the main acid which is found in every citrus species and Darjeeling Mandarin is not an exception. It was found that the sample from Samsing, Kalimpong had highest vitamin C content (45.36 mg/100 g), followed by Silugaon, Mirik (41.6 mg/100 g), Mirik Busty, Darjeeling (39.6 mg/100 g). Overall, the higher vitamin C content in the selected samples highlights their potential nutritional advantage and may serve as superior candidates for further propagation techniques for revival aimed at enhancing the antioxidant profile and nutritional quality of Darjeeling mandarin. Besides, the presence ascorbic acid, many acids like citric, malic, oxalic, pantothenic, folic, and hydro cinnamic acids are also present in citrus species. So, total acidity of the samples was evaluated. The findings revealed that sample from Mirik Busty, Darjeeling had lower acidic value (0.9 %) followed by Sittong-1, Darjeeling (1.087%). The lower acidic value makes these sample more approachable to consumers. However, higher acidity makes the fruit sample less vulnerable towards pathogens which lead towards increased of shelf life (Dissanayake, 3). Total phenol, an additional factor which signifies the presence of polyphenols and flavonoids of fruits which reduces the oxidative stress by scavenging free radicals (Phuyal *et al.,* 15). The highest total phenolic content was found in Ladba, Mungpoo (98.5 µg GAE/ml) followed by Mirik Busty, Darjeeling (89.17 mg µg GAE/ml). Average of all the location-based data of biochemical analysis are provided in Table 5.

The location-based correlation analysis showed that all samples are significantly correlated with each other (Table 6). However, the samples from Sittong-1, Darjeeling are significantly correlated with Mirik Busty, Darjeeling, followed by Samsing, Kalimpong with Mirik Busty, Darjeeling and Silugaon, Mirik.

Principal Component Analysis (PCA) was employed to reduce data dimensionality and examine associations between fruit traits and sampling locations (Silugaon, Labda, Sittong-1, Mirik Busty, and Samsing). The PCA biplot (Fig. 2) illustrates

Table 5: Average biochemical properties of mandarin samples from different locations.

	Silugaon, Mirik	Labda, Mungpoo	Sittong-1, Darjeeling	Mirik Busty, Darjeeling	Samsing, Kalimpong
Juice Volume (ml)	49.87	42.9	33.01	40.33	39
TSS (ºBrix)	10.86	11.3	11.23	11.26	10.67
pH	4.06	4.04	4.03	4.08	4.09
Vit C (mg/100g)	41.6	37.63	36.61	39.6	45.36
Total acidity (%)	1.215	1.288	1.087	0.9	1.1
Total phenol (µg GAE/ml)	69.94	98.5	66.2	89.17	68.8

Table 6: Correlation of samples between the location.

	Silugaon, Mirik	Labda, Mungpoo	Sittong-1, Darjeeling	Mirik Busty, Darjeeling	Samsing, Kalimpong
Silugaon, Mirik	1.0000				
Labda, Mungpoo	0.9677	1.0000			
Sittong-1, Darjeeling	0.9692	0.9868	1.0000		
Mirik Busty, Darjeeling	0.9797	0.9870	0.9964	1.0000	
Samsing, Kalimpong	0.9871	0.9645	0.9850	0.9905	1.0000

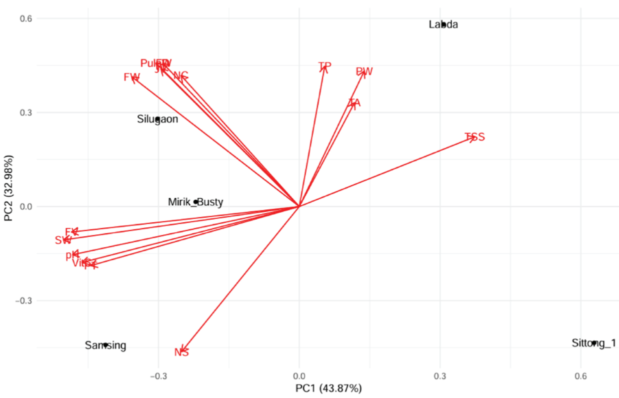

Fig. 2. Principal Component Analysis (PCA) biplot showing the distribution of five fruit sample locations based on 15 fruit morphological and biochemical traits [Fruit weight (FW), Fruit volume (FV), Peel weight (PW), Peel thickness (PT), Pulp weight (PulpW), Fruit diameter (FD), No. of segments (NC), No. of seeds (NS), Seed weight (SW), Juice volume (JV), TSS, Ph, Vit C, Total acidity (TA), Total phenol (TP)]

both sample distribution and trait loadings, revealing considerable phenotypic and biochemical variability among *Citrus reticulata* genotypes across regions. The first two principal components explained 76.85% of the total variance, with PC1 and PC2 accounting for 43.87% and 32.98%, respectively. PC1 was primarily associated with fruit size–related traits, including fruit weight, volume, pulp weight, and juice volume, resulting in a clear separation of Sittong-1, which exhibited a distinct morphological profile. In contrast, Samsing was positioned negatively along PC1, reflecting comparatively lower fruit size attributes, while Mirik Busty occupied an intermediate position. PC2 mainly captured biochemical variation, driven by total acidity, TSS, vitamin C, and total phenolic content. Labda (Mungpoo) exhibited the highest PC2 score, indicating superior biochemical and nutraceutical attributes despite moderate fruit size, suggesting the influence of a favorable microclimate on secondary metabolite synthesis. Labda and Silugaon were primarily differentiated along PC2, whereas Silugaon and Mirik Busty clustered near the center, indicating stable and balanced phenotypic expression. The direction and length of the red vectors represent the contribution and correlation of each trait to the principal components. Traits like fruit weight, fruit volume, peel weight, and seed weight cluster together, indicating positive correlations among them. Some previous studies on Citrus also showed some positive correlation among fruit weight, fruit volume, peel weight and seed weight (Yacomelo *et al.,* 19; Gaikwad *et al.,* 6). Traits such as seed number and peel thickness showed negative associations with certain yield and biochemical parameters, while shorter vectors (e.g., pH and vitamin C) contributed less to overall sample discrimination. Overall, PCA distinguished contrasting genotypic extremes, with Sittong-1 characterized by distinct morphological traits and Labda by enhanced nutraceutical quality, while Silugaon and Mirik Busty represented balanced phenotypes. These findings provide valuable insights for germplasm selection and location-specific cultivation planning for mandarins under Himalayan agro-ecological conditions.

The present study demonstrated considerable morphological and biochemical variability among Darjeeling mandarin fruits collected from different locations, indicating the influence of local agro-ecological conditions on fruit quality. Despite this variability, all samples exhibited appreciable levels of vitamin C, total phenolic content, and total soluble solids, reflecting their nutritional and commercial importance. Among the studied locations, Silugaon (Mirik) produced fruits with superior fruit weight, pulp weight, and juice volume, whereas samples from the Mungpoo region, particularly Labda, showed better performance for peel weight, number of segments, seed number, total soluble solids, and total phenolic content. Principal component analysis further identified location-specific superiority and highlighted the potential for selecting elite germplasm. Overall, these findings provide useful information for germplasm selection, propagation, and location-specific cultivation strategies to improve fruit quality, support sustainable mandarin production, and enhance the cultivation and export potential of Darjeeling mandarin in the Himalayan region.

AUTHORS' CONTRIBUTION

Conceptualization and designing of experiment (SS, AG and NG); Lab experiments and data collection (SS, AG, WT and NG); data interpretation and preparation of manuscript (SS, AG, SM and WT), statistical analysis (SS, AG and SM).

DECLARATION

The authors declare that they don't have any conflict of interest.

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