FLUORESCENCE DETERMINATION OF THE OPTIMUM HARVESTING TIME OF GREEN BEAN AND GREEN PEAS USING ARGON LASER

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ABSTRACT

Argon laser with wavelength 488nm (visible light) and with power 128 mW was used in the laser-induced fluorescence technique in Egypt for determining the optimal harvesting time of green beans (Phaseolus vulgaris L. var Paulista) and Sweet peas (Pisum Sativum L. var Sugar Lays) depending on maturity, which presented a non-destructive methods and very accurate method to check the maturity. The obtained results are summarized as follows:

1) The fluorescence curve from green bean pods shows two peaks, the higher band was about 677 nm and followed by a smaller one centered at about 755 nm in the red region of the spectrum. Moreover, the higher intensity band was found to associate with a shoulder at 700 nm, 2) The fluorescence curve for sweet pea pods shows two peaks, the higher band was about 680 nm and followed by a smaller one centered at about 722 nm in the red region of the spectrum. Moreover, the higher intensity band was found to associate with a shoulder at 710 nm, and 3) Criteria for optimal harvest time for green beans and sweet peas are maximum intensity of fluorescence, 3.91 E-01a.u. and 2.23 E-02 a.u. at 22 days from the appearance of the flower, respectively.

Key Words: Optimal harvesting time, Green beans, Sweet peas, Laser Induced Fluorescence.

INTRODUCTION

Saito (2007) stated that LASER-induced fluorescence (LIF) is emitted from leaf pigments irradiated with LASER. LIF contains lots of information on living plants, so LIF spectroscopy, also known as the LIF technique, is a powerful tool for plant investigation.

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The most important feature of plants which discriminates them from other living organisms is photosynthesis. Photosynthesis is activated by the reaction of plant pigments by photons (light), i.e., it is a light-dependent reaction.

Astafurova et al. (2001) studied the LASER-induced fluorescence of birch, pine, and aspen trees. The fluorescence of birch leaves, excited with a Xe–Cl LASER at a wavelength of 308 nm, was measured under laboratory conditions. A persistent directly proportional dependence was found between the measured fluorescence signals and the chlorophyll content.

Schuerger et al. (2003) summarized that healthy and stressed plants were measured with two hyperspectral imagers, LASER-induced fluorescence spectroscopy (LIFS), and LASER-induced fluorescence imaging (LIFI) systems in order to determine if the four handheld remote sensing instruments were equally capable of detecting plant stress and measuring canopy chlorophyll levels in bahia grass.

Wulf et al. (2005) stated that LASER-induced fluorescence spectroscopy (LIFS) was non-destructively applied to apple fruit and carrot for determining changes in fruit and vegetable pigment contents. The samples were excited by short LASER pulses emitting at 337 nm, and recordings of fluorescence spectra were carried out directly on the tissue surface and in fruit extracts in a wavelength range from 350 to 820 nm.

Saito et al. (2006) stated that Broad LIF spectrum 400 nm to 800 nm gave information about pigments inside the leaves. Plant leaves can emit fluorescence in response to LASER irradiation which is called LASER-induced fluorescence (LIF). The LIF spectrum varies its shape depending on molecule species and concentration containing in the leaves.

Buschmann and Lichtenthaler (1997) used a laser-induced florescence imaging system, which can be used as a non-invasive tool to obtain images of leaves.

Bodria et al. (2002) mentioned that among the several changes that a fruit undergoes during ripening, chlorophyll degradation is responsible for degreasing of ground color, that is a well established maturity indicator for several species.

Takeuchi et al. (2002) reported that growth monitoring of agricultural products and prediction of their harvest time were tried by applying laser-induced fluorescence as a nondestructive and in vivo method. Two kinds

of lettuce grown outside were used. The laser-induced fluorescence measurement system was constructed with a 355 nm pulsed laser and a multi-spectral detection system. The fluorescence spectra showed peaks at 460, 520, 685 and 740 nm.

Kameoka and Hashimoto (2003) considered that the fluorescence around 532 nm have the information on carotene etc. and 685 nm fluorescence that of chlorophyll. In addition, the information on sucrose, chlorogenic acid, saccarides and polyphenol are included in the shorter wavelength fluorescence.

Corp et al. (2003) summarized that in vivo fluorescence emissions can occur in the wavelength region from 300 to 800 nm and are dependent on the wavelength of illumination for corn (Zea mays L.). These light emissions have been grouped into five primary bands with maxima most frequently received from corn at 320 nm (UV), 450 nm (blue), 530 nm (green), 685 nm (red), and 740 nm (far-red).

Mercure et al. (2004) stated that UV-induced chlorophyll (ChlF UV) and blue-green fluorescence (BGF) emitted by leaves have been proposed as useful indicators of plant physiological status under stress conditions. They investigated the effects of nitrogen (N) deficiency on ChlFUV and BGF emissions of leaf sections in relation to plant growth inhibition and accumulation of phenolic metabolites in barley leaves. The main objectives of this research are to study Laser Induced Fluorescence (LIF) technique in Egypt for determining the optimal harvest time of green beans and green peas depending on maturity.

The main objective of the present investigation: 1) The developed setup was used to measure the fluorescence properties by using Argon laser in Laser-Induced Fluorescence (LIF) from green bean and green peas pods, and 2) Establish a criterion to identify Fluorescence properties to determine the optimal harvesting time of green bean and green peas pods according to optical emission.

MATERIAL AND METHODS

Fluorescence system: It consists of Argon laser, lens, monochromator, detector and computer (Fig. 1).

1. Argon Laser: The Argon (Ar^+) laser (Lexel laser 95 ion laser, USA) in the visible light (wavelength 488 nm) was used as a continuous wave (CW) with power 125mW. The laser was directed to a mirror with vertical holder to a pod.

2. Monochromator: The monochromator (Spex 750m) is an optical element that is used to isolate the different spectral components of light beam. Mononchromator has two main utilities in optical spectroscopy experiments **(Garica Sole et al., 2004):**

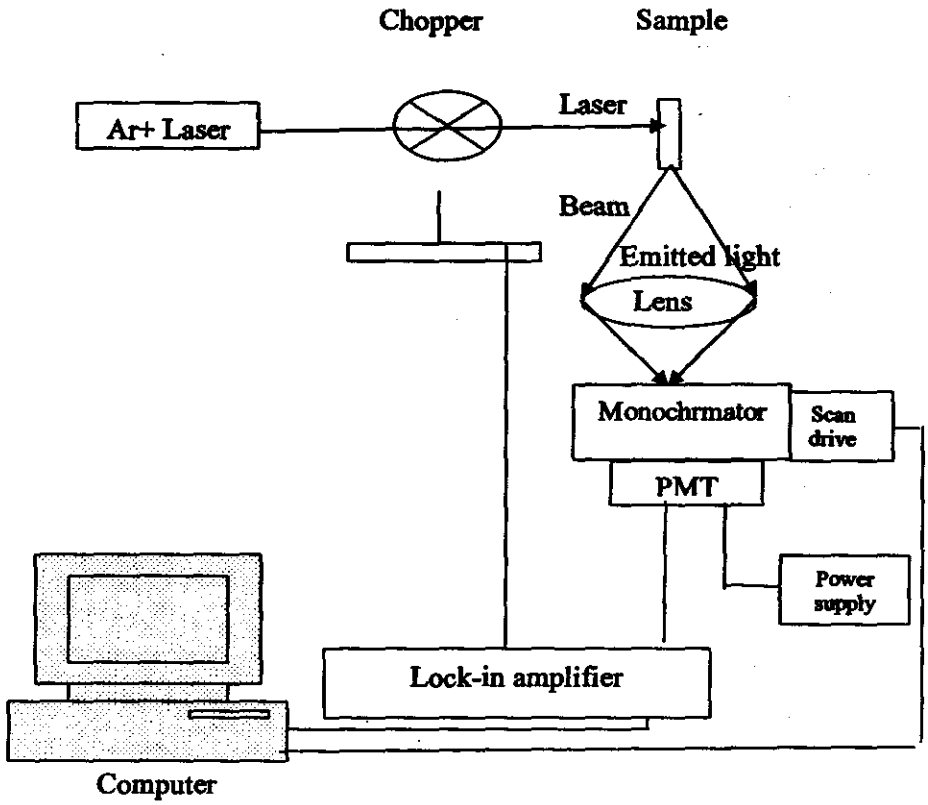
i) To transform the polychromatic beam generated by lamps into a monochromatic beam for selective excitation.

ii) To analyze the light emitted or scattered by any material after some kind of excitation. The emitted or scattered light usually extends over some range of the spectrum.

3. Spectrophotometer: It was used to measure the absorbance of light at different wavelengths to determine the concentration of the pigments at the green beans pods and green pea's pods. It has the following characteristics as shown in Table (1).

Table (1): Specifications of Spectrophotometer.

No.	Item	Specifications
1	Source of manufacture	U K
2	Model	6300
3	Wavelength: nm • Range • Resolution • Accuracy • Bandwidth	 320 – 1000 1 ± 2 8
4	Factor	0 to 199.9, 1000 to 99999
5	Photometric Noise Levels, %	<1
6	Photometric Stability	1% after 1 h warm-up
7	Readout	Custom LCD Graphics display
8	Outputs	Analogue (0-1.999V d.c.)/RS232 serial port
9	Light Source	Tungsten Halogen 20W 12V
10	Input Voltage	115/230 Vac – 20% + 10%
11	Input power, W	<50
12	Size, mm	365 (w) x 272 (d) x 160 (h)
13	Mass, Kg	6



a) The schematic setup of optical emission experiment.

1: Source of Argon laser, 2:Chopper, 3: Sample, and 4: Monochromator
b) The experimental setup of optical emission.

Fig. (1): Measuring the fluorescence of pods using the developed setup.

RESULTS AND DISCUSION

1.Green bean's fluorescence: Fig. (2) illustrates the fluorescence curve of green bean pods at 10 days age (for example). It shows two peaks, the higher band is about 677 nm (due to the chlorophyll fluorescence) and followed by the smaller one centering at about 755 (due to the carotenoid fluorescence) in the red region of the spectrum. Moreover, the higher intensity band was found to associate with a shoulder at 700 nm.

The same trend was found at different ages but with different intensities (Fig.3). The highest intensities were found for ages 19 and 22 days, but the smallest intensity was found at age 31 days.

Comparing the maximum values of intensity at different ages (Table 2 and Fig. 4), it is found that the maximum values of the fluorescence intensity increased from 3.70E-02 to 3.91E-01 a.u. at pods ages from 10 to 19 days and reached the maximum value 3.91E-01 a.u. at ages 19 and 22 days and then the intensities decreased from 3.91E-01 to 1.14E-02 a.u. at ages from 22 to 31 days, respectively. Also, Table (2) and Fig. (5) show the intensity values at wavelength 755 nm, the smallest band, values increased from 1.43E-02 to 5.82E-02 a.u. at ages from 10 to 19 days and the maximum value 9.65E-02 a.u. was at 22 days and then the values decreased to 4.18E-03 a.u. at age 31 days.

Using the mean values shown in Table (2), the following general equations were deduced to express the relationships between the value of maximum fluorescence intensity (F_{max}) and the fluorescence intensity at 755nm (F_{755}) for green beans pods at different ages from the appearance of the pods.

For the first age (pods at 10 days): $F_{max}=0.386\ F_{755}$.....(1)

For pods at 19 days: $F_{max}=0.149\ F_{755}$.....(2)

For pods at 22 days: $F_{max}=0.247\ F_{755}$.....(3)

For the last age (pods at 31 days): $F_{max}=0.367\ F_{755}$.....(4)

Because the fluorescence related to the quantity of chlorophyll a (chl.a) and total carotenoids (car), we studied the quantity of chl.a, chl.b, and car (Table 3). It is found that the quantity of chl.a decreased from 0.1115 to 0.0367 mg/g at pod's age 10 and 31 days respectively. Also, chl.b decreased from 0.0208 to 0.0072 mg/g for ages 10 and 31.

But, total car increased from 0.02945 to 0.04531 at the same ages. Therefore, the green color was the dominant at the first ages as a result of increasing chl.a and chl.b, then it would be light green as a result of increasing the other pigments (carotinoids). (Fig 6).

Table (2): The maximum intensities and the intensities at 755 nm for green bean pods at different ages from the appearance of the pods from flowers.

Age, day	Max. Intensity for green bean pods, a.u.*	Intensity at 755 nm for green bean pods, a.u.*
10	3.70E-02	1.43E-02
13	4.02E-02	1.30E-02
16	1.94E-01	3.54E-02
19	3.91E-01	5.82E-02
22	3.91E-01	9.65E-02
25	1.25E-01	2.67E-02
28	1.93E-02	5.40E-03
31	1.14E-02	4.18E-03

* a.u. = arbitrary unit

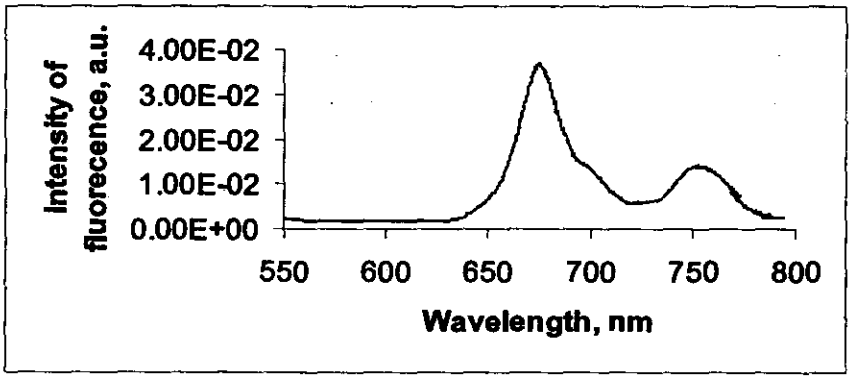


Fig (2): Green bean's pods fluorescence at age 10 days from the appearance of the pods from flowers.

Table (3): The quantity of chlorophyll a and b and total carotinoids for green bean's pods at different ages.

Age, day	A_{663}	A_{647}	A_{476}	Chl.a		Chl.b		Total Chl		Total Car	
				mg/l	mg/g	mg/l	mg/g	mg/l	mg/g	mg/l	mg/g
10	1.883	0.619	1	22.300	0.1115	4.151	0.0208	26.2947	0.1315	5.8897	0.02945
13	1.588	0.538	1.09	18.762	0.0938	3.832	0.0192	22.4612	0.1123	6.1955	0.03098
16	1.151	0.389	1.365	13.601	0.0680	2.758	0.0138	16.2632	0.0813	6.9923	0.03496
19	1.111	0.371	1.496	13.141	0.0657	2.569	0.0128	15.6178	0.0781	7.4860	0.03743
22	1.022	0.343	1.701	12.084	0.0604	2.399	0.0120	14.3975	0.0720	8.3212	0.04161
25	0.888	0.289	1.782	10.525	0.0526	1.897	0.0095	12.3481	0.0617	8.4722	0.04236
28	0.818	0.27	1.846	9.684	0.0484	1.826	0.0091	11.4424	0.0572	8.7331	0.04367
31	0.621	0.208	1.952	7.344	0.0367	1.449	0.0072	8.7409	0.0437	9.0628	0.04531

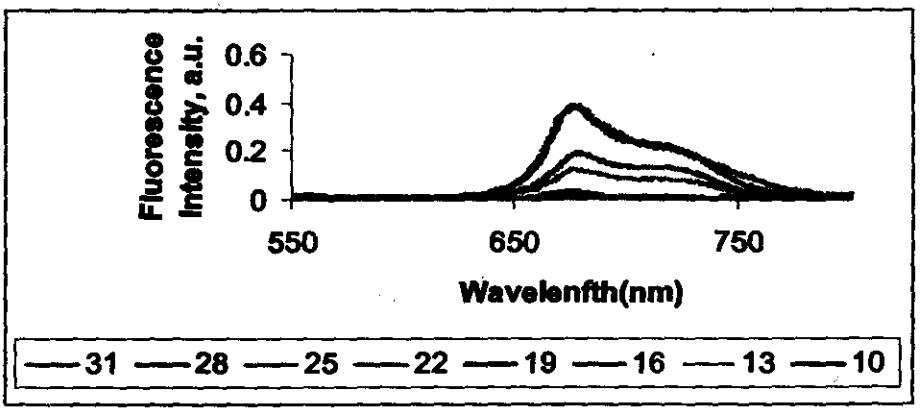


Fig (3): Green bean's pods fluorescence at different ages from the appearance of the pods from flowers.

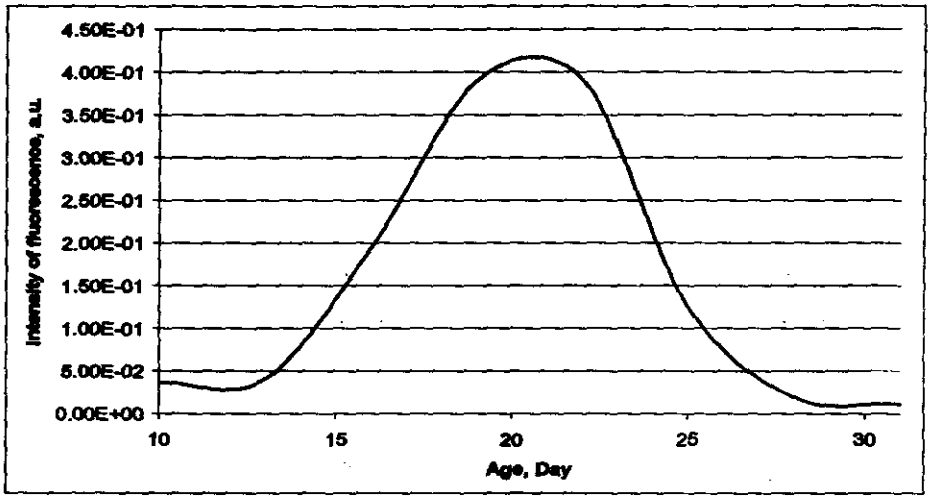


Fig. (4): The maximum intensities of fluorescence for green beans at different ages from the appearance of the pods from flowers.

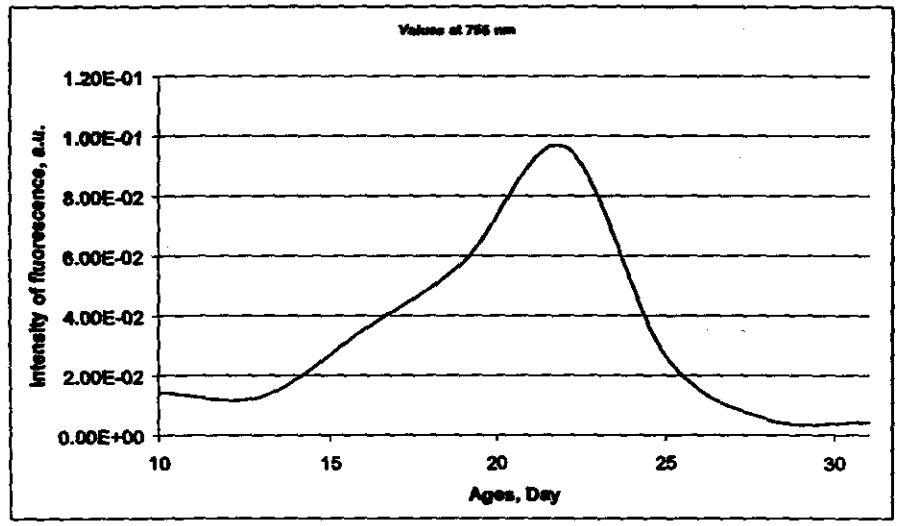


Fig. (5): The intensities at 755 nm for green beans fluoresce at different ages from the appearance of the pods from flowers.

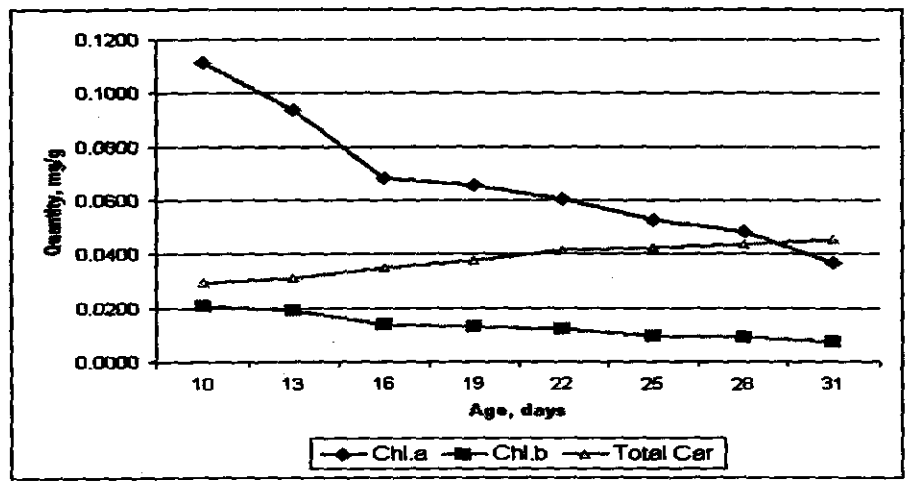


Fig. (6): Quantity of chlorophyll and carotenoids for green beans

2. Sweet pea's fluorescence:

Fig. (7) illustrates the fluorescence curve for sweet pea's pods at 6 days age (for example). The fluorescence curve shows two peaks, the higher band was about 680 nm (due to the chlorophyll fluorescence) and followed by a smaller one centered at about 722 (due to the carotenoid fluorescence) in the red region of the spectrum. Moreover, the highest intensity band was found to associate with a shoulder at 710 nm.

The same trend was found at different ages but with different values of intensities (Fig. 8). The highest intensities were found at age 18 days, while the smallest intensity was found for age 27 days.

Comparing the maximum values of intensity at different ages (Table 4 and Fig. 9), it is found that the maximum values of fluorescence intensity increased from 1.56E-02 to 2.23E-02 a.u. at ages from 6 to 18 days , then the intensities decreased from 2.23E-02 to 1.24E-02 a.u. at ages from 18 to 27 days. Also, Table (4) and Fig. (10) show the intensity values of fluorescence at wavelength 722 nm. The smallest band, values increased gradually from 7.87E-03 to 1.09E-02 a.u. for ages from 6 to 18 days, respectively and then the values decreased. Using the mean values shown in Table (4), the following general equations were deduced to express the relationships between the value of maximum fluorescence intensity (F_{max}) and the fluorescence intensity at 722nm (F_{722}) for sweet pea pods at different ages from the appearance of the pods.

For the first age (pods at 6 days): $F_{max}=0.504\ F_{722}$.....(5)

For pods at 15 days: $F_{max}=0.589\ F_{722}$.....(6)

For pods at 18 days: $F_{max}=0.489\ F_{722}$.....(7)

For the last age (pods at 27 days): $F_{max}=0.531\ F_{722}$.....(8)

Table (5) illustrates the quantity of ch.a , chl.b and total car, the quantity of chl.a and chl.b decreased from 0.1242 to 0.0184 mg/g and from 0.0242 to 0.0057 mg/g when pod's age increased from 6 to 33 days, respectively. However, total car increased from 0.0288 to 0.0440 mg/g when pod's age increased from 6 to 33 days, respectively. Therefore, the green color was dominant at the first ages as a result of increasing chl.a and chl.b, then it would be light green as a result of increasing the other pigments (carotinoids). (Fig 11).

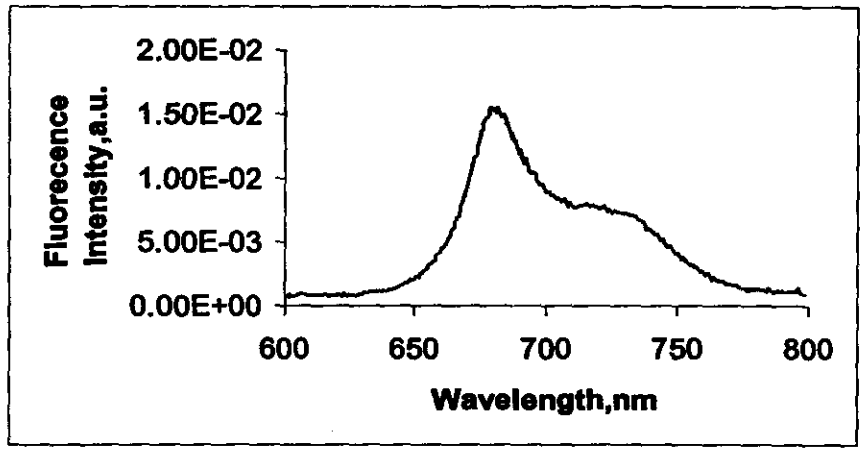


Fig (7): Sweet pea's pods fluorescence at age 6 days from the appearance of the pods from flowers.

Table (4): Maximum intensities and the intensities at 722 nm for sweet pea pods at different ages from the appearance of the pods from flowers.

Age, day	Max. Intensity for sweet pea pods, a.u.*	Intensity at 722 nm for sweet pea pods, a.u.*
6	1.56E-02	7.87E-03
9	1.56E-02	8.30E-03
12	1.60E-02	9.25E-03
15	1.42E-02	8.36E-03
18	2.23E-02	1.09E-02
21	1.25E-02	6.50E-03
27	1.24E-02	6.58E-03

- a.u. = arbitery unit

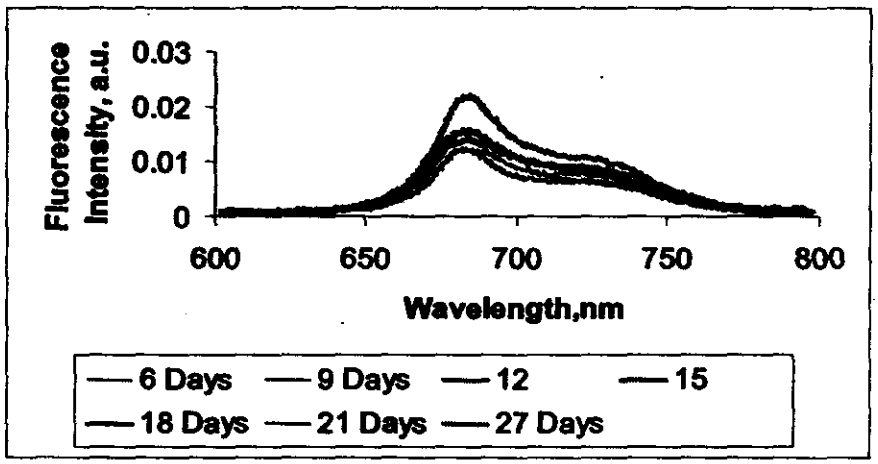


Fig (8): Sweet pea's pods fluorescence at different ages from the appearance of the pods from flowers.

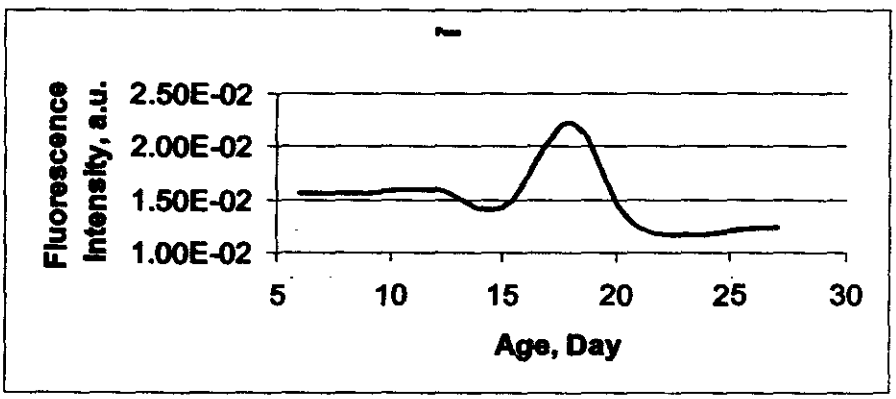


Fig. (9): The maximum intensities for sweet peas at different ages from the appearance of the pods from flowers.

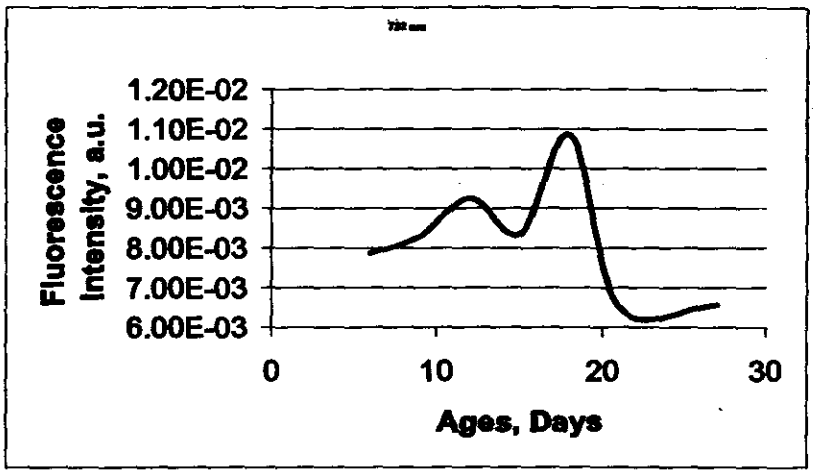


Fig. (10): The intensities at 722 nm for sweet peas pods at different ages from the appearance of the pods from flowers.

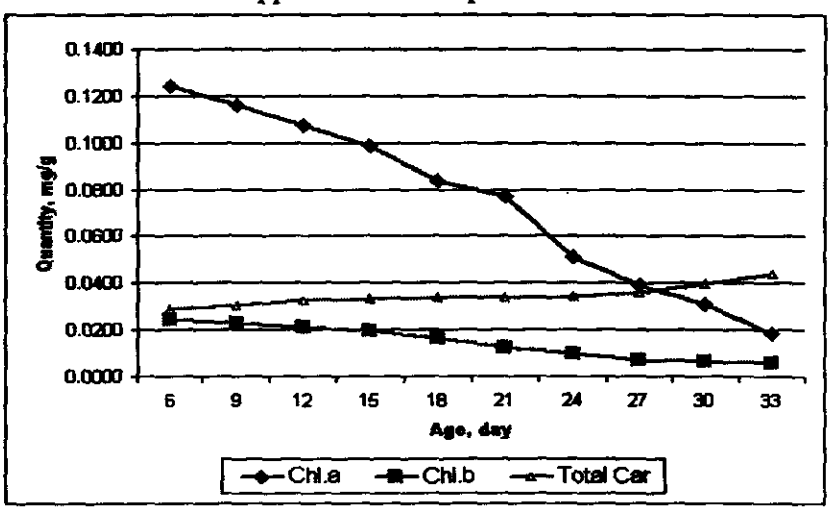


Fig. (11): Quantity of chlorophyll and carotenoids for sweet pea pods.

Table (5): The quantities of chlorophyll a and b, and total carotinoids for sweet pea's pods at different ages.

Age, day	A_{663}	A_{647}	A_{476}	Chl. a		Chl. b		Total Chl		Total Car	
				mg/l	mg/g	mg/l	mg/g	mg/l	mg/g	mg/l	mg/g
6	2.1	0.7	0.91	24.84	0.124	4.83	0.024	29.5	0.1475	5.768	0.0288
9	1.96	0.65	1.00	23.23	0.116	4.54	0.023	27.61	0.1381	6.051	0.0303
12	1.82	0.61	1.13	21.52	0.107	4.28	0.021	25.64	0.1282	6.551	0.0328
15	1.67	0.56	1.18	19.75	0.098	3.95	0.019	23.56	0.1178	6.635	0.0332
18	1.41	0.47	1.26	16.69	0.083	3.28	0.016	19.85	0.0993	6.734	0.0337
21	1.3	0.41	1.33	15.44	0.077	2.53	0.013	17.86	0.0893	6.745	0.0337
24	0.86	0.28	1.39	10.25	0.051	1.93	0.009	12.04	0.0602	6.815	0.0341
27	0.65	0.21	1.51	7.76	0.038	1.4	0.007	9.11	0.0456	7.125	0.0356
30	0.52	0.18	1.69	6.14	0.031	1.35	0.007	6.16	0.0308	7.893	0.0395
33	0.31	0.12	1.9	3.67	0.019	1.14	0.006	4.78	0.0239	8.803	0.0440

CONCLUSION

1) The developed setup was used to measure the fluorescence properties by using Argon laser in Laser-Induced Fluorescence (LIF) technique.
2) The fluorescence curve from green bean pods shows two peaks, the higher band was about 677 nm and followed by a smaller one centered at about 755 nm in the red region of the spectrum. Moreover, the higher intensity band was found to associate with a shoulder at 700 nm.
3) The fluorescence curve for sweet pea pods shows two peaks, the higher band was about 680 nm and followed by a smaller one centered at about 722 nm in the red region of the spectrum. Moreover, the higher intensity band was found to associate with a shoulder at 710 nm.
4) Criteria for optimal harvesting time for green beans and sweet peas are maximum intensity of fluorescence, 3.91 E-01a.u. and 2.23 E-02 a.u. at 22 days from the appearance of the flower, respectively

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الملخص العربى

التفلور الضوئي لتحديد الوقت الأمثل لحصاد الفاصوليا والبسلة الخضراء باستخدام ليزر الأرجون

أحمد الراعى امام سليمان[1] يحي عبد الحميد بدر[2] حلمى السيد حسن[3] راقيا خميس ابراهيم[4]

الهدف الرئيسي لهذا البحث هو دراسة تقنية الليزر لتحديد الوقت الأمثل لحصاد الفاصوليا (صنف (بوليستا) والبسلة الخضراء (صنف شوجر ايز) باستخدام ليزر الأرجون اعتمادا على درجة النضج بطريقة الفلورة (أنبعاث وميض ضوئي من أسطح الخضروات) المناسب للتبريد والتخزين والنقل خلال دورة السوق والتصدير.

أجريت التجربة الرئيسية فى معمل فيزياء الليزر بالمعهد القومى لعلوم الليزر- جامعة القاهرة ، وتم الحصول على العينات من صوب مزرعة المعمل المركزى للمناخ الزراعى – مركز البحوث الزراعية – دقى – جيزة خلال موسم زراعة ٢٠٠٥/٢٠٠٦ حيث تم جمع العينات كل ثلاثة أيام منذ خروج القرن من الزهرة وحتى مرحلة الجفاف والاصفرار ، تم تجميع جهاز لاستخدامه فى تقدير الخصائص الفلورية باستخدام ليزر الأرجون.

ويمكن ايجاز النتائج التى تم الحصول عليها فى الاتى:

١- أعطى منحنى الفلورة لقرون الفاصوليا الخضراء قمتين الأعلى عند طول موجى ٦٧٧ نانومتر متبوعة بأخرى أصغر عند طول موجى ٧٥٥ نانومتر فى المنطقة الحمراء من الطيف الضوئى . بالاضافة الى وجود منكب عند الطول الموجى ٧٠٠ نانومتر.

٢- بالنسبة لقرون البسلة الخضراء أعطى منحنى الفلورة قمتين الأعلى عند طول موجى ٦٨٠ نانومتر متبوعة بأخرى أصغر عند طول موجى ٧٢٢ نانومتر فى المنطقة الحمراء من الطيف الضوئى . بالاضافة الى وجود منكب عند الطول الموجى ٧١٠ نانومتر.

٣- تحدد الوقت الأمثل لحصاد قرون الفاصوليا والبسلة الخضراء طبقا لهذه الدراسة والمواصفات القياسية للجودة المعتمدة على الفلورة عند اعلى كثافة للفلورسنت ٣,٩١ X ١٠ $^{-١}$ وحدة وعند ٢,٢٣ X ١٠ $^{-٢}$ وحدة للقرون عند ٢١ ، ١٨يوم منذ ظهورها من الزهرة على التوالي.

١- أستاذ الهندسة الزراعية – كلية الزراعة – جامعة القاهرة – مصر.

٢- أستاذ فيزياء الليزر- المعهد القومى لعلوم الليزر- جامعة القاهرة – مصر.

٣- أستاذ مساعد تطبيقات الليزر فى الهندسة الزراعية - المعهد القومى لعلوم الليزر- جامعة القاهرة – مصر.

٤- باحث بمعهد بحوث الهندسة الزراعية – مركز البحوث الزراعية – الدقى – مصر.