Indian Journal of Experimental Biology
Vol. 60, April 2022, pp. 263-268

Potential of X-ray imaging to detect citrus granulation in different cultivars with progress in harvesting time

Swarajya Laxmi Nayak[1], Shruti Sethi[1]*, Rabi Narayan Sahoo[2], Anil Kumar Dubey[3] & Swati Goel[4]

[1]Division of Food Science and Postharvest Technology; [2]Division of Agricultural Physics; [3]Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute (IARI), New Delhi-110 012, India
[4]RMSI Pvt. Ltd., Noida-201301, Uttar Pradesh, India

Received 17 February 2021; revised 04 February 2022

Granulation, a physiological disorder of citrus is manifested by shriveled juice sacs and internal dryness. Extractable juice in granulated tissue is drastically reduced as a consequence of gelatinization and secondary epidermis formation. Since, the defect cannot be detected externally it leads to consumer dissatisfaction and poor returns to farmers. Processing industry also faces huge economic loss due to reduction in the juice recovery from granulated fruit. In this context, here, we studied the possibility of developing an image processing algorithm through MATLAB software to detect granulation with advancement of maturity via X-ray micrographs. Fruit of eight citrus cultivars comprising of granulation susceptible and tolerant varieties harvested at four different intervals were exposed to X-rays. Voltage of 46 kV and current of 6.5 mA given to fruit for an exposure time of 320 mAs gave the best X-ray image contrasts. The developed algorithm could effectively distinguish the healthy and granulated fruit with an accuracy of 90% as validated by subsequent destructive analysis when estimated for four different harvesting dates. The imaging technique can be employed by the processors to determine the severity of granulation and to sort out fruit online which will help in saving economic losses.

Keywords: *Citrus sinensis*, Gelatinization, On-tree storage, Sweet orange

Citrus (Fam. Rutaceae) is one of the popular and extensively grown fruit crops in tropical and subtropical regions of the world. It is considered as the third most important fruit crop in India after mango and banana with a current production of 14.56 million MT for an area of 1075 thousand ha[1]. It possesses a distinct aroma and is savoured for its taste and abundant amount of nutrients[2,3]. Granulation is an important physiological disorder causing havoc in the whole citrus industry, affecting most of the cultivars, resulting in huge economic loss to the farmers as well as the processors[4]. The disorder is accompanied by reduction in nutritive value, loss of taste, deterioration of several organic acids and enzymes for which the relevant mechanism is still obscure. Granulation commences at the stem end region and then gradually extends towards the stylar end of the citrus fruit[5]. It significantly impairs the sugar/acid balance as a consequence of which the fruit become organoleptically unacceptable. Farmers favour to prolong the on-tree storage of fruit for staggered marketing[6]. The disorder cannot be detected until the fruit are peeled or cut open. Hence, the grower is not able to decide the on-tree-storage of fruit for extended marketable period. Moreover, defective fruit may be passed on to the consumers leading to distrust. It also hampers the processing line as the yield of juice is significantly reduced and may be a cause of economic loss in business[7].

Diversified techniques are being employed by various researchers worldwide to detect granulation through non-destructive techniques such as hyperspectral imaging[8], Visible/Near-Infrared Spectroscopy[9,10] and Beam Scanning Reflectometer[11] to sort out the low-quality granulated citrus fruit. X-ray imaging is also one such non-destructive technique that can be used to detect granulation. It is one of the most promising techniques to evaluate quality of fruit without damaging them and is comparatively cheaper than other major subsurface imaging techniques[12,13]. X-rays are electromagnetic radiations (shorter than ultraviolet and microwaves) with wavelength ranging between 10 and 0.01 nm. When X-rays are passed through an object, they physically interact with the object producing an X-ray radiograph in a 2D plane. When citrus fruit are exposed to radiation, X-rays pass through dense and

*Correspondence:
E-Mail: docsethi@gmail.com

lighter areas of citrus fruit pulp to reach the X-ray-sensitive film and deliver images/micrographs of the fruit. Application of X-ray on food products has been proven safe with respect to human safety and nutritional quality of irradiated food[14]. Scanty work is available for detection of granulation disorder in citrus using X-ray imaging technique. Considering the widespread nature of this disorder across citrus species, in this study, we investigated eight citrus cultivars at four different harvesting stages using X-ray imaging to check their susceptibility to the disorder and to determine the stage of onset of granulation in a particular cultivar. We developed an algorithm to avoid the subsequent labour-intensive destructive sampling while preventing accompanied losses.

Materials and Methods

Procurement of fruit

Eight commonly cultivated genotypes of citrus (Sweet orange: 'Itaborai', 'Hamlin', 'Mosambi'; Grapefruit: 'Duncan', 'Marsh Seedless'; Pummelo: 'Pummelo Red Flesh', 'Pummelo White Flesh' and Tangerine: 'Dancy') were harvested from ICAR-Indian Agricultural Research Institute, New Delhi, India. Fruit were harvested from healthy trees budded on *Jatti Khatti* rootstock at four different harvesting dates, starting from the date at which they obtain the recommended Total Soluble Solids (TSS): acid ratio (≥ 12)[15] except for grapefruit where TSS: acidity ratio is ≥10 followed by three harvests at an interval of 20 days each. Thus, four harvesting stages were defined as; Stage I: Optimum stage of harvest; Stage II: Stage I + 20 days; Stage III: Stage I + 40 days; Stage IV: Stage I + 60 days. The fruit were exposed to optimized X-ray radiation and then destructive analysis was done by cutting the fruit transversely to validate the X-ray images obtained.

Exposure to X-ray

Citrus fruit were exposed to X-rays, using 'Heliophos D/ Klinoskop-H/Siemens Ltd, Japan X-ray machine at Dr. Doda's Diagonostic and Health Care, Pusa Road, New Delhi. Different combinations of voltage, current and time in the range of 30 to 60 kV, 4 mA to 8 mA, 100 to 400 mAs, respectively were used in a trial and error manner to develop good contrast X-ray micrographs. For each exposure, fruits were placed on one frame for X-ray scanning. The information was generated in the form of digital grey scale JPEG images from sensitive elements (Fig. 1 C and D).

Detection algorithm

The X-ray scanned images of eight citrus cultivars harvested at different stages were then analyzed by MATLAB R2017b (The Mathworks, Inc. US) and an algorithm was developed to differentiate healthy and granulated fruit. MATLAB, a proprietary multi-paradigm programming language and numeric computing environment, is a useful tool for digital image processing since it represents images as matrices and allows easy matrix manipulation[16]. An Images Processing Toolbox offers a comprehensive set of reference-standard algorithms and workflow apps for digital image processing, analysis, visualization, and algorithm development[17]. The algorithm we developed to segregate healthy fruit from granulates ones.

When the X-ray images were processed using the developed algorithm, it gave a clear pattern of differentiated areas, that defined granulated areas in the fruit. The processed images of healthy fruit showed no dots whereas granulated portion showed a dotted pattern. The combination of both prewitt operator and range filter gave differentially pooled values of percentage edge threshold which clearly classified healthy and granulated portions within the fruit. Subsequently, the fruit were examined for granulation incidence after cutting to validate the data obtained through non-destructive technique. On the X-ray radiographs, granulation was visible as dark regions in the fruit flesh, as the granulated region has comparatively lighter density than the healthy region. Furthermore, the rind of different citrus cultivars was excluded by using ENVI software (Environment for

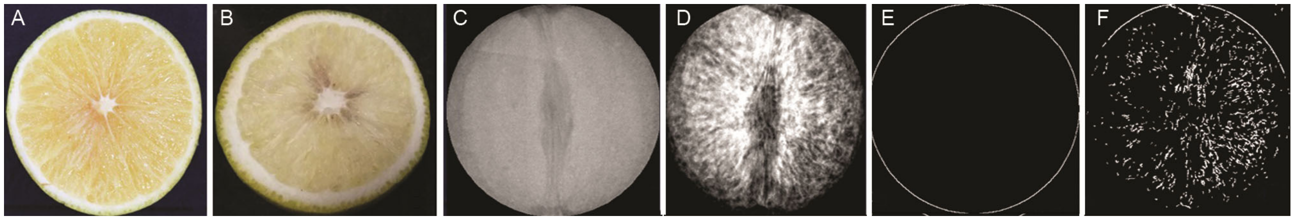

Fig. 1 — Healthy (A, C & E) and granulated (B, D & F) Citrus fruit. (A and B) Transversely cut opened; (C and D) X-ray image processed through ENVI software; and (E and F) Image processed through MATLAB software.

Visualizing Images) to get a uniform size image. The healthy tissue was easily segmented from the granulated tissues non-destructively by applying a fixed grey value threshold on the image. Furthermore, the total tissue (affected + healthy tissue) was estimated by identifying the bounding ellipse of the tissue, excluding the rind and albedo. The threshold value of the fruit was determined by using the following equation[18]:

$$\text{Threshold value} = \frac{\text{No. of white pixel}}{\text{Total no. of pixel}} \times 100$$

Granulation index (GI)

The method of Sharma et al[19] was followed to determine the GI of the fruit. The fruit of each cultivar exposed to X-rays were hand peeled and transversely cut in the laboratory. The individual fingers were observed for portion affected by granulation to calculate the GI using the following formula:

$$\text{Granulation index (destructive)} = \frac{\text{Granulated portion}}{\text{Total fruit}} \times 100$$

Results and Discussion

Exposure to X-ray

Penetration and attenuation of X-rays depends on composition, density and thickness of the material[20]. Depending on the energy received by the sensor from X-rays, each pixel transmits a discrete value indicating the quantity of energy received. If the X-rays cross a non-dense area, the pixel receives a high quantity of energy, which produces a strong signal, whereas if the X-rays cross a dense region, the pixel receives a low level of energy, which is converted into a weak signal[21]. Of the different combinations of voltage, current and time of X-ray exposure tried, the best X-ray images were developed when citrus fruit were exposed to a voltage of 46 kV, current 6.5 mA, and an exposure time of 320 mAs. This X-ray condition was kept constant for all the cultivars studied. When the fruits were exposed to X-rays, the rays traversed through the dense and non-dense regions within the fruit before reaching the X-ray sensitive film to generate the micrographs. The X-ray upon passing through a healthy fruit generated a micrograph with an entire light region (Fig. 1C). In contrast, X-ray micrographs of granulated fruit (Fig. 1D) revealed discrete light regions (indicating healthy) and dark regions (indicating granulated portion). The cultivars which are prone to granulation displayed dark regions in the X-ray image in the fruit indicating the sclerification of juice sacs. The X-ray images of healthy and granulated fruits were then processed through MATLAB software to yield images as shown in Figs 1 E and F, respectively wherein the dark region depicted the healthy portion of fruit while the white dotted region signified the granulation.

Fig. 2 gives the MATLAB generated images of the eight citrus genotypes harvested at four different stages. As evident, grapefruit and pummelo were less affected to granulation disorder even after prolonged on-tree storage extended upto a month. Sweet orange cultivars, 'Itaborai', 'Hamlin' and 'Mosambi' and the tangerine ('Dancy') were affected by granulation when harvested after 40 days (stage III) from their optimum harvest time. Since, all cultivars were budded on the same rootstock and were cultivated following same horticultural practices, such variation in incidence of granulation may be due to the genetic variability. When we ran the algorithm, it clearly differentiated between different stages of granulation based on the extent of granulation in the fruit. The threshold value (health score) of <2.7 (Table 1), indicated the fruit to be healthy which is also evident from the micrographs. Furthermore, if the threshold value ranged between 2.7 to 6.0, the particular fruit can be rated as partially granulated and/if the value goes beyond 6.0, then the fruit is considered to be highly granulated. These interpretations were highly correlated with the visual observations obtained after destructive evaluation. Sweet orange cultivars such as 'Itaborai', 'Hamlin', 'Mosambi' and the 'Dancy' tangerine showed more than 20 % granulation on the stage III and $\geq$50% on the stage IV of harvest. Thus, these cultivars can be considered as partial and fully granulated, respectively through non-destructive X-ray imaging technique. Similar observations were made when the fruits were cut and examined. Further, few citrus genotypes such as 'Marsh Seedless' grapefruit and 'Pummelo Red Flesh' and 'Pummelo White Flesh' showed no granulated tissue and were considered as least affected (tolerant) varieties as these recorded threshold values of <2.7, irrespective of the harvesting stage. Thus, the suggested algorithm can be effectively applied in packaging line to discard the granulated fruit and screen out healthy, juicy fruit for beverage industry. The developed algorithm could effectively distinguish the healthy and granulated fruit with an accuracy of 90% as validated by subsequent destructive analysis when calculated for different citrus cultivars at four different harvesting dates.

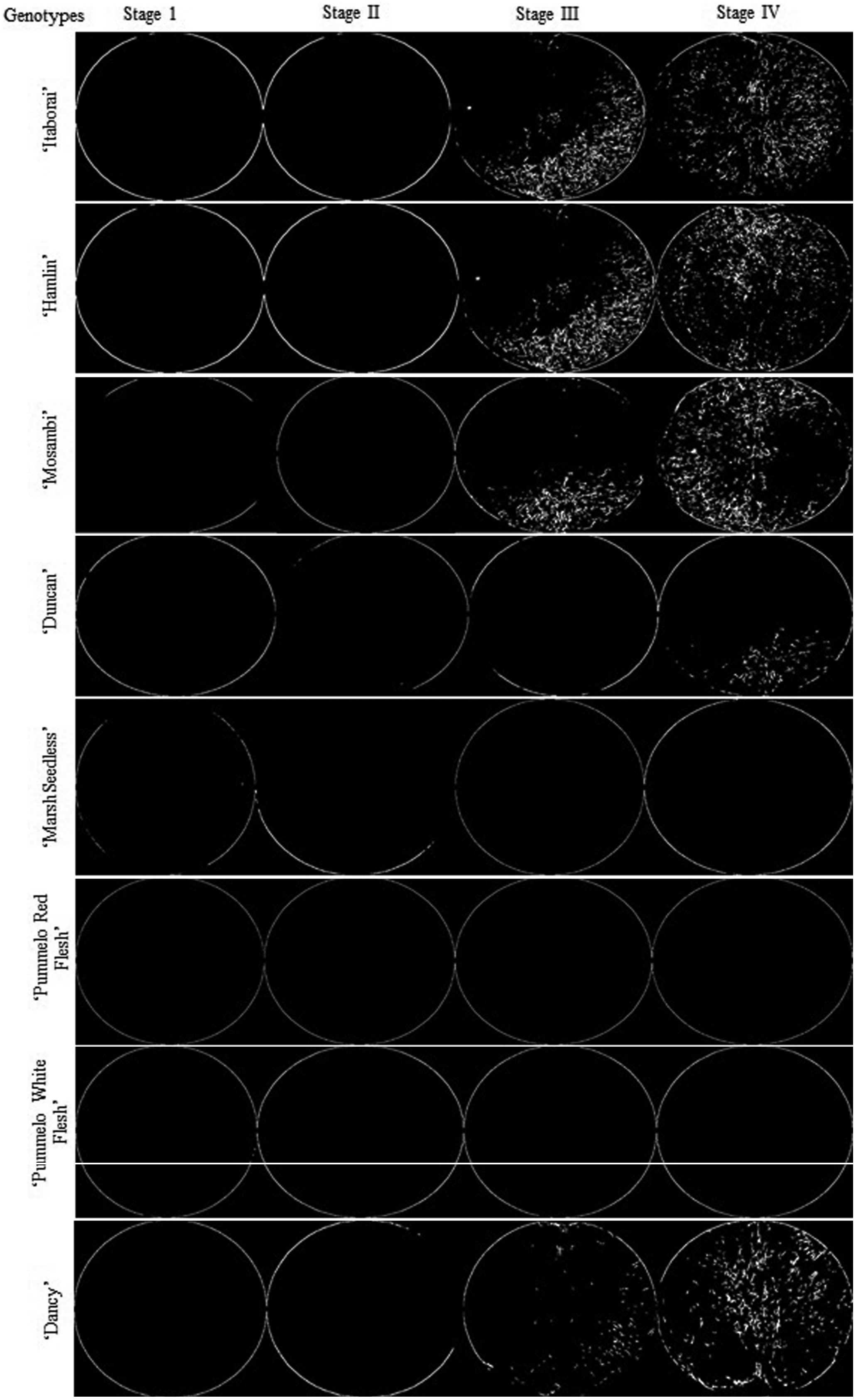

Fig. 2 — Images of fruits of citrus genotypes during different harvesting intervals, processed through MATLAB software

Table 1 — Granulation index of different citrus genotypes and their corresponding threshold value

Genotype/Stage	I		II		III		IV	
	Granulation index*	Threshold value**	Granulation index	Threshold value	Granulation index	Threshold value	Granulation index	Threshold value
'Itaborai'	0	2.44	8	2.53	45	3.74	60	8.77
'Hamlin'	0	2.26	0	2.31	35	3.27	56	10.26
'Mosambi'	0	0.61	9	1.65	41	5.82	58	12.38
'Dancy'	0	1.82	9	1.79	43	2.72	59	9.01
'Marsh Seedless'	0	0.89	0	0.91	0	1.76	16	1.31
'Duncan'	0	1.95	0	1.84	0	1.18	21	2.83
'Pummelo White Flesh'	0	1.56	0	1.30	0	1.64	6	1.86
'Pummelo Red Flesh'	0	1.24	0	1.11	0	1.28	4	1.38

[*As measured by destructive analysis; and **As obtained by MATLAB software]

Granulation index

Table 1 summarizes the granulation index of the eight citrus genotypes with respect to their stage of harvest and their corresponding threshold values obtained through MATLAB R2017b software. The data clearly show that the citrus genotypes viz., sweet orange and tangerine were susceptible to granulation whereas pummelo and grapefruit genotypes were less affected (tolerant) to this malady. Cultivars 'Itaborai', 'Mosambi' and 'Dancy' showed incidence of granulation at stage II of harvest (i.e., 20 days later to optimum harvest date) whereas 'Pummelo White Flesh' and 'Pummelo Red Flesh' and 'Marsh Seedless' grapefruit were least affected by granulation even at the stage IV of harvest (i.e., 60 days later to optimum harvest date). The variability among the studied cultivars for degree of granulation might be due to genetic differences[22]. Interestingly, the literature on citrus granulation reveals that in general, all sweet orange cultivars are extremely susceptible to granulation than grapefruit and pummelo[19,20]. This confirms that non-destructive determination of granulation through X-ray technique is a promising tool to detect the disorder. Furthermore, our study also indicated that sweet orange cultivars such as 'Itaborai', 'Mosambi', 'Hamlin' and 'Dancy' tangerine can only be stored for up to 20 days on-the-tree. In contrast, prolonged on-tree storage is possible for 'Pummelo White Flesh' and 'Pummelo Red Flesh' and grapefruit 'Marsh Seedless' and 'Duncan' as these are least affected by granulation. This study has practical application as well because farmers prefer to harvest citrus fruit in a staggered manner so as to prolong their marketing period. Storage space constraints and lack of proper infrastructure further add to the woes of the farmer which can be overcome by delayed harvesting.

Conclusion

Granulation is an internal citrus fruit disorder that cannot be detected through external appearance of the fruit. The X-ray imaging technique used in this study was able to detect the incidence of granulation non-destructively in different citrus cultivars. The best contrast X-ray images could be developed with a voltage of 46 kV, current 6.5 mA, and an exposure time of 320 mAs. The algorithm developed could successfully differentiate granulated fruit from healthy ones. X-ray micrographs revealed that onset of granulation was dependent upon the time of harvest and the cultivar suggesting that sweet orange and tangerine should be timely harvested by farmers. . The non-destructive prior evaluation will help to authentically determine the fruit quality in industries for the end user thus, preventing huge losses to the citrus processing industry. This method is fast, robust and has the advantage of processing large quantities of fruit through X-ray based systems at one time. Further, it can be applied to any existing inline X-ray radiograph equipment.

Acknowledgment

We thank Dr. Doda's Diagnostics and Healthcare, New Delhi for providing the X-ray facility.

Conflict of interest

Authors declare no competing interests.

References

1 Area and Production of Horticulture Crops. Department of Agriculture and Farmers' Welfare agricoop.nic.in/sites/default/files/2019-20282nd20Advance 20Estimates291.pdf.

2 Kalal P, Sharma RM, Dubey AK, Kamil D, Lekshmy S, Kumar A & Awasthi OP, Tolerance mechanism in hybrid citrus rootstock progenies against *Phytophthora nicotianae* Breda de Haan. *Indian J Exp Biol*, 59 (2021) 202.

3 Chhikara N, Kour R, Jaglan S, Gupta P, Gat Y & Panghal A, *Citrus medica*: nutritional, phytochemical composition and health benefits–a review. *Food Funct*, 9 (2018) 1978.

4 Li Q, Yao S, Deng L & Zeng K, Changes in biochemical properties and pectin nanostructures of juice sacs during the granulation process of pomelo fruit (*Citrus grandis*). *Food Chem*, 376 (2022) 131876.

5 Yao S, Cao Q, Xie J, Deng L & Zeng K, Alteration of sugar and organic acid metabolism in postharvest granulation of Ponkan fruit revealed by transcriptome profiling. *Postharvest Biol Technol*, 139 (2018) 2.
6 De-Miguel MD, Caballero P & Fernández-Zamudio MA, Varietal change dominates adoption of technology in Spanish citrus production. *Agron*, 9 (2019) 631.
7 Lacerna MM & Bayogan EV, Rind color change and granulation in pummelo [*Citrus maxima* (Burm. ex Rumph.) Merr.] fruit as influenced by 1-methylcyclopropene. *Int Food Res J*, 25 (2018) 1483.
8 Jie D, Wu S, Wang P, Li Y, Ye D & Wei X, Research on *Citrus grandis* granulation determination based on hyperspectral imaging through deep learning. *Food Anal Methods*, 14 (2021) 280.
9 Xu S, Lu H, Ference C, Qiu G & Liang X, Rapid Nondestructive Detection of Water Content and Granulation in Postharvest 'Shatian' Pomelo Using Visible/Near-Infrared Spectroscopy. *Biosensors*, 10 (2020) 41.
10 Theanjumpol P, Wongzeewasakun K, Muenmanee N, Wongsaipun S, Krongchai C, Changrue V, Boonyakiat D, Kittiwachana S. Non-destructive identification and estimation of granulation in 'Sai Num Pung' tangerine fruit using near infrared spectroscopy and chemometrics. *Postharvest Biol Technol*, 153 (2019) 13.
11 Kittiyanpunya C, Leekul P, Phongcharoenpanich C & Krairiksh M, Beam-scanning reflectometer for detecting granulated fruits. *IEEE Sensors J*, 17 (2017) 1277.
12 Kotwaliwale N, Singh K, Kalne A, Jha SN, Seth N & Kar A, X-ray imaging methods for internal quality evaluation of agricultural produce. *J Food Sci Tech*, 51 (2014) 1.
13 Arendse E, Fawole OA, Magwaza LS & Opara UL, Non-destructive prediction of internal and external quality attributes of fruit with thick rind: A review. *J Food Engg*, 217 (2018) 11.
14 Krishna KR, Sharma RR, Kar A & Srivastav M, Non-destructive detection of jelly seed disorder in Amrapali and Pusa Surya varieties of mango (*Mangifera indica*). *Indian J Agric Sci*, 89 (2019) 95.
15 Nayak SL, Sethi S, Sharma RR, Dubey AK & Bhowmik A, Variation in fruit quality traits and health promoting compounds of citrus fruits grown in semi-arid region. *Indian J Hort,* 77 (2020) 635.
16 Nguyen G, Dlugolinsky S, Bobak M, Tran V, Garcia AL, Heredia I, Malík P & Hluchy L, Machine learning and deep learning frameworks and libraries for large-scale data mining: a survey. *Artif Intell Rev*, 52 (2019) 77.
17 Sun X, Xu S & Lu H, Non-Destructive Identification and Estimation of Granulation in Honey Pomelo Using Visible and Near-Infrared Transmittance Spectroscopy Combined with Machine Vision Technology. *Appl Sci,* 10 (2020) 5399.
18 Dael MV, Lebotsa S, Herremans E, Verboven P, Sijbers J, Opara UL, Cronje PJ & Nicolai BM, A segmentation and classification algorithm for online detection of internal disorders in citrus using X-ray radiographs. *Postharvest Biol Technol*, 112 (2016) 205.
19 Sharma RR, Awasthi OP & Kumar K, Pattern of phenolic content, antioxidant activity and senescence-related enzymes in granulated vs non-granulated juice-sacs of 'Kinnow' mandarin (*Citrus nobilis* x *C. deliciosa*). *J Food Sci Tech*, 53 (2015) 1525.
20 Jiang JA, Changa HY, Wu KH, Ouyang CS, Yang MM, Yang EC, Chen TW & Lin TT, An adaptive image segmentation algorithm for X-ray quarantine inspection of selected fruits. *Comput Electron Agric*, 60 (2008) 190.
21 Deshpande A, Pillai MP & Cheeran AN, X-ray imaging for non-destructive detection of spongy tissue in *Alphonso* mango. In: *Computer Vision and Information Technology: Advances and Applications,* (Eds. Kale KV, Mehrotra SC & Manz RR; I.K. International Publishing House Pvt. Ltd., New Delhi), 2010, 252.
22 Sharma RR, Singh R & Saxena SK, Characteristics of citrus fruits in relation to granulation. *Scientia Hortic*, 111 (2006) 91.