Life Science Informatics Publications
Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences
Journal Home page http://www.rjlbpcs.com/
RJLBPCS
ISSN 2454-6348

Original Research Article **DOI: 10.26479/2018.0404.17**

NON-DESTRUCTIVE DETECTION OF FROST DAMAGE IN SWEET LEMON USING IMAGE PROCESSING AND ULTRAVIOLET RADIATION

Rouhallah Abedi Firouzjaei, Saeid Minaei*, Babak Beheshti

Department of Agricultural Systems Engineering, Science and Research Branch,

Islamic Azad University, Tehran, Iran.

ABSTRACT: Citrus peel is more resistant to frost damage compared to the intercellular flesh. Thus, it may be possible that the internal tissues of apparently healthy fruits are damaged and devoid of juice. Frost damage under UV radiation appears as light green spots on the fruit skin. The purpose of this study was to propose a fast, non-destructive method for sweet lemon frost damage detection and to evaluate the extent of frost damage using image processing technique and UV radiation. This study was done on 135 sweet lemons. The samples at 3 levels of temperature (0, 7 and -7°C) were exposed to cold treatment (6, 12 and 24hours). Sample images were captured under UV light 12, 24 and 36 hours post treatment. The images analyzed using MATLAB software. "Green Spot Index" or GSI was used to show the extent of fruit frost damage. Results were compared with the conventional visual method of frost damage assessment by a team of experts. Results showed that the percentage of GSI is significant at the 1% level considering main, double and triple interaction effects of all treatments. GSI increased significantly by decreasing the temperature to -7°C. By increasing the duration of cold treatment from 6 to 24 hours and increasing the waiting period from 12 to 36 hours, the green spot index increased dramatically. The detection accuracy of frost damage based on the percentage of green spots on the lemon fruit skin was acquired to be equal to 92.6% compared to manual assessment.

KEYWORDS: sweet lemon, freezing temperatures, UV radiation, computer vision.

Corresponding Author: Dr. Saeid Minaei Ph.D.

Department of Agricultural Systems Engineering, Science and Research Branch,

Islamic Azad University, Tehran, Iran.

Email Address: minaee@modares.ac.ir

1.INTRODUCTION

Citrus is a native of Southeast Asia [1]. The origin of that region is between India, South China and Indonesia [2]. Lemon belongs to the citrus family and is generally produced in moderate as well as in dry, hot climates. Ranked after orange and tangerine, sweet lemon (Citrus limetta) is the third important citrus fruit [3]. Frost damage in citrus fruits is one of the major reasons for degradation of fruits in the citrus industry. Assessment of frost damage in citrus fruits is very challenging since it cannot be detected visually with the naked eye [4, 5, 6]. Chilling and cold conditions cause damage in citrus (the tree as well as the fruits). Damaged citrus fruits are not suitable for consumption due to their poor taste and low juice content as frost damage results in fruit degradation [7, 8]. The destructive effects of chilling have been observed in citrus growing countries such as Spain, Italy, Greece, Turkey, America and Japan [9]. During the first weeks after the chilling incidence, the evaluation of damage level to fruits is done by inspectors through careful investigation of flesh segments using visual and segment cut method [10]. This method is destructive and time-consuming. A wide variety of instruments has been tested to detect the frost damage in citrus fruits. For instance application of chromatography and mass spectrometry for the non-destructive measurement of greenhouse volatile gases emitted from healthy and damaged oranges for one hour, shows that the ethanol, ethylene botanoate, methyl hexanuate and ethyl octanoate emissions increase in completely frozen Navel oranges [11]. A gas sensor was used for frost damage detection in oranges. In this method, the level of ethanol gas in the headspace of the sample preservation container was used for the assessment. The accuracy of the mentioned method was 100 and 37 percent for healthy and partially frozen fruits, respectively [12]. In order to increase the accuracy of this method, a hand-held (manual) ethanol sensor with higher sensitivity compared to the one used in Tan et al, [13] study was applied for measuring the ethanol level in oranges with the aim of frost damage detection [14, 15].In another research, Nuclear Magnetic Resonance (NMR) spectrometer was utilized for frost damage investigation in the flesh segment of citrus [5, 16]. Making use of NMR is very complex and expensive and requires extensive studies. A smell sensor has been used for quality evaluation of oranges. This sensor is sensitive to a wide range of volatile materials such as aromatic mixtures, alcohols and aldehydes [17]. Making use of ultraviolet radiation researchers have found a non-destructive method for frost damage detection. This kind of damage has its effects on citrus peel oil in the form of observable compounds on the skin surface. The results showed that the visual appearance of frozen oranges is different and a vivid pattern of yellow spots in the range of 1-2 mm width can be observed when placed under UV light (365nm) [18,19]. This experiment was conducted on California Navel Oranges. A research was performed to find the best excitation wavelength for scattering the fluorescence of citrus peel under UV light (300-700nm) on 15 species with the aim of frost damage detection in citrus fruit. Results showed that the appropriate excitation wavelength to achieve the best scattering of fluorescence is in the ranges of 350-380 nm and 490-

540nm [18, 20]. Overtime, frost damage leads to fruit spoilage and if not identified in time, it can spread and cause the spoilage of adjacent fruits. Thus, the citrus industry requires a fast and non-destructive method in order to distinguish frost damage in citrus fruits. The goal of this study is to employ a non-destructive and relatively fast procedure to identify frost damage in sweet lemon fruits and evaluate the extent of damage utilizing image processing techniques.

2. MATERIALS AND METHODS

In order to conduct the experiments, 135 sweet lemon fruits were harvested on November 2014 from an orchard located in Ghaemshahr, Mazandaran Province in Iran. The samples were stored in a refrigerator for 48 hours at 10°C before the experiments. Fruits which had diameters of around 6cm were not chemically or physically treated postharvest. Freezing conditions for sweet lemon was simulated in the laboratory using a cooled incubator (Model Panasonic MIR-154-PE). Statistical evaluation of the results was performed using a completely randomized factorial design with 5 replications for each treatment. The independent variables included temperature, chill duration and tempering period (holding time at room temperature 25±2°C after treatment) the levels of which are shown in Table 1.

Table 1: Levels of independent variables in frost damage investigation.

Treatment	1	2	3
Temperature (° C) A	7	0	-7
Chill duration (hours) B	6	12	24
Tempering time (hours) C	12	24	36

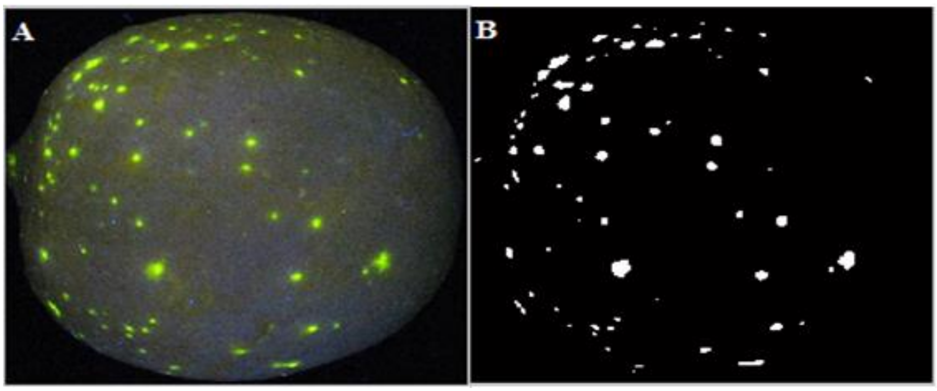

Fig.1. (A) light green spot pattern visible in freeze-damaged sweet lemons 12 hours after thawing when viewed under 365nm illumination, (B) Automatically segmented image of sweet lemon in part showing light green spot extraction (green spot differentiation by image processing).

In order to investigate frost damage using the machine vision system, sweet lemon fruits were digitally imaged according to Table 1. To do this, an imaging platform and an illuminating system were employed. The fruit was located in the middle of the light box directly under camera. The illuminating system includes two ultraviolet radiation lamps (365 nm, Black light, model TL 8W

BLB 1FM, Philips Poland) which are located at 2 corners symmetrically. The images were captured using a digital camera (Canon powershot-SX30 IS, 14.1 Megapixel sensor with DIGIC 4 processor) and sent to a personal computer for processing and recording the data. Sweet lemons were then evaluated for freeze damage using the conventional segment cut method by 3 inspectors and classified into 4 quality groups [10]. At the time of inspection, the inspectors were not aware of the type of chill treatment that lemons were subjected to. In this study, investigation of the digital image features and optimization of their quantities were done using the image processing tool in MATLAB analytical software. Then, the image of each sweet lemon fruit was investigated by MATLAB software and the damaged fruits were separated from the healthy ones based on the presence or absence of green spots on fruit skin. In addition, the extent of damage was designated by calculating a new indicator called the Green Spot Index or GSI (ratio of the green spot area to the total surface area) of the image. The system accuracy and its proper functioning were determined based on the capability of precise discrimination between healthy and freeze-damaged fruits. Comparison of the means was performed using Duncan's multiple range test (at 99% confidence level). All the analyses were carried out using SAS ver. 9.1.

3. RESULTS AND DISCUSSION

Using image processing technique, GSI was calculated after separating the object from the background image and detecting the light green spots on the sweet lemon surface (Fig.2).

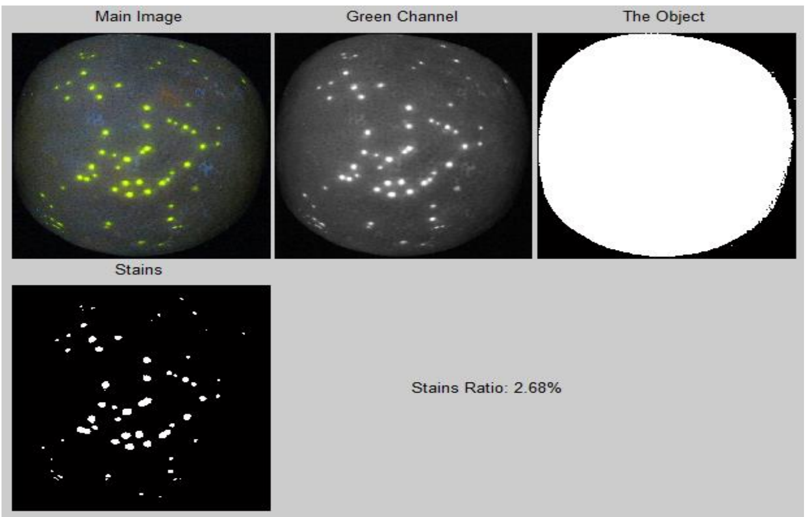

Fig.2. Results of image processing using MATLAB software

According to the results of UV radiation and image processing, all samples were classified based on visual surface damage score and GSI given by machine vision automatically into 4 groups: 1- no damage, 2- slight damage, 3- moderate damage and 4- severe damage. The 24-hour freeze-treated sweet lemons at -7°C and tempering time of 36 hours were classified as frozen (severe damage) according to USDA segment cut method. Moreover, almost no green spots were observed on sweet lemons refrigerated at 7°C for which GSI was equal to zero. In order to investigate the effects of temperature, chilling duration and tempering time on GSI, the data acquired by image processing were analyzed based on completely randomized factorial design.

Table 2: Results of analysis of variance for green spot percentage on sweet lemon as affected surface by the independent variables.

Source variations	DF	Mean Square
Temperature (A)	2	6052.398**
Chill duration (B)	2	1619.859**
Tempering time (C)	2	674.909**
interaction (A*B)	4	1607.789**
interaction (A*C)	4	671.729**
interaction (B*C)	4	138.956**
interaction (A*B*C)	8	137.994**
Error	108	15.644

**: significant at the%1 level

Results of the analysis of variance (Table 2) showed that the percentage of green spots on the fruit surface is significant for all the main effects, double and triple interaction effects (α = %1). Comparison of the means for GSI due to double and triple interaction effects are shown in figures 3, 4, 5 and 6. Here, different levels of temperature (A), chill duration (B) and tempering period (C) are according to Table 1. Comparison of the means using Duncan's multiple range tests showed that by decreasing the temperature from 7 to -7°C, the percentage of green spots on fruit skin representing the extent of frost damage would increase dramatically (Table 3).

Table 3: Comparison of the means for GSI on sweet lemon surface (Duncan's multiple range test).

Temperature (°C)	% green spots
7	0 b
0	0.039 b
-7	20.11 a
Chill duration(hours)	
6	2.72 b
12	3.8 b
24	13.61 a
Tempering time (hours)	
12	3.20 c
24	6.07 b
36	10.86 a

Mean values with the same letter are not significantly different (α = 0.01).

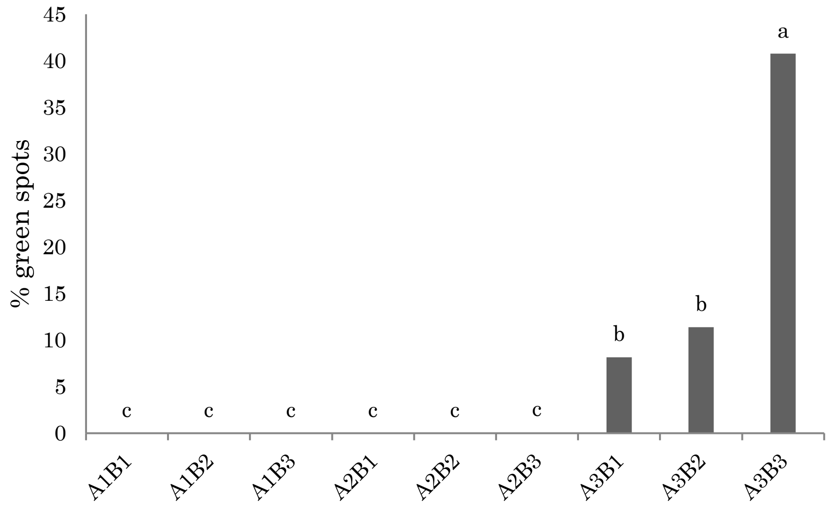

Fig.3. Comparison of the means for interaction effects of temperature and chill duration (A×B).

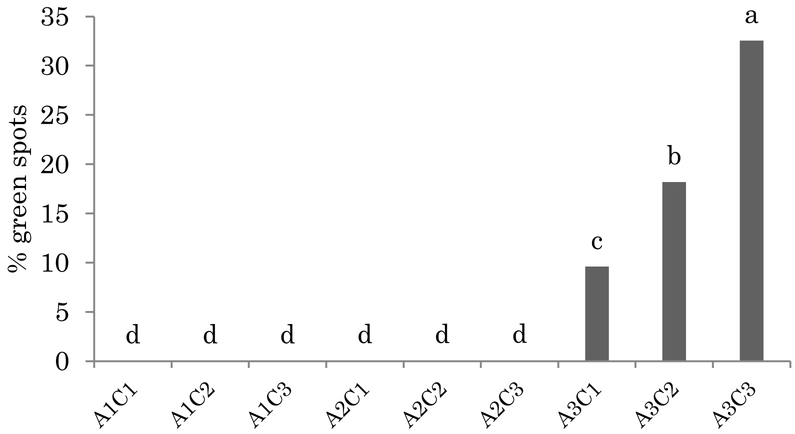

Fig.4. Comparison of the means for interaction effects of temperature and tempering time (A×C).

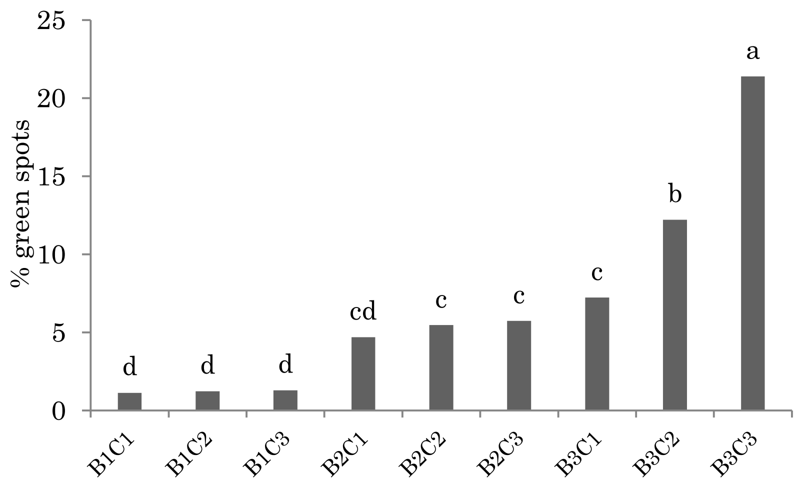

Fig.5. Comparison of the means for interaction effects of chill duration and tempering time (B×C).

Freezing causes oil glands (and eventually the citrus peel to rupture) leading to the formation of watery spots on the fruit peel surface. These spots spread overtime. The presence of fluorescence material in the peel's oil is responsible for appearance of green spots under UV radiation. At 7°C, GSI (under all chill durations and tempering periods) was equal to zero and there was no significant effect at this temperature (Fig.6). In other words, above zero temperatures which do not cause freezing, naturally do not lead to frost damage. In frost condition, the percentage of green spot on lemon peel increased with chill duration and tempering period in which a significant effect was observed (Fig.6).

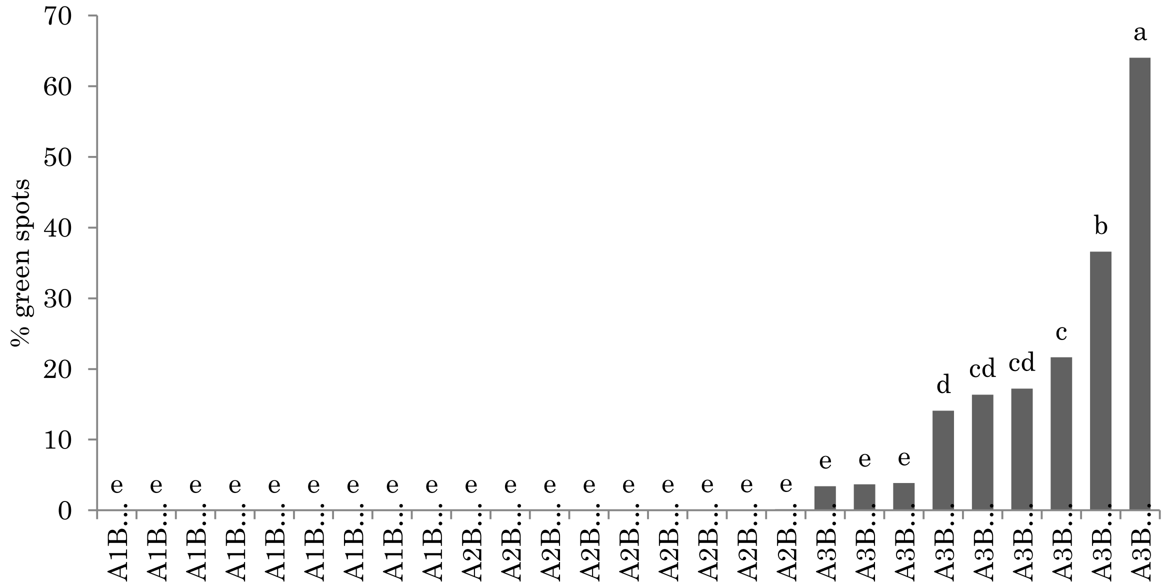

Fig.6. Comparison of the means for triple interaction effects of temperature, chill duration and tempering time (A×B×C).

Diagnostic analysis was done based on the percentage of green spots on fruit surface in order to develop a predictive model from group memberships.

Table 4: The results of discriminant analysis

Original Groups	Predicted Group Membership				Total
	1	2	3	4	
1	90	0	0	0	90
2	2	13	0	0	15
3	0	3	9	0	12
4	0	0	5	13	18
1	100	0	0	0	100
2	13.3	86.7	0	0	100
3	0	25	75	0	100
4	0	0	27.8	72.2	100

According to Table 4, it was found that 100% of the undamaged sweet lemons have been placed in the no-damage group. Two, three and five lemons with slight, moderate and severe damage were classified as no-damage, slight and severe damage, respectively. Damage classification accuracy is presented in Table 4 as a percentage. Finally, by investigating the data and based on the obtained coefficients, estimation accuracy of the frost damage level based on the percentage of green spots detected by image processing was determined to be equal to 92.6%. Compared to non-destructive methods for frost damage detection such as chromatography [11] and ethanol sensor for measuring the ethanol level of oranges [12], making use of image processing and UV illumination with the ability of real-time monitoring is a suitable method for frost damage detection in sorting and grading lines of citrus packaging. This is while, chromatography and ethanol sensor methods are only appropriate for the evaluation of small groups. Another advantage of image processing technique and UV illumination (compared to other methods) is that they are fast and cost-effective such that citrus damage detection monitoring can be implemented in a very short time.

4. CONCLUSION

The light green spot pattern visible on the surface of sweet lemons when illuminated by long wave UV radiation (365nm) can be utilized to identify slight to severe levels of frost damage. Results of the analysis of variance showed that the percentage of green spots on fruit peel surface under main, double and triple interaction effects is very significant (α = %1). Reducing the temperature to -7°C, caused the number of green spots to increase significantly. By increasing the chill duration from 6 to 24 hours and tempering period from 12 to 36 hours, green spot density increased dramatically. Based on diagnostic analysis, a 92.6% prediction accuracy of frost damage level based on the percentage of green spots on sweet lemon surface was obtained using UV image processing technique.

5. CONFLICT OF INTEREST

There is no conflict of interest

REFERENCES

1. Olsen M, Matheron M, McClure M, Xiong Z. Diseases of Citrus in Arizona. Plant Disease Publications, Cooperative Extension, College of Agriculture & Life Sciences, University of Arizona, Tucson, AZ. 2000.
2. Timmer L.W, Garnsey S.M. Broadbent P. Diseasesof citrus. In: Diseases of Tropical Fruit Crops (ed. R.C. Ploetz), 2003; pp. 197–226.
3. Iglesias D.J, Cercós M, Colmenero-Flores J.M, Naranjo M.A, Ríos G, Carrera E, Ruiz-Rivero O, Lliso I, Morillon R, Tadeo F.R. Talon M. Physiology of citrus fruiting. Braz. J. of Plant Physiol. 2007; 19: 333–362.
4. Gambhir P.N, Choi Y.J, Slaughter D.C, Thompson J.F, McCarthy M.J, Proton spin–spin relaxation time of peel and flesh of navel orange varieties exposed to freezing temperature. J. Sci. Food Agric. 2005; 85: 2482–2486.
5. Zhang L, McCarthy M.J. NMR Relaxometry Study of Development of Freeze Damage in Mandarin Orange. J Sci Food Agric. 2016; 96(9):3133-9.

6. Pavia D.L, Lampman, G, Kriz G.S, Vayvyan J.R. Introduction to Spectroscopy. Western Washington Univercity. United States of America. 2009.
7. Syvertsen J.P. Dehydration of freeze-damaged oranges. Hort Science. 1982; 17: 803–804.
8. Zhao X, Burks T, Qin J, Ritenour M. Digital microscopic imaging for citrus peel disease classification using color texture features. Applied Engineering in Agriculture. 2009; 25(5):769-776.
9. Davis S, Albrigo G. Citrus. Crop Production Science in Horticulture Series. CAB. International, Wallingford, UK. 1994.
10. USDA. Arizona California Citrus Loss Adjustment Standards Handbook. FCIC-25040 (11-1999). USDA, Washington, DC. 1999.
11. Obenland D.M, Aung L.H, Bridges D.L, Mackey B.E. Volatile emissions of navel oranges as predictors of freeze damage. J. Agric. Food Chem. 2003; 51: 3367–3371.
12. Tan E.S, Slaughter D.C, Thompson J.F. Freeze damage detection in oranges using gas sensors. Postharvest Biol. Technol. 2005; 35: 177–182.
13. Thompson J.F, Slaughter D.C, McCarthy M.J. Survey of Sensing Methods for Detection of Freeze Damage-Annual Progress Report for Project CRB No. 5600-17. California Citrus Research Board, Visalia, CA. 2006.
14. Thompson J.F, Slaughter D.C. Survey of Sensing Methods for Detection of Freeze Damage-Annual Progress Report for Project CRB No. 5600-17. California Citrus Research Board, Visalia, CA. 2005.
15. Gambhir N, Choi J, Slaughter C, Thompson F, McCarthy J. Proton spin–spin relaxation time of peel and flesh of navel orange varieties exposed to freezing temperature. J. Sci. Food Agric. 2005; 85: 2482–2486.
16. Natale C.D, Macagnano A, Martinelli E, Paolesse R, Proietti E, D'Amico A. The evaluation of quality of post-harvest oranges and apples by means of an electronic nose. Sensors and Actuators B. 2001; 78: 26–31.
17. Slaughter D.C, Obenland J.F, Thompson M.L, Arpaia D.A. Non-destructive freeze damage detection in oranges using machine vision and ultraviolet fluorescence. Postharvest Biology and Technology. 2008; 48(3): 341-346.
18. Momin Abdul M, kondo N, Kuramoto M, Ogawa Y, Investigation excitation wavelength for fluorescence emission of citrus peel based on uv-vis spectra, J. of EAEF. 2012; 5(4):126-132.
19. Kondo N, Kuramoto M, Shimizu H, Ogawa Y, Kurita M, Nishizu T, Chong V.K, Yamamoto K. Identification of Fluorescent Substance in Mandarin Orange Skin for Machine Vision System to Detect Rotten Citrus Fruits. J. of EAEF. 2009; 2: 54-59.
20. Aleixos N, Blasco J, Navarron F, Molto E. Multispectral inspection of citrus in real-time using machine vision and digital signal processors, J. of Comput. Electron. Agric. 2002; 33: 121-137.