

Identification of Fluorescent Substance in Mandarin Orange Skin for Machine Vision System to Detect Rotten Citrus Fruits

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Abstract

As basic research to develop a machine vision system to detect rotten mandarin orange, the extraction and identification of fluorescent substances contained in rotten parts of mandarin orange were conducted, and the excitation and fluorescence wavelengths of the substance were determined. Although it has been reported that damaged orange fruit skins are often fluoresced by UV light, it was suggested that fluorescent substances exist not only in the rotten parts of skins but also the normal parts of skins from this research. The fluorescent substances were extracted from 1kg of mandarin peel, and NMR analysis and mass spectrometry were conducted. From this experiment, it was found that the fluorescent substance was quite possibly heptamethylflavone and that the excitation and fluorescent wavelengths of one of the substances were 360 to 375nm and 530 to 550nm, respectively.

[Keywords] excitation wavelength, fluorescent wavelength, extraction, fruit grading, ultraviolet

I Introduction

It is very important to properly evaluate fruit quality when a small amount of high quality fruits are produced, as in East Asian countries. Many researches on fruit defect detection using a color TV camera (Aleixos et al., 2002; Andrew 2002; Ishii et al., 2003; Kurita et al., 2006; Leemans et al., 2002; Leemans and Destain, 2004), X-ray imaging device (Ogawa et al., 2005a; Ogawa et al., 2005b; Njoroge et al., 2002), or near infrared inspection system (Lu and Ariana, 2002) have been reported so far and sensors for defect detection are actually used in cooperative fruit grading systems in Japan. The systems can assign various data: fruit size, color, shape, bruising, rind puffing, sugar content, acidity and etc; however, it is still difficult to recognize rotten parts of fruit, because the external color is very similar to normal parts of fruit. The rotten part mostly introduces mold and is too soft to be conveyed or transported by mechanical systems, and can burst, which is not good for the grading machine itself and from

hygienic aspects. Many human operators are therefore required to work in the fruit grading facilities in order to eliminate rotten fruit on sorting conveyors before and after grading operations.

Uozumi et al. (1987) reported that the rotten parts of mandarin orange fruit fluoresced when excited by UV light. Since fluorescent light from rotten parts is a greenish color, it is considered that a color TV camera used for color imaging can also be used for fluorescence imaging; however, the fluorescence substances have not been identified so far. Because the fluorescent light energy is small, it is necessary to construct an efficient machine vision system which can capture a higher gray level image by exciting the most suitable wavelength. The objectives of this paper were to identify the fluorescence substances in mandarin fruit skin, and to investigate the excitation wavelengths of the substances to determine the most efficient lighting device specification.

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II Materials and Methods

1. Preparation of samples

The orange fruit used in this project was mandarin orange cv. "Tokumori wase", and was harvested on October 19, 2007 and graded at an orange fruit grading facility on October 23, 2007 in Ehime Prefecture, Japan. Rotten samples were collected from mandarin oranges graded as substandard because of rotten skins as shown in Fig.1. The sample of rotten fruit looked similar to healthy fruit. Normal samples were collected from oranges classified as the upper grade without damage to their skins.

The visual condition of the rotten part of the skin in Fig.1 looks similar to normal healthy fruit, and generally it is not easy to distinguish rotten fruit from normal fruit by eye.



Fig.1 Sample of rotten skin

2. Comparison of spectra in fluorescent substances between rotten fruit and normal fruit

Before analysis of the fluorescence substances, the difference of substances between rotten and normal (healthy) parts was investigated to confirm whether some substances were created during rotting or not. The rotten (13.9g) and normal peels were mashed and placed in flasks, respectively. The fluorescent substance was extracted using 45 ml of hexane as a solvent, and the absorption spectrum, fluorescence spectrum, and excitation spectrum were measured after one day. A visible-ultraviolet absorption spectrometer (U-4000, Hitachi, Ltd., Tokyo, Japan) was used to measure the absorption spectrum and a visible-ultraviolet fluorospectrophotometer (U-4500, Hitachi, Ltd., Tokyo, Japan) was used for excitation and fluorescence spectra. The procedure of the extraction described above is called "Extraction Method A" in this paper.

3. Fluorescent substance extraction for ingredient identification

One kilogram of peel, including the flavedo layer of

mandarin orange, was finely ground using a centrifugal mill, and solvent was extracted using hexane as a solvent and was soaked for one day. The mixture was filtered with cotton and vacuum concentration was performed using evaporator (Rotary Vacuum Evaporator Type N-1, EYELA). Next, 200ml of methanol and a separation funnel (500ml) were used to separate the hexane and methanol layers. The methanol extract was separated by column chromatography on SiO₂. Finally, the fluorescent active fractions were combined and purified by preparative thin layer chromatography (PTLC). Nuclear magnetic resonance (NMR) analysis with a heavy chloroform solvent and liquid chromatography mass spectrometry/ mass spectrometry (LC- MS/MS) analysis were performed. The above procedure is summarized in Figure 2. The excitation procedure is called "Extraction Method B" in this paper.

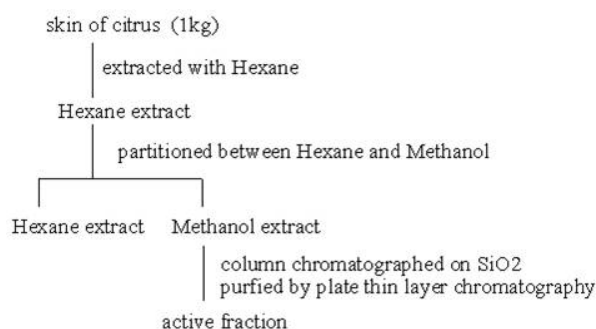


Fig.2 Procedure of fluorescent substance extraction

III Results and Discussion

1. Comparison of the spectra of the rotten and normal parts by hexane extraction

Components from the rotten and normal parts of mandarin orange skin were extracted with "Extraction Method A", respectively, and absorption, excitation and fluorescent spectra were measured as shown in Fig.3. In the normal part, the absorption wavelength had a peak near 325nm and decreased as the wavelength became longer. The minimum intensity was found at around 400nm, and a smaller second peak was recognized at nearly 440nm. Although the rotten part showed almost the same pattern, intensity was overall smaller than the case of the normal part, in the range of 300 to 350nm, the spectrum curve sloped more gently than the normal part and the peak near 325nm could not be confirmed; however, it is suppressed that these are the same substances because the fundamental shape of these two spectra was almost the same. In the fluorescent spectrum, although there was no distinctive trend from 400 to 500nm in

both parts, a rapid rise was observed from 520nm and the peak was reached near 540nm. The peak value of the rotten part was about 20% smaller than the normal part. After 540nm, it decreased gradually and reached the same intensity level in the range 400 to 500nm, near 580nm. The excitation spectrum in the normal part increased linearly from 300nm, peaked near 360nm, and subsequently maintained the same value at near 380nm. The minimum value was at 400nm and was almost the same until 500nm.

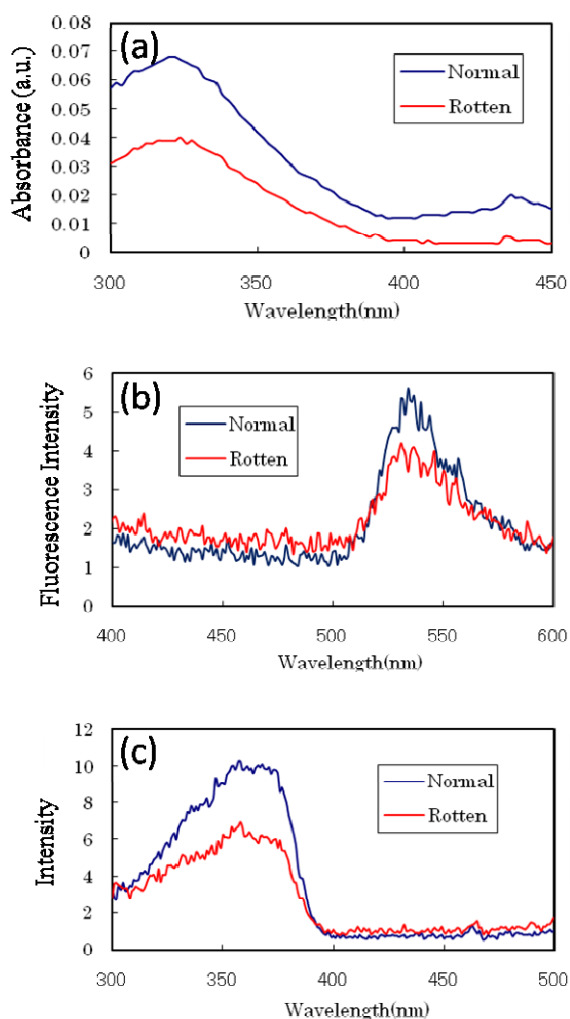


Fig.3 Spectra in the normal and rotten parts by rough extraction (a) absorption spectrum, (b) fluorescent spectrum ($\lambda_{EX}=320$ and 322 nm for normal and rotten parts, respectively), (c) excitation spectrum.

As mentioned above, the tendency of the absorption, fluorescence, and excitation spectrum distributions were the same although there was a small difference in the intensity itself, and it was suggested that the fluorescent substance of the normal and the rotten parts is a substance generally contained in the peel of mandarin orange. Therefore, it was

clear that the fluorescent substance in the skin comes out to the surface by rotting, and can then be detected, and that this fluorescent substance is excited with the light from 320 to 390nm and emits the fluorescence of 520-570 nm.

2. Spectra of the extracted substance

Fluorescent substances extracted from the normal and the rotten parts were the same. The fluorescent substance was extracted from only the normal part of 1kg peel in mandarin orange because it was difficult to collect 1kg rotten peel.

The fluorescent substance was extracted as shown in Fig.2, and 5.33g and 1.37g of extraction liquids were obtained from hexane and methanol layers, respectively. The methanol extract was separated by column chromatography on SiO_2 .

The results of the absorption, fluorescence, and excitation spectra of the extracted substance are shown in Fig.4. It was clear that substances extracted by "Extraction Method B" (Fig.4) and "Extraction Method A" (Fig.3) were the same because the absorption spectrum of the extraction substance (Fig.4) became 322 nm, almost corresponding to the result of Fig.3. Moreover, the excitation spectrum peaked near 370 nm after rapidly rising from 300 nm, and then decreased rapidly. It became clear that the fluorescence spectrum remained low value from 400 to 500nm, increased rapidly from 520nm, and peaked at 531nm. The absorption, excitation and fluorescence spectra well agreed with the results of "Extraction Method A" (Fig.3), and the spectral characteristics of the fluorescent substance in the peel of mandarin orange were clarified.

In order to conduct more detailed analysis, the nuclear magnetic resonance (NMR) spectrum of this extracted substance was measured (Fig.5). First, the signal observed at 0.8 to 2.5 ppm is a fatty acid impurity. A set of single peaks at 3 to 4 ppm is methyl ether. A signal characteristic of an aromatic series is observed at 6.5 to 8 ppm. Moreover, this activity substance took on a light-yellow color.

As mentioned above, it was strongly suggested that the extracted substance belongs to a type of flavones which do not have sugar but have a methyl ether group. Searching the previously published papers, it was found that the extracted substance was a type of poly-methoxy flavone, among which, nobiletin, sinenstien, tangeretin, heptamethoxyflavone could be candidates, because it is known that their properties meet the substance conditions and they are present in mandarin orange skin. Structural formulae of these components are shown in Fig.6, and the content of each component in mandarin orange is shown in Table 1.

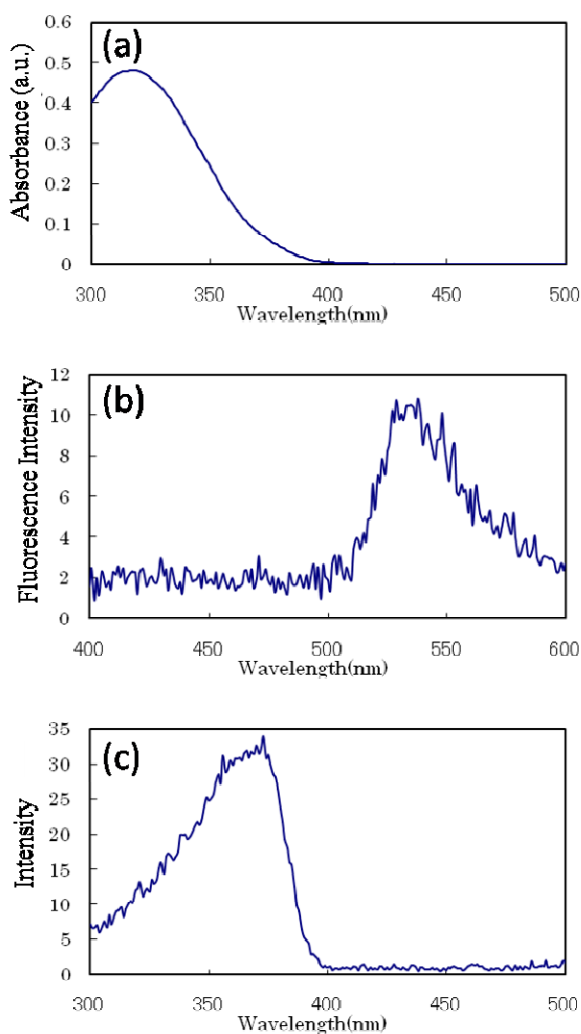


Fig.4 Spectra of peel in mandarin orange by "Extraction Method B" extraction. (a) absorption spectrum, (b) fluorescent spectrum ($\lambda_{EX}=322\text{nm}$) (c) excitation spectrum.

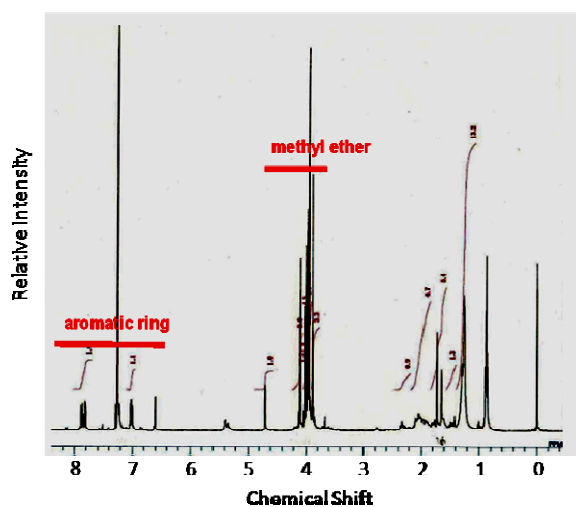
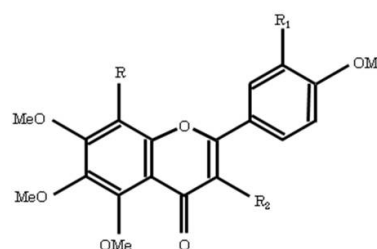


Fig.5 NMR spectrum of extracted substance



Polymethoxylated flavone

Sinensetin $R=H, R_1=OMe,$

$R_2=H$

Nobiletin $R=R_1=OMe, R_2=H$

Tangeretin $R=OMe, R_1=R_2=H$

Heptamethoxyflavone $R=R_1=R_2=OMe$

Fig.6 Structures of Polymethoxylated Flavone

Table 1 Components of mandarin fruit

Name	Content(mg/100g)			λ_{max} (nm)
	Flavedo	albedo	peel	
Nobiletin	10.8	1.8	6.4	No data
Sinensetin	2.2	0.0	0.0	240,265,326
Tangeretin	7.6	1.9	4.8	271,322
Heptamethoxy-flavone	3.8	1.0	2.4	No data

λ_{max} is UV absorption max

The component mentioned in Fig.6 was contained in flavedo, as shown in Table 1. In order to identify the extracted substance, an authentic standard was prepared by mixing 100ppm of sinensetin, 200ppm of nobiletin, and 100ppm of tangeretin, and each spectrum was analyzed using methanol as the solvent. The comparison of spectra in the authentic standard (indicated as 'authentic' in Fig.7) and the extracted substance (indicated as 'sample' in Fig.7) is shown in Fig.7. For the excitation wavelength, the authentic standard had a single-peaked pattern with the maximum intensity at nearly 362nm. On the other hand, the sample showed a gentle rise from 300, peaked around 360 to 375nm, and then decreased rapidly after the peak. Although the shape of spectra of the authentic standard and the sample itself differed, the maximum wavelength was mostly in agreement, 360 to 375nm. In contrast, the fluorescent wavelengths differed greatly in the authentic and the sample.

agreement, 360 to 375nm. In contrast, the fluorescent wavelengths differed greatly in the authentic and the sample. The maximum value in the authentic standard occurred near 440nm and in the sample it was near 540nm. Although fluorescence wavelengths differed greatly in the authentic standard and sample and the peak was near 540nm in the sample, near 440nm was the peak in the authentic standard.

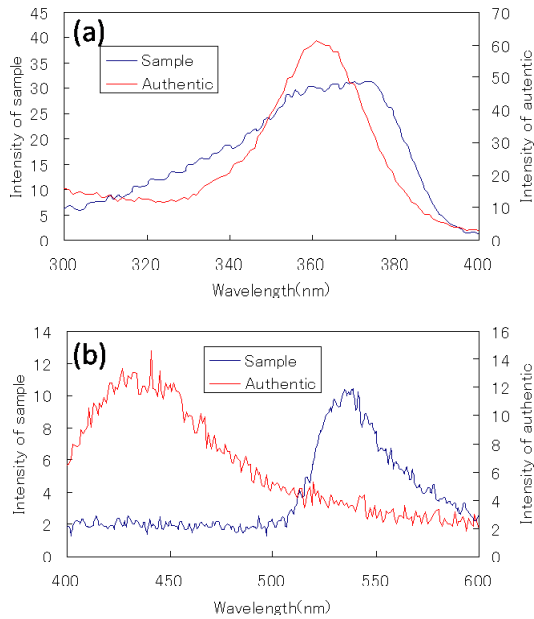


Fig.7 Comparison of spectra between authentic standard and extracted substance. (a) excitation spectrum, (b) fluorescent spectrum.

From these results, it was observed that although the candidates were fluorescence substances, they were different from the substance in the sample liquid. Because it was confirmed that not only one but multiple substances fluoresced, it is important to detect suitable wavelengths to excite the fluorescence substances to obtain the highest brightness from rotten or injured skin. It is considered that further separation of the sample liquid was required. Mass spectrometry was conducted for the extracted substance and the atomic mass number was $m/z = 433$ ($M+H$), 455 ($M+Na$), as shown in Fig.8.

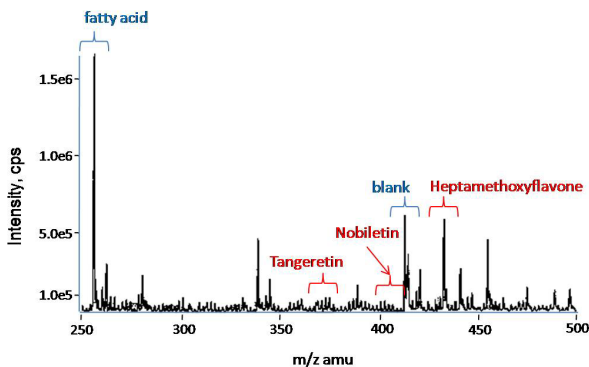


Fig.8 MS Spectral Data of FL-Active Fraction

It was suggested that the extracted substance was heptamethylflavone, which has seven methyl groups.

In this research, it became clear that the excitation wavelength of mandarin orange peel was near 360 to 375nm. Slaughter et al. (2008) conducted an experiment to detect freeze damage to Californian navel orange illuminated with 365nm light; the 360 to 375nm excitation wavelength in this research agreed with the 365nm wavelength used by Slaughter et al. Although Slaughter did not identify the substance, this fluorescent substance was suggested to be heptamethylflavone in this research.

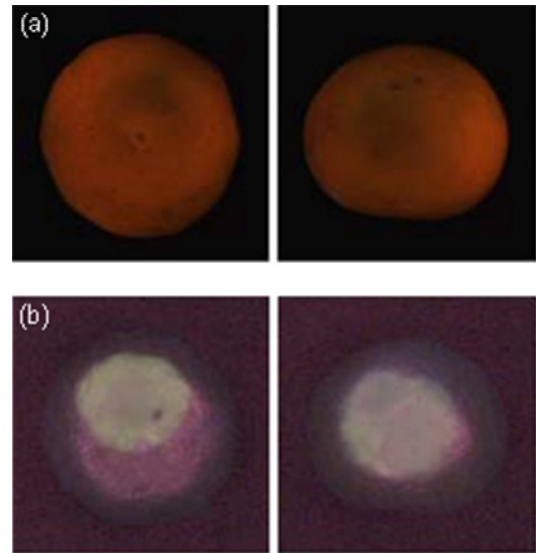


Fig.9 Detection of rotten part of mandarin orange. (a) external appearance in visible range. (b) fluorescent image of the rotten part excited by 365nm wavelength.

On the basis of these findings, the result of detecting the rotten part of mandarin oranges using 365nm excitation wavelength, which is almost the mean value of 350 to 375nm, is shown in Fig.9. Fig.9(a) shows the external appearance of rotten mandarin oranges in the visible range, and Fig 9(b) shows the fluorescent images excited by 365nm and the white part is rotten. It became clear that the rotten part of mandarin orange, which is difficult to detect in the visible range, can be clearly detected by fluorescent images excited with 365nm UV light.

V Summary and Conclusions

mandarin oranges, which are also difficult to detect by eye. Spectrum analysis revealed that a fluorescent substance detectable in the rotten part exists also in the normal part, and was not produced by rotting.

The substance was identified, and it was found that the fluorescent substance extracted from the peel of mandarin oranges had the basic structure of flavones as the mother nucleus, and it was quite possibly heptamethylflavone. The excitation and fluorescent wavelengths of the fluorescent substance were 360 - 375nm and 530-550nm, respectively. The meaning of the excitation wavelengths used up to now for other citrus fruits could be logically explained due to specification of the fluorescent substance. These findings are applicable for the development of a machine vision system to detect rotten mandarin oranges that are not easy to detect by eye.

Although a substance excited at 360 to 375 nm has been specified in this research, it is suggested that more than one fluorescent substance is present in the skin of citrus. Further studies will be needed to identify other fluorescent substances and their excitation wavelengths to construct the optimum system by combining these wavelengths to detect rotten citrus fruits.

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References

- Aleixos, N., J. Blasco, F. Navarron, and E. Molto. 2002. Multispectral inspection of citrus in real-time using machine vision and digital signal, *Computers and Electronics in Agriculture*, 33: 121-137.
- Andrew, W. 2002. Vision system grades moving food products. *Vision System Design*. June 2002: 26-29.
- Davenel, A., Guizard, C., Labarre, T., and F. Sevila. 1998. Automation Detection of Surface Defects on Fruit by Using a Vision System, *J. agric. Engng Res*, 41: 1-9
- Ishii, T., Toita, H., Kondo, N., Tahara, N. 2003. Deciduous Fruit Grading Robot(Part 2) —Development of Image Processing System—(in Japanese). *J. JSAM* 65(6):173-183.
- Kondo, N. 2006. Machine Vision Based on Optical Properties of Biomaterials for Fruit Grading System, *Environment Control in Biology*, 44(3): 3-11.
- Kurita, M., N. Kondo, and K. Ninomiya. 2006. Defect Detection for Tomato Grading by use of Six Color CCD Cameras, *Journal of Science and High Technology in Agriculture*, 18 (2): 135-144.
- Leemans, V., H. Magein, M. F. Destain. 2002. On-line Fruit Grading according to their External Quality using Machine Vision, *Biosystems Engineering*, 83(4): 397-404.
- Leemans, V., M. F. Destain. 2004. A real-time grading method of apples based on features extracted from defects, *Journal of Food Engineering*, 61: 83-89.
- Lu, R. and D. Ariana. 2002. A near-Infrared Sensing Technique for Measuring Internal Quality of Apple Fruit, *Applied Engineering in Agriculture*, 18(5):585-590.
- Njoroge, J. B., K. Ninomiya, N. Kondo, and H. Toita, 2002. Automated Fruit Grading System Using Image Processing. In :*Proc 2002 SICE*, Osaka. 1290-1295.
- Ogawa, Y., N. Kondo and S. Shibusawa. 2005a. Internal Quality Evaluation of Fruit with Soft X-ray CT, *Journal of the JSAM*, 67(3): 114-121.
- Ogawa, Y., N. Kondo and S. Shibusawa. 2005b. Internal Quality Evaluation of Fruit with Soft Transparent Image, *Journal of Science and High Technology in Agriculture*, 17(2):75-83.
- Slaughter, D.C., D.M. Obenland, J.F. Thompson, M.L. Arpaia and D.A. Margosan. 2008. Non-destructive freeze damage detection in oranges using machine vision and ultraviolet fluorescence. *Postharvest Biological Technology* 48: 341-346.
- Uozumi, J., Kohno, Iwamoto, and Nishinari. 1987. Spectrophotometric System for the Quality Evaluation of Unevenly Colored Food, *Journal of the JSFST*, 34(3):163-170.

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