INTERNATIONAL JOURNAL OF APPLIED AND EXPERIMENTAL BIOLOGY
(ISSN PRINT: 2790-6523; ISSN ONLINE: 2790-6531)
2025, Vol. ???, NO.???, ???
doi: https://doi.org/10.56612/ijaaeb.v1i1.150
ARK: https://n2t.net/ark:/71142/IJAaEB.v1i1.150
https://eminentscientists.com/

Chlorophyll and carotenoid quantification: An MS Excel-based calculator

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Abstract
Quantification of photosynthetic pigments, including chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids, is one of the potential determinants in plant physiology and related disciplines. Spectrophotometric methods employing the Arnon's equations are widely used for this purpose following pigment extraction in 80% acetone. However, manual calculation of these parameters is often a tedious and error-prone process, particularly for researchers analyzing large numbers of samples. To address this challenge and enhance efficiency in plant physiology laboratories, we have developed a user-friendly Chlorophyll Calculator as an MS Excel spreadsheet. This technical note describes the development of the calculator and highlights its advantages for efficiently quantifying the photosynthetic pigments. This simple software tool named *Chlorophyll Calculator v 1.2* was written in Microsoft® Excel 2010 for the mathematical calculations of the most fundamental photosynthetic pigments. This spreadsheet is based on the most popular methods of Arnon (1949) for chlorophyll estimation and of Davis (1976) for carotenoid determination. It can calculate up to four different types of photosynthetic pigments (chlorophyll *a, b*, total, and carotenoids). All estimates carried mean values and standard errors. The tool has been tested under different Microsoft® Excel for Microsoft Windows® environments. It is available free of charge for use in teaching and research. It can be obtained from the supplementary data of this article.

ARTICLE TYPE
Technical Note (TN)

SECTION
Plant Biology (PB)

HANDLING EDITOR
İbadullayeva, S.J. (PB)

ARTICLE HISTORY
Received: 30 Dec, 2024
Accepted: 21 Feb, 2025
Online: 6 Mar, 2025
Published: ?????????

KEYWORDS
Carotenoids;
Chlorophyll;
Microsoft Excel;
Pigment quantification;
Plant physiology;
Spectrophotometry

Introduction

Chlorophylls and carotenoids are essential photosynthetic pigments that play critical roles in light absorption and energy conversion in plants (Hashimoto et al., 2016; Maslova et al., 2021). Accurate quantification of these pigments is crucial for a wide range of plant physiological studies, including assessment of plant health, stress responses, photosynthetic efficiency, and pigment metabolism (Fernández-Marín et al., 2018). The spectrophotometric method, based on absorbance measurements, of pigment extracts in 80% acetone and subsequent calculations using equations devised by Arnon (1949), remains a standard technique in plant science laboratories globally (Manolopoulou et al., 2016; Salem et al., 2016).

Determination of photosynthetic pigments is one of the most important aspects of understanding plant growth and development mechanisms (Zeng et al., 2021). Moreover, with a major focus on uncovering underlying mechanisms of plant stress physiology and developmental biology in nineteenth century, determination of plant pigments has attained considerable importance. Now-a-days, changes in photosynthetic pigments are considered as an important component of our desire to

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CITATION (APA): Ahmad, M. S. A., Riffat, A., Rani, W. (2025). Chlorophyll and carotenoid quantification: An MS Excel-based calculator. *International Journal of Applied and Experimental Biology* ??(??):??????.

study stress effects and tolerance mechanisms in plants. Although, a number of sophisticated wet-lab based methods are available for the determination of photosynthetic pigments including HPLC (Picazo et al., 2013), laser fluorometry (Chekalyuk and Hafez, 2013) and other instruments that use non-destructive direct estimations (Laura et al., 2022) of photosynthetic pigments, the classical spectrophotometric methods of Arnon (1949) for chlorophyll and that of Davis (1976) for carotenoid estimation are still popular among plant scientists, because these methods are very convenient, cost-effective and reliable.

Despite its widespread use, the manual calculation of chlorophyll *a*, chlorophyll *b*, total chlorophyll, chlorophyll *a/b* ratio, carotenoids, and carotenoid/chlorophyll ratios using Arnon's and Davis's equations is a cumbersome and time-consuming task (Biswas, 2022). In fact, it involves multiple steps, including substitution of absorbance values at specific wavelengths (typically 663 nm, 645 nm, and 480 nm), along with acetone volume and fresh leaf weight, into several formulas (Arnon, 1949; Davis, 1976). This multi-step process is prone to human error, especially when processing large datasets, which is common in plant physiological experiments. Furthermore, many students and researchers, while proficient in experimental techniques, may find the direct implementation of these formulas in spreadsheet software like MS Excel or other calculation platforms, leading to considerable inaccuracies or inefficiencies.

This paper deals with an up-to-date and very comprehensive software tool that can calculate four basic photosynthetic parameters that are otherwise considered tedious. This method also minimizes the chances of human errors in mathematical calculations.

Extraction of chlorophylls and carotenoids

The fresh material is ground and chlorophyll is extracted in 80% acetone solution. The absorbance is read at 645 nm, 480 nm and 663 nm using a spectrophotometer.

Equations used for calculations

Chlorophyll *a, b* and total chlorophyll are calculated by the methods of Arnon (1949) following these equations:

$$\text{Chl. } a \text{ (mg/g leaf fresh wt)} = [12.7 \text{ (OD663)} - 2.69 \text{ (OD645)}] \text{ v/1000W}$$

$$\text{Chl. } b \text{ (mg/g leaf fresh wt)} = [22.9 \text{ (OD645)} - 4.68 \text{ (OD663)}] \text{ v/1000W}$$

$$\text{Total Chl. (mg/g leaf fresh wt)} = [20.2 \text{ (OD645)} - 8.02 \text{ (OD663)}] \text{ v/1000W}$$

Carotenoids are calculated by the method of Davis (1976) using the following equation:

$$\text{Carotenoids (mg/g leaf fresh wt)} = [\{\text{OD 480} + 0.114 \text{ (OD663)} - 0.638 \text{ (OD645)}\}/ \text{ EM}]\ 1000$$

Where, OD = optical density, v = volume of acetone used for chlorophyll extraction; W = fresh weight of leaf sample taken for chlorophyll extraction; EM is specific absorption extinction coefficient (Lichtenthaler, 1987) and is used for the calculation of carotenoids. The reference EM value is calculated at 10% and the default value in this worksheet is set at 2500. Users of this worksheet may enter EM values ranging from 1% to 100%. This worksheet will automatically recalculate carotenoid contents. It should be kept in mind that these calculated values assume a linear relationship between the absorption (OD) and EM value, which might not always be the case. However, this calculation generally provides a reasonable estimate.

Development of the MS Excel chlorophyll calculator

To overcome these limitations and provide a readily accessible and user-friendly tool, an MS Excel-based Chlorophyll Calculator has been developed. This spreadsheet tool is designed to automate the calculation process, minimizing manual efforts, and reducing the risk of computational errors.

Input matrices

Two types of inputs are required for these software-based calculations:

Input 1 requires the total volume of the extract (mL), fresh weight of sample material (g), number of

replications (1-10), blank OD (not necessary, but it may be used for old single beam spectrophotometers), and EM in case of carotenoid calculation.
Input 2 requires optical densities read at 480 nm, 645 nm and 663 nm by a spectrophotometer.

Entering data into input matrices

The Chlorophyll Calculator is structured with clearly labeled cells where users can input the following data for each sample:

(i) Absorbance at 663 nm: Spectrophotometric reading at 663 nm
(ii) Absorbance at 645 nm: Spectrophotometric reading at 645 nm
(iii) Absorbance at 480 nm: Spectrophotometric reading at 480 nm
(iv) Volume of 80% acetone (mL): The volume of 80% acetone used for pigment extraction
(v) Fresh leaf weight (g): The fresh weight of the leaf tissue used for extraction

Output matrix

The output matrix calculates four basic photosynthetic pigments including chlorophyll *a*, *b*, total chlorophyll and carotenoid contents. In addition, it can also calculate chlorophyll *a/b* ratio for individual replications, and it provides mean values and SE for each component. Upon entering the values in Input 1 and 2, the Excel sheet automatically calculates and displays the following output parameters in designated cells, using pre-programmed Arnon's equations:

(i) Chlorophyll a concentration (mg/mL or µg/mL): Calculated chlorophyll *a* concentration in the extract
(ii) Chlorophyll b concentration (mg/mL or µg/mL): Calculated chlorophyll *b* concentration in the extract
(iii) Total chlorophyll concentration (*mg/mL* or *µg/mL*): Sum of chlorophyll *a* and chlorophyll *b* concentrations
(iv) Chlorophyll a/b ratio: Ratio of chlorophyll *a* to chlorophyll *b*
(v) Carotenoid concentration (mg/mL or µg/mL): Calculated total carotenoid concentration in the extract
(vi) Carotenoids/Chlorophyll ratio: Ratio of total carotenoid content to total chlorophyll content

Units

The units of output matrix are mg g^{-1} fresh weight for chlorophyll *a, b*, total chlorophyll, and carotenoid contents.

Testing and Error

The formulas and coding of this spreadsheet have also been tested manually and the results were found satisfactory (**Figure 1**).

Software validation

Two of our recently published datasets (Ahmad et al., 2023; Attiq et al., 2024) were used to validate the calculations. The comparison of the accuracy of data entry and outputs for computation of chlorophyll calculations was done both manually and by using the software (**Table 1**). Standard deviations for Chl *a* and *b* values calculated both by the software and manually were computed. The results showed standard deviation values of 0.0007 (Chl *a*), and 0.0008 (Chl *b*) for dataset 1, and 0.0004 (Chl *a*), and 0.0009 (Chl *b*) for dataset 2. These findings confirmed that the calculations done by the software tool are highly reliable and can be used for calculation of photosynthetic pigments by researchers.

Software specifications

The tool runs on a personal computer and is written in Microsoft® Excel 2010. It is tested in Microsoft Windows 10, however, it can also work equally well on other older and new operating systems and versions of Excel.

Chlorophyll calculator v 1.2

Input 1	
DataSet name	Sample 1
Volume of extract (ml)	10
F. wt. of sample (g)	0.2
Number of replicates	3
Blank OD	0
EM for carotenoids (%)	10

Date: 2/21/2025

Input 2 (OD in nm)				Output (mg g^{-1})					
Rep	OD (480)	OD (645)	OD (663)	Chl a	Chl b	Total Chl	Chl a/b	Carot.	Chl a+b
R1	2.940	1.324	2.850	1.632	0.849	2.480	1.922	0.968	2.481
R2	2.126	1.214	2.974	1.725	0.694	2.419	2.485	0.676	2.419
R3	2.426	1.045	2.674	1.557	0.571	2.128	2.728	0.826	2.128
R4				0.000	0.000	0.000	0	0.000	0.000
R5				0.000	0.000	0.000	0	0.000	0.000
R6				0.000	0.000	0.000	0	0.000	0.000
R7				0.000	0.000	0.000	0	0.000	0.000
R8				0.000	0.000	0.000	0	0.000	0.000
R9				0.000	0.000	0.000	0	0.000	0.000
R10				0.000	0.000	0.000	0	0.000	0.000
Mean				1.638	0.705	2.342	2.379	0.823	2.343
SE				0.45742	0.20015	0.655208	0.23896	0.233	0.655375

Chlorophyll calculator v 1.2

Input 1	
DataSet name	Sample 2
Volume of extract (ml)	10
F. wt. of sample (g)	0.2
Number of replicates	3
Blank OD	0
EM for carotenoids (%)	10

Date: 2/21/2025

Input 2 (OD in nm)				Output (mg g^{-1})					
Rep	OD (480)	OD (645)	OD (663)	Chl a	Chl b	Total Chl	Chl a/b	Carot.	Chl a+b
R1	2.567	1.012	2.123	1.212	0.662	1.873	1.831	0.865	1.874
R2	2.119	1.128	2.359	1.346	0.740	2.085	1.820	0.667	2.086
R3	2.459	1.310	2.567	1.454	0.899	2.352	1.617	0.766	2.353
R4				0.000	0.000	0.000	0	0.000	0.000
R5				0.000	0.000	0.000	0	0.000	0.000
R6				0.000	0.000	0.000	0	0.000	0.000
R7				0.000	0.000	0.000	0	0.000	0.000
R8				0.000	0.000	0.000	0	0.000	0.000
R9				0.000	0.000	0.000	0	0.000	0.000
R10				0.000	0.000	0.000	0	0.000	0.000
Mean				1.337	0.767	2.104	1.756	0.766	2.104
SE				0.37443	0.21641	0.590325	0.06971	0.2154	0.590486

Figure 1: The spreadsheet tool for calculation of the most fundamental photosynthetic pigments, displaying two sample sets of input and output data (dataset 1 and dataset 2 used in Table 1). This spreadsheet is available free of charge from article supplementary data. Reference EM is set at 10% (2500). However, it can be entered in a range from 1% to 100% as desired.

Table 1: A comparison of the calculations performed manually with the software

Rep.	Data value			Manually calculated		Calculated with software		Data source
	OD (480)	OD (645)	OD (663)	Chl. *a*	Chl. *b*	Chl. *a*	Chl. *b*	
Sample dataset 2								
R1	2.940	1.324	2.850	1.631	0.849	1.632	0.849	
R2	2.126	1.214	2.974	1.725	0.695	1.725	0.694	Attiq et al., 2024
R3	2.426	1.045	2.674	1.557	0.570	1.557	0.571	
Mean	-	-	-	1.638	0.705	1.638	0.705	
SE	-	-	-	0.45054	0.20963	0.45473	0.20015	
Sample dataset 2								
R1	2.567	1.012	2.123	1.211	0.662	1.212	0.662	
R2	2.119	1.128	2.359	1.347	0.740	1.346	0.740	Ahmad et al., 2023
R3	2.459	1.310	2.567	1.455	0.900	1.454	0.899	
Mean	-	-	-	1.338	0.767	1.337	0.767	
SE	-	-	-	0.20514	0.1186	0.37443	0.21641	

Advantages of the MS Excel chlorophyll calculator

The MS Excel Chlorophyll Calculator offers following advantages for researchers and students involved in plant pigment analysis:

Ease of Use: The spreadsheet is designed with a simple and intuitive interface, requiring minimal Excel proficiency. Users only need to input the measured values into clearly marked cells.

Reduced Calculation Errors: Automation eliminates the potential for manual calculation errors, ensuring accuracy and consistency in data processing, especially when dealing with large datasets.

Time Efficiency: The calculator significantly reduces the time spent on calculations, freeing up researchers to focus on experimental designs, data interpretation, and other critical aspects of their work.

Accessibility and Availability: MS Excel is widely available in research institutions and universities, making the calculator readily accessible to a broad range of users. The spreadsheet can be easily shared and distributed among lab members and collaborators.

Customization and Adaptability: While pre-programmed with Arnon's equations, the Excel sheet can be readily adapted to incorporate other sets of equations for chlorophyll and carotenoid calculations, or to modify output units as per specific research needs.

Frequently asked questions

Question: What should one do if only a single beam spectrophotometer is available?
Answer: No matter if someone is using a single beam spectrophotometer. A blank can be run for the extraction procedure and then the blank OD can be entered in the *Input 1* matrix. This spreadsheet can subtract the blank optical density from those at all wavelengths.

Question: Can a blank be run on a double beam spectrophotometer?
Answer: Yes, because it is necessary to minimize chances of turbidity during the process of extraction. However, most of double beam spectrophotometer models have the function of *Auto Zero* to subtract blank reading automatically. If you are using “auto zero” function in a double beam spectrophotometer, do not enter blank OD in the *Input 1* matrix.

Question: What could be done if there are less than 10 replicates?
Answer: The number of replicates can be entered in the *Input 1* matrix so that all calculations are dependent on them. However, a maximum of 10 replicates are allowed in this spreadsheet. In addition, correct number of replicates must be entered for attaining accurate results.

Question: Can this software calculate other method-based estimations?
Answer: No. Currently, only methods of Arnon (1949) and Davis (1976) are supported in this spreadsheet. However, other method-based calculations may be incorporated in later versions of this spreadsheet.

Question: What is the range of EM values for carotenoids?
Answer: Users of this worksheet can enter EM values ranging from 1 to 100. This worksheet validates the entered data values and prompts an error message, if values are not entered within the permissible range.

Distribution

This software tool is available free of charge for use and distribution in teaching and research. It can be obtained from the supplementary data of this article.

Conclusion

The MS Excel-based Chlorophyll Calculator provides a practical and efficient tool for the efficient quantification of photosynthetic pigments in plant physiology research. By automating the calculation process based on spectrophotometric measurements and established equations, this calculator minimizes manual efforts, reduces errors, and enhances the overall efficiency of chlorophyll and carotenoid analysis in plant science laboratories. This tool is particularly beneficial for students and researchers who may find manual calculations cumbersome or error-prone, thereby promoting more accurate and reliable data generation in plant pigment studies.

Author(s), Editor(s) and Publisher's declarations

Source of funding
None declared.

Conflict of interest
The authors declare no conflict of interest.

Contribution of authors
Development of software tool: MSAA. Testing for errors: AR. Write up of first draft of the manuscript: MSAA, AR. Revision of the manuscript: ER. Reading of the final proof: All authros.

Ethical approval
This study does not involve human/animal subjects, and thus no ethical approval is required.

Handling of bio-hazardous materials
Since this is a technical note, so it does not involve any experimentation or use of any types of materials/chemicals.

Supplementary material
The information on usage of supplementary material can be found on the journal website.

Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher/Journal management. These are available with the corresponding author (s) and/or with the co-author(s) as declared by the corresponding author (s) of this manuscript.

Authors' consent
All authors have critically read this manuscript and agreed to publish in IJAaEB.

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Declaration of generative AI and AI-assisted technologies in the writing process

It is declared that the authors did not use any AI tools or AI-assisted services in the preparation, analysis, or creation of this manuscript submitted for publication in the International Journal of Applied and Experimental Biology (IJAaEB).

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