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Classification of ripening period and development of colour grade chart for Neelam mangoes using multivariate cluster analysis

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ABSTRACT

A study was carried out for scientific classification of ripening period of mango into different stages and develop a colour grade chart. The physico-chemical properties (TSS, titrable acidity), external and internal colour values and textural characteristic (peel strength, stiffness and flesh firmness) were measured throughout the ripening period. PCA along with Hierarchical clustering with Ward method were used in this study and the ripening period of mango fruits were classified into five stages, *viz.* unripe, early ripe, partially ripe, ripe and over ripe. Based on the ripening stages, a colour grade chart was developed for Neelam mango along with physico-chemical, textural and colour values during five stages. The developed colour grade chart can be used as a useful rapid, non-destructive grading method for Indian mangoes at pack houses and pulping industries.

Key words: Colour grade chart, Neelam mangoes, ripening period.

INTRODUCTION

Scientific classification of ripening period is essential for determining the optimal post harvest strategies for handling and marketing of the fruits and for developing colour chart. Very limited scientific reports are available about classification of different stages of ripening period in case of tropical fruits. Von Loesecke (18) described the different stages of ripening with related to pigment changes within the peel of banana. The commercial colour grade charts for banana and mango like SH Pratt & Co., Chiquita® and Splendid products™ are available and purely based on external peel colour (Tapre and Jain, 16; Boudhrioua *et al.*, 3). These colour charts were developed purely based on the external appearance, while internal changes were not considered.

Mango (*Mangifera indica* L.) is an important tropical fruit having huge demand in world market. In 2013-14, India exported around 42 thousand tonnes of fresh mangoes and 1.75 lakh tonnes of mango pulp, which is valued to Rs. 1,000 crores (Anon, 2). Presently, mango fruits are graded with the localized empirical knowledge by external peel colour. Mango packhouses require an easy non-destructive tool to grade the fruits based on ripening before packaging. The pulping industry needs it to select the fruits at optimum ripening stage to get desired pulp characteristics. Colour grade chart would fulfil the above purposes, but at the same time it should contain the information about quality parameters like physico-chemical properties and textural properties.

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Vásquez-Caicedo *et al.* (17) proposed a ripening index (RPI). Changes in colour were not considered in RPI. Joas *et al.* (7) proposed ripening class index (Rci) based on respiration rate. The Rci does not account the changes in internal and external quality parameters. These characterizations and classifications may not be suitable for commercial colour grade chart preparation. Scientific reports on ripening index or the classification of ripening stages based on physico chemical, colour and textural characteristics of mangoes are scanty and hence this study was carried out to classify the ripening period of mango into different stages and develop colour grade chart for Neelam mangoes.

MATERIALS AND METHODS

Raw Neelam mangoes were collected from different locations, *viz*. Tamil Nadu Agricultural University orchard, Coimbatore, and from farmer's orchards in Krishnagiri and Theni Districts of Tamil Nadu at 20 day interval in order to include the zonal variations in the results. The matured mangoes (100-105 DFFB) were harvested manually in the early morning and desapped meticulously. Desapped mangoes were transported to the laboratory on the same day. The mangoes after washing and shade drying for 30 min. were treated with ethylene at 200 ppm for 24 h at 20°C with 85% RH in the ripening chamber. After the treatment, the treated mangoes were kept in the chamber (at 20°C with 85% RH) for regular analysis. Two batches of mangoes were made, one set for imaging and another one for physico-chemical analysis. The set of mangoes used for imaging were kept in the chamber throughout the study period. From the second set, three fruits were taken randomly for physio-chemical analysis every day. The experiments were continued till the decay of fruit. The decay stage was decided by visual inspection when 50 per cent of the fruits reached the spoilage.

Homogenized mango pulp was obtained with laboratory mixer to determine TSS, pH and titrable acidity as suggested by Vásquez-Caicedo *et al.* (17). Every measurement was taken thrice on each fruit, and the average value was used. The textural characteristics of the mangoes were measured using the Texture Analyzer (TA-HDi, Stable Micro Systems, UK) by the method proposed by Camps (8) 4 mm cylindrical probe (P/4) was used with 1 $mm.s^{-1}$ pre-test speed, 0.1 $mm.s^{-1}$ test speed, 1 $mm.s^{-1}$ post-test speed, and 10 mm penetration depth to obtain force displacement curve. Textural values were measured at three points in a mango and the average value was used. Colour coordinates were recorded both for external (Le*, ae*, be*) and internal (L_i*, a_i*, b_i*) surface of the mango using Hunter LAB colour meter (Hunter Associates Laboratory, Inc. USA).

Initially ANOVA was carried out to check the variability between zones. Then all the observed data were analyzed with Pearson correlation to find out the correlation between variables. Then PCA was carried out to predict the variability. Hierarchical clustering analysis using Ward method was carried out in order to group the whole data into 5 stages of ripening. All the statistical analysis was carried out in JMP (SAS Institute Inc., USA).

RESULTS AND DISCUSSION

The decay stage for Neelam mangoes reached on 22nd day. The results showed that, the changes in physico-chemical, textural and colour properties between the different zones were highly non-significant (P>0.40) during ripening. First 10 days of ripening period had contributed for the major changes in all quality parameters, hence it could be perceived that 75 per cent of ripening had reached within 10 days (Fig. 1-4). Then the remaining ripening period did not have significant influence on most of the physico-chemical and textural properties.

Changes in total soluble solids in Neelam fruits during ripening is shown in the Fig. 1. The TSS was continuously increasing up to 17th day from 13.3 ± 1.3 to 24.1 ± 0.67°Brix. Increase of TSS could mainly be due to hydrolysis of starch into soluble sugars such as sucrose, glucose and fructose (Agravante and Kitagawa, 1; Cordenunsi and Lajolo, 5) After 17th day, decrease in TSS was observed till 22nd day (Fig. 1). It could be inferred that the decay of fruit or over ripening caused the reduction in TSS.

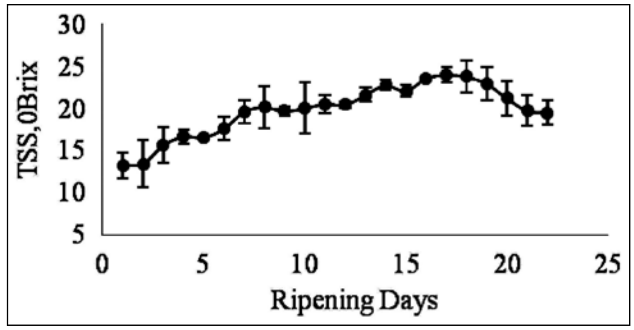

Fig. 1. Changes in TSS during ripening of Neelam mangoes.

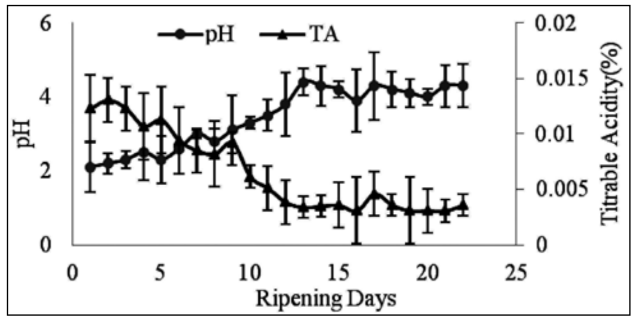

Fig. 2. Changes in pH and Titrable acidity during ripening of Neelam mangoes.

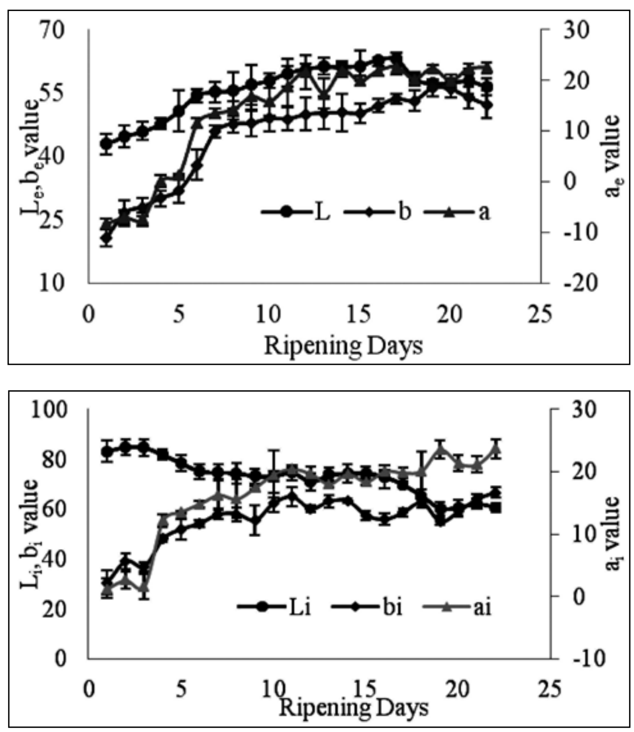

Fig. 3. Changes in external and internal colour during ripening of Neelam mangoes.

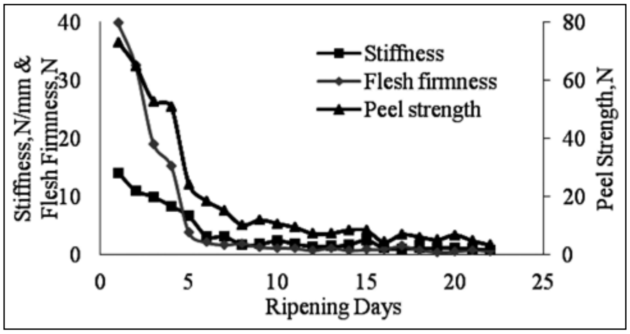

Fig. 4. Changes in textural characteristics during ripening of Neelam mangoes.

Similar to TSS, the pH was linearly increasing up to 13th day from 2.1 ± 0.67 to 4.4 ± 0.8. After that not much changes was observed (Fig. 3). But contractedly titrable acidity (TA) was decreasing up to 13th day from 0.01 to 0.003 g/100 g of sample and slight increase in TA was found on the last day (22nd day) of ripening. During ripening, chlorophyll present in peel starts degrade, which subsequently revealed the yellow carotenoid pigments (Marriott and Lancaster, 8; Seymour *et al.*, 14; Rathore *et al.*, 10). These changes causes significant increase or decrease in *L*, *a* and *b* colour coordinates during ripening. The changes in internal (L_i*, a_i*and b_i*) and external (L_e*, a_e*and b_e*) colour values are shown in L_e* value was increasing up to 17th day from 42.9 ± 1.6 to 63.04 ± 2.5. Thereafter a decreasing trend was observed till last day. The external a_e* and b_e* values were increasing from -8.26 ± 2.23 to 22.5 ± 4.5 and 20.66 ± 0.54 to 56.32 ± 2.3, respectively. Similar to L_e*, after 19th day the be* started decreasing till last. Similar trends were reported either or all of the *L*, *a* and *b* coordinates during ripening in banana (Boudhrioua *et al.*, 3; Shahir and Visvanathan, 15; Salvador *et al.*, 12), in Dashehari mango (Rathore *et al.*, 10; Jha *et al.*, 6), and in guava (Reyes and Paull, 11). At the same time, different trends were observed in internal colour change during ripening. The a_i and b_i values were increasing and the L_i values were decreasing till end of ripening (Fig. 3). L_i* value decreased from 83.27 ± 2.5 to 60.55 ± 1.9. Major changes in a_i and b_i values was observed between 5th to 10th day of ripening. This may be due to the acceleration in ripening of mango in the 2nd week. a_i* and b_i* values increased from 1.35 ± 1.4 to 23.71 ± 1.78 and 30.51 ± 2.5 to 66.78 ± 1.98, respectively.

Textural characteristics, *viz.* peel strength, fruit stiffness and flesh firmness were extracted from the graphs and the changes in the textural characteristics during ripening were presented in Fig. 4. Decreasing trend was found in all the three parameters during ripening (Fig. 4). The reduction in fruit firmness was due to alteration in cell wall structure by degrading enzymes (*e.g.* polyglacteronase) and also by degradation of starch (Seymour, 14) and breakdown of starch, cellulose and hemicellulose (Salunkhe and Kadam, 13).

During first week of ripening, drastic reduction was observed in all the three parameters and after that, not many changes were observed in textural parameters. The decreasing trend was observed up to 8th day. The maximum and minimum peel strength of raw and ripe mango was 73.3 ± 1.3 N and 2.20 ± 0.25, respectively. Drastic reduction was observed in stiffness up to 8th day and after 8th day no significant changes in stiffness was observed. Raw mangoes exhibited higher stiffness of 14.14 ± 0.49 N and minimum stiffness was observed in ripe mango and the values was 0.7 ± 0.03 N. Maximum reduction was observed in flesh firmness in the first 5 days. Raw mangoes had higher flesh firmness of 39.91 ± 5.16 N and the final flesh firmness was recorded as 0.57 ± 0.3 N.

From the Pearson correlation matrix (Table. 1) it can be observed that the ripening period (RD) had either highly negative or positive correlation with the quality parameters. Further exploration of Table 1 leads to conclusion that colour coordinates, TSS and pH were inversely correlated with textural characteristics and acidity. From correlation study, it could be observed that all the measured variables were correlated each other and they were well redundant upon the ripening process. Hence, PCA would be best suitable for further analysis. It was noticed from the PCA results that PC1 explained about 92 per cent of total variations occurred during ripening of Neelam mangoes. PC2 explained about 4 per cent of total variations occurring during ripening. From these PCA results, it could be concluded that two principal components (PC1 and PC2) were sufficient to explain about more than 96 per cent of the total variations during ripening. Two plots *viz.* score graph and loading graph were obtained from PCA results and shown in Fig. 5. The total variations occurred during the ripening period were explained in PCA score graph (Fig. 5a). The loading plot, displays information about the variables in the PCA model. Since the internal and external colour coordinates were found dispersed over the PCA loading graph, both PC1 and PC2 had important role to explain the variation in colour values, at the same time, PC2 explains more about the colour values. PC1 was sufficient to explain variation in TSS and acidity during ripening. The textural characteristics were encircled and shown in PCA loading graphs (Fig. 5b).

Then the ripening period was classified into five clusters using hierarchical clustering with Ward method which was encircled and denoted in alphabets in Fig. 5a. Those five clusters represents five stages, *viz.*

Table 1. Pearson's correlation matrix of quality parameters for Neelam mangoes.

Parameter	*RD*	L_e*	a_e*	b_e*	L_i*	a_i*	b_i*	Acidity	pH	TSS	Peel strength	Stiffness	Pulp firmness
RD	1.000												
L_e*	0.749	1.000											
a_e*	0.872	0.932	1.000										
b_e*	0.891	0.913	0.952	1.000									
L_i*	-0.926	-0.624	-0.803	-0.850	1.000								
a_i*	0.822	0.821	0.926	0.895	-0.825	1.000							
b_i*	0.747	0.845	0.905	0.863	-0.707	0.940	1.000						
Acidity	-0.908	-0.884	-0.943	-0.918	0.792	-0.857	-0.816	1.000					
pH	0.930	0.858	0.904	0.896	-0.784	0.818	0.784	-0.960	1.000				
TSS	0.792	0.929	0.903	0.917	-0.678	0.804	0.765	-0.891	0.867	1.000			
Peel strength	-0.796	-0.926	-0.934	-0.946	0.777	-0.911	-0.909	0.840	-0.803	-0.876	1.000		
Stiffness	-0.801	-0.924	-0.938	-0.961	0.784	-0.907	-0.910	0.844	-0.812	-0.882	0.986	1.000	
Pulp firmness	-0.676	-0.856	-0.835	-0.858	0.671	-0.850	-0.864	0.723	-0.681	-0.808	0.962	0.941	1.000

RD = ripening days, subscript e = external, subscript, I = internal

unripe, early ripe, partially ripe, ripe, over ripe/decay during ripening. The first two stages were grouped as pre-climacteric phase, second two stages as climacteric and the last stage as senescence phase. First three days were grouped as a first stage then 4th and 5th days as a second stage then 6 to 9th day as third stage. The period from 10th to 17th day were designated as fourth stage. Then the fifth stage was grouped from 18th day to 22nd day. The second stage existed to short stint (only for two days). The fourth stage was lengthier stage from 10th to 17th day of ripening period. 3rd and 4th stage had longer period (8-10 days) out of five stages. The decay or over ripen stage existed for 5 days. The colour grade chart was prepared based on the five clusters with five ripening stages and shown in Fig. 6.

Images of mangoes of each stage were given in colour chart along with the corresponding ripening period. In addition to this information, internal and external quality parameters for the corresponding ripening stage were given in the Table 2.

Table 2. Physico-chemical, textural and colour values for different ripening stages of Neelam mangoes (mean ± SD).

Phase	Pre-climacteric		Climacteric		Senescence
Stage	Unripe	Early ripe	Partially ripe	Ripe	Over ripe
Ripening period (days)	1-6	7-8	9-14	1-6	7-8
Parameter / Stage	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
L_e*	44.51 ± 1.52	49.22 ± 2.16	55.54 ± 1.04	61.00 ± 1.63	57.44 ± 0.70
a_e*	-7.56 ± 0.63	0.79 ± 0.86	11.44 ± 3.33	19.97 ± 2.44	21.03 ± 1.97
b_e*	25.05 ± 3.86	30.99 ± 1.25	44.84 ± 4.66	50.54 ± 1.59	54.30 ± 1.70
L_i*	84.33 ± 0.92	80.09 ± 2.39	74.40 ± 0.83	73.03 ± 1.73	61.78 ± 2.10
a_i*	1.88 ± 0.68	12.89 ± 0.98	15.09 ± 1.21	18.62 ± 2.00	21.58 ± 1.97
b_i*	35.53 ± 4.48	50.07 ± 2.71	54.97 ± 4.01	60.79 ± 3.25	61.53 ± 4.57
TSS	14.17 ± 1.33	16.65 ± 0.07	19.33 ± 1.14	21.96 ± 1.50	21.51 ± 1.91
Acidity	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.001	0.009 ± 0.001	0.008 ± 0.001
Peel strength	63.73 ± 10.31	37.68 ± 18.89	14.04 ± 3.64	7.91 ± 1.88	5.28 ± 1.35
Stiffness	11.73 ± 2.15	7.54 ± 1.21	2.49 ± 0.81	1.73 ± 0.54	1.10 ± 0.18
Pulp firmness	29.28 ± 12.61	11.51 ± 10.84	1.84 ± 0.35	1.09 ± 0.25	0.70 ± 0.16

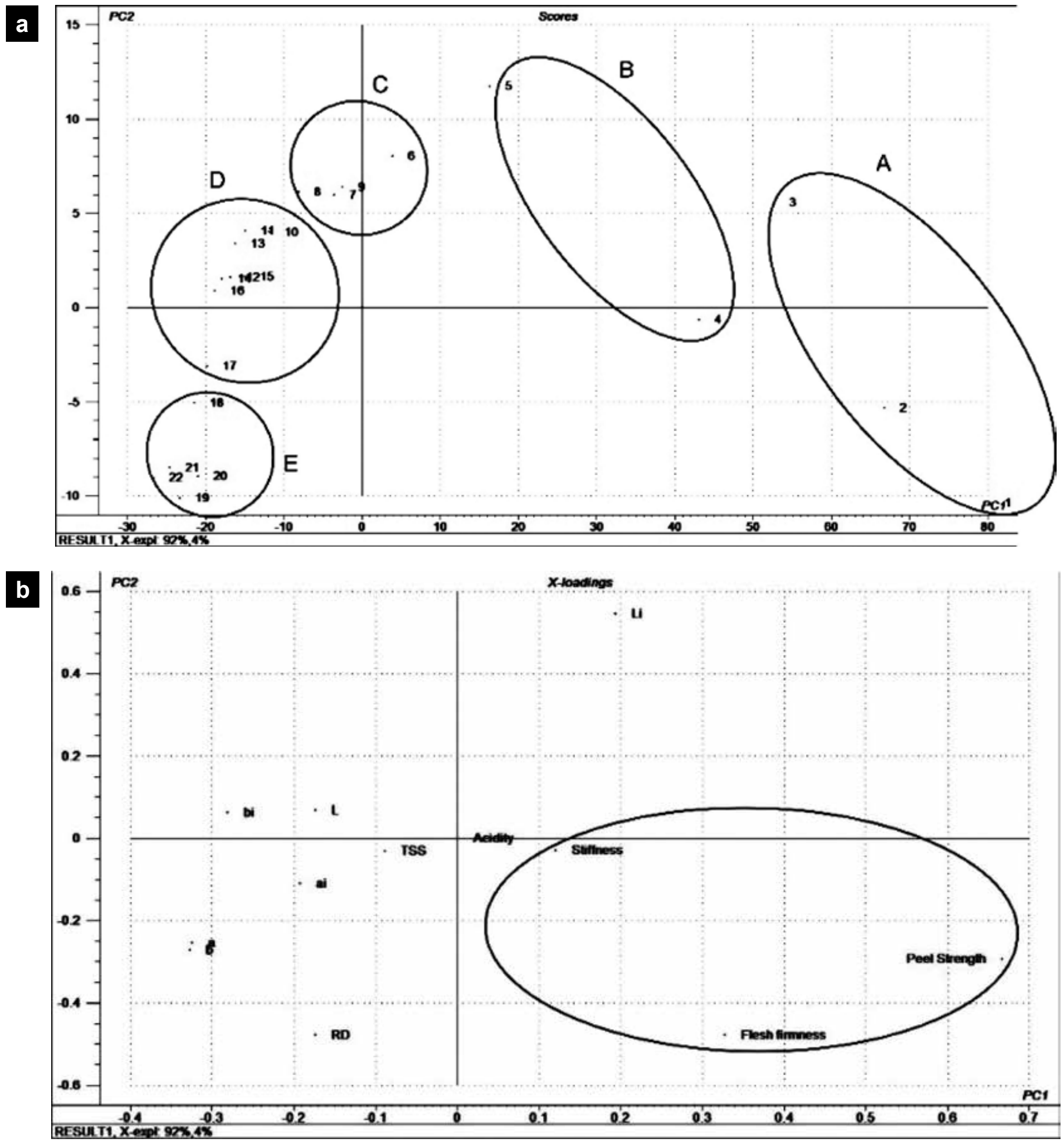

Fig. 5. PCA graphs for Neelam mangoes during ripening. a. Scores graph and b. Loading graph.

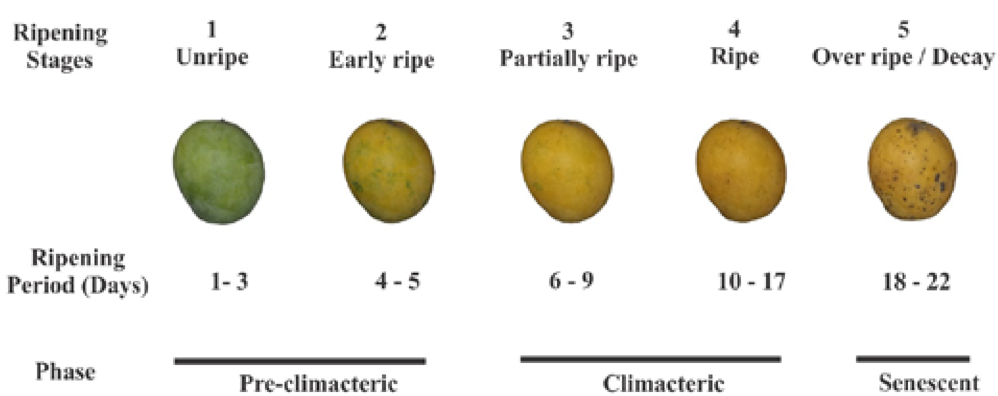

Fig. 6. Different ripening stages and colour chart for Neelam mangoes.

A scientific colour grade chart was developed with five stages, *viz.* unripe, early ripe, partially ripe, ripe, over ripe/ decay in Neelam mango using PCA and hierarchal cluster analysis. For the export of fresh whole mango, the stage at which the fruits to be packed may be decided according to its transit period. The first and second stage would be more suitable for long and third stage would be suitable of short distance. For the pulping industry, the fourth stage would be more suitable to get good quality finished product.

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