DETERMINATION OF CHLOROPHYLLS AND CAROTENES CONTENT IN SOME VEGETABLES OF ETHIOPIABY USING UV-VIS SPECTROSCOPY

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Abstract
Several research results have demonstrated that vegetable pigments play important roles in health. They have biological functions to health benefit, such as strengthening the immune system, reducing the risk of degenerative diseases, antioxidant properties, cancer chemo protection and anti obesity/hypolipidemic activities and others, Therefore information on availability and consumption of vegetables are vital in designing sustainable interventions to prevent micronutrient deficiencies. Hence, this study aimed to assess the chlorophylls and carotenes content of seven different vegetables by using spectrophotometric method, testing three organic solvents to obtain the best extraction solution. The carotenoid (C_{x+c}) *and chlorophyll* (Ch_a&Ch_b) *pigments extracted in acetone, methanol and ethanol were calculated according to "trichromatic equations" of Lichtentaler and Wellburn on the absorbance of three wavelengths: (470 nm, 661.6 nm and 645 nm), (470 nm, 665.2 nm and 652.4 nm) and (470 nm, 664 nm and 649 nm) respectively. The chlorophylls amount in collard green, Swiss chard, lettuce vegetables and carotenoids content in carrot, Swiss chard, collard green vegetables were found the highest. From Swiss chard, lettuce, cabbage, collard green, tomato, green pepper and carrot vegetables the maximum extractant solvent was methanol for chlorophylls and acetone for carotenoids. The experiment obtained appreciable results and the vegetables were graded in the order of their respective chlorophylls and carotenes content.*

Keywords: Vegetables, Chlorophylls, Carotenoids, Extraction, Spectrophotometry

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1. INTRODUCTION

Vegetables play an important role in human diets, as they support the normal functioning of the different body systems. They provide our cells with vitamins, minerals, fibber, essential oils and phytonutrients. Vegetables contain low amounts of fat and calories(Banerjee et al., 2012).
Leafy vegetables also contain several types of photosynthetic pigments that are chlorophylls and carotenoids. The composition of these pigments produces specific coloration of the food, which is one of the assessed visual quality attributes(Xu et al., 2009). In addition, chlorophyll and carotenoid concentration correlate to the photosynthetic potential of plants giving some indication of the physiological status of the plant. However, the content of pigments in plants is important, not only due to the coloration and physiological function, but also due to their acknowledged roles in health. For example, carotenes are the sources of vitamin A, Lutein and zeaxanthin are important factors for human vision (Znidarcic et al., 2011).Carotenoids and chlorophylls have an important role in the prevention of various diseases associated with oxidative stress, such as cancer, cardiovascular diseases and other chronic diseases(Sangeetha et al., 2010). The interest in new data on carotenoids in edible plants is increasing due to a more extensive use of natural compounds in the food, following the directives of Ethiopian Community in favour of natural rather than synthetic compounds.
The present study was undertaken with an aim to evaluate the pigments content of vegetables commonly consumed in the Gamo zone of Ethiopia, using three organic solvents, in order to obtain thebest extraction solution. Absorptive properties of the pigments were

followed up in quantitative analysis using a spectrophotometric method. Thechlorophyll pigments have broadabsorption band from blue to red. Also the coextracted carotenoids have maximum absorptionin blue band so that the chosen determination method is based on measuring the absorbance atthree wavelengths for each type of matrix and then calculate chlorophyll and carotene usingthe three "trichromatic equations" of Lichtentaler and Wellburn (Lichtenthaler et al., 1985; Wellburn,1994). The results of our study can be used as fundamental data for dietary recommendation to help the consumers to select appropriate types of vegetables to meet their nutrient and health needs.

2. MATERIALS AND METHODS

Instrumentation, Apparatus and Reagents

The common apparatuses are mortar and Pestle, centrifuge, grinder, Cuvette, UV-Visible spectrophotometer (Doublebeam, spectral range from 190 to 1100 nm,Specord 50 plus analytik Jena Germany), 25-100mLVolumetric flasks, whatman filter paper No.42 and filtering funnel. The common reagents used in the work were 95% Ethanol, Methanol, Acetone and distilled water.

Collection of Vegetable Samples

In this study, we selected seven types of vegetables (Swiss Chard, lettuce, cabbage, collard green, tomato, green pepper and carrot) for experimental purpose. These vegetables were collected from four sites of Gamo zone, Ethiopia in 200-300g portions. Freshvegetable samples were wash thoroughly first in tap water followed by distilled water in the laboratory, kept to dry in room temperature (20^0C) and analyzed for the determination of chlorophylls (Ch-a &Ch-b) and carotenoids content. UV spectrophotometer (Specord 50 plus Analytik JenaGermany) was used for the absorbance measurements.

Analytical Procedure

Accurately weighted 2 g of fresh vegetable samples was taken, and homogenized in tissue homogenizer with 40 ml of different extractant solvent. Homogenized sample mixture was centrifuge for 6,000 rpm for 20min at 8^0C (Costache et al.,2012;Lakra et al., 2018).The supernatant were separated and 2ml of it is mixed with 10ml of the respective solvent. The solution mixture was analyzed for Chlorophyll-a, Chlorophyll-b and carotenoids absorbances were read at 400-700 nm on UV spectrophotometer (Specord 50 plus Analytik Jena). The quantification of Chlorophyll-a, Chlorophyll-b, and Carotenoids by different extractant solvents was calculated according to the formulas of (Lichtentaler and Wellburn, 1985) the formulas were showed in Table 1; and spectral absorbance for Chlorophyll-a, Chlorophyll-b and Carotenoids for various solvents are represented in table 2.

Table 1: Equations to determine concentrations of Pigments (µg/ml) by different extractant solvents in Spectrophotometer

Solvents	Equations / Formula
Acetone	$Ch_a = 11.24\,A_{661.6} - 2.04\,A_{644.8}$ $Ch_b = 20.13\,A_{644.8} - 4.19\,A_{661.6}$ $C_{x+c} = \frac{(1000A_{470} - 1.90Ch_a - 63.14\,Ch_b)}{214}$
95% Ethanol	$Ch_a = 13.36A_{664} - 5.19A_{649}$ $Ch_b = 27.43\,A_{649} - 8.12A_{664}$ $C_{x+c} = \frac{(1000A_{470} - 2.13Ch_a - 97.63Ch_b)}{209}$
Methanol	$Ch_a = 16.72A_{665.2} - 9.16A_{652.4}$ $Ch_b = 34.09\,A_{652.4} - 15.28A_{665.2}$ $C_{x+c} = \frac{(1000A_{470} - 1.63Ch_a - 104.96Ch_b)}{221}$

Quality Control

Analytical reagents used during the extraction process were of AR grade (Marck). Milli Q water was used for preparation of intermediate solution and for dilution purpose (where ever needed). Quartz cuvette ($1cm^2$) was used and corresponding solvent was taken as reference during spectrophotometric observation. Every procedure (for each vegetable sample and extracting solvent) was triplicated formaintaining the precision of analytical results.

3. RESULT AND DISCUSSION

Analyses performed on some vegetables showed a large variety of chlorophylls and carotenes content were presented in Table 3 and Graph.1-3.

Table 2: Spectrophotometric absorbance value of Chlorophyll-a (Cha), Chlorophyll-b(Chb) and Carotenoids (C_{x+c}) of vegetables by using different organic solvents

Extractant Solvent	Swiss Chard			Lettuce			Cabbage			Collard Green		
	Cha	Chb	C_{x+c}	Cha	Chb	C_{x+c}	Cha	Chb	C_{x+c}	Cha	Chb	C_{x+c}
Acetone	1.2136	0.71	1.17	1.1221	0.7576	1.1721	0.4488	0.3251	0.5474	1.4267	0.9747	1.5807
Ethanol	0.8622	0.5117	0.9294	0.8345	0.6635	1.1621	0.4259	0.3285	0.6704	1.1351	0.8265	1.3807
Methanol	1.5276	1.0032	1.7279	1.2558	0.8653	1.325	0.5747	0.3822	0.6245	1.6336	1.1035	1.8806
	Tomato			Green Pepper			Carrot					
Acetone	0.4656	0.375	0.562	1.0076	0.6576	1.0714	0.6539	0.6573	1.2893			
Ethanol	0.4257	0.3099	0.622	0.9108	0.5062	1.0149	0.5361	0.5416	1.1613			
Methanol	0.4016	0.3087	0.5975	1.2694	0.846	1.3734	0.8757	0.5733	1.3715			

Table 3: Quantification of Chlorophyll-a, Chlorophyll-b and Carotenoids (µg/ ml) of vegetables using different organicsolvents

Extractant Solvent	Swiss Chard			Lettuce			Cabbage			Collard Green		
	Cha	Chb	C_{x+c}	Cha	Chb	C_{x+c}	Cha	Chb	C_{x+c}	Cha	Chb	C_{x+c}
Acetone	12.1925	9.2073	2.6425	11.0669	10.5489	2.2664	4.3813	4.6638	1.1430	14.0477	13.6428	3.2365
Ethanol	8.8633	7.0349	1.0704	7.7054	11.4237	0.1454	3.9851	5.5525	0.5733	10.8754	13.4539	0.2107
Methanol	16.3522	10.8574	2.5414	13.0708	10.3095	1.0028	6.1080	4.2478	0.7633	17.2057	12.6569	2.3714
	Tomato			Green Pepper			Carrot					
Acetone	4.4683	5.5979	0.9349	9.9839	9.0156	2.2579	6.0089	10.4916	2.8759			
Ethanol	4.0790	5.0439	0.5784	9.5411	6.4894	1.7274	4.3514	10.5030	0.6059			
Methanol	3.8871	4.3871	0.5914	13.4750	9.4437	1.6300	9.3903	6.1631	3.2096			

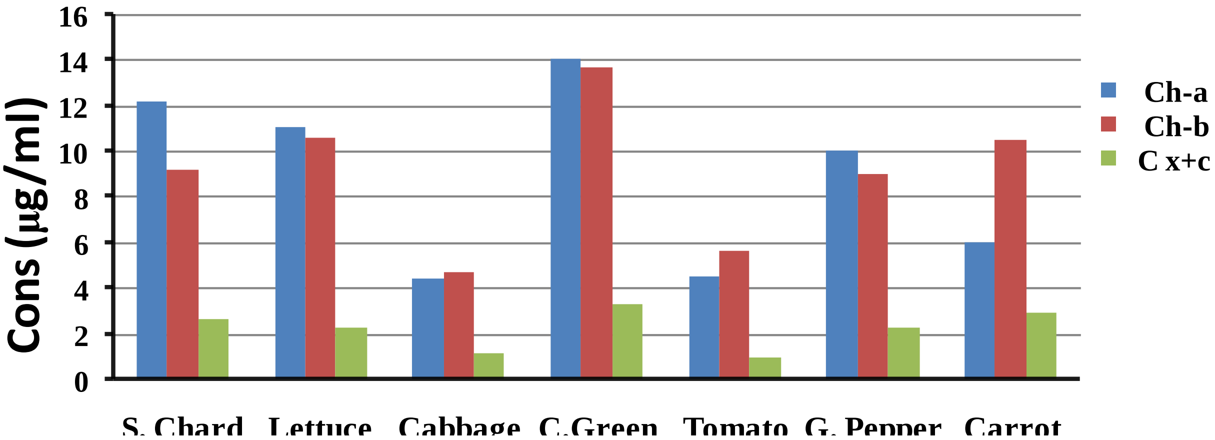

Fig.1. Pigment contents in vegetables using Acetone extraction

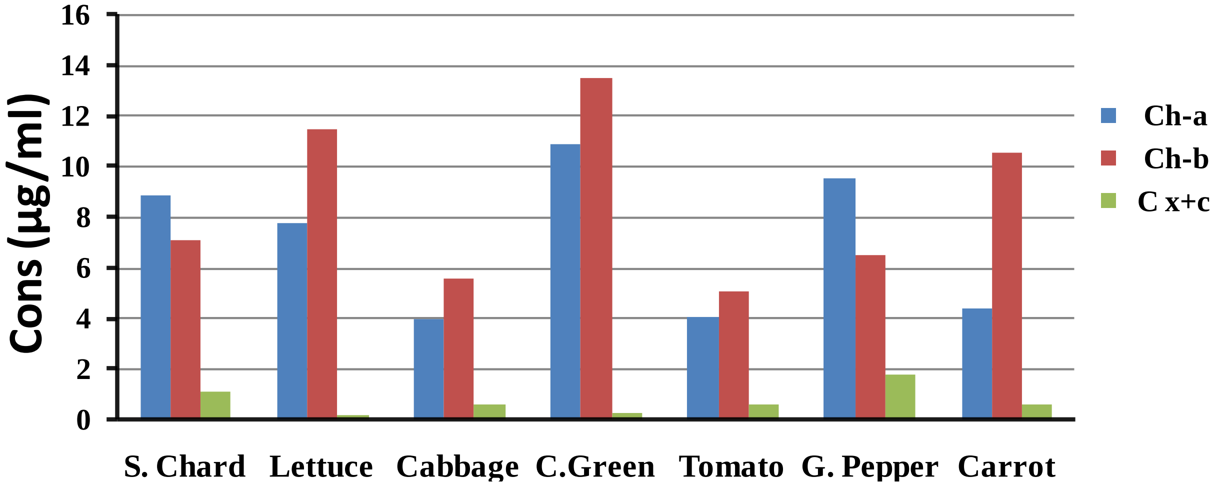

Fig. 2. Pigment contents in vegetables using Ethanol extraction

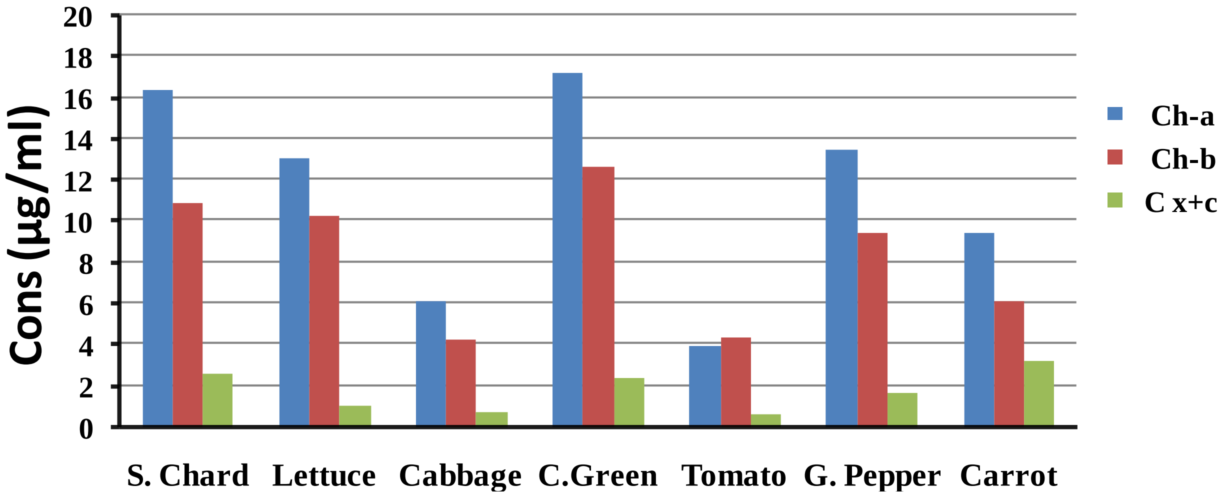

Fig.3. Pigment contents in vegetables using Methanol extraction
Note: S. Chard = Swiss Chard, C. Green = Collard Green, G.Pepper = Green Pepper

Chlorophylls Concentration in Vegetables

Chlorophyll-a is recognized as the main pigments which convert light energy into chemical energy. Chlorophyll-b as accessory pigments acts indirectly in photosynthesis by transferring the light it absorbs to chlorophyll-a. The chlorophyll molecule has Mg^{2+} at its center which makes it ionic and hydrophilic, and a ring that is hydrophobic in nature with a carbonyl group at its tail which makes it polar (Lichtenthaler et al., 1985; Costache et al., 2012).

The solvents used were important in the pigment extraction. In the case of Acetone extraction The average concentration of chlorophyll-a in the vegetables was the Collard Green had the highest concentration (14.05 μg/ml) followed by Swiss Chard(12.20μg/ml), and next by Lettuce (11.10μg/ml) while Cabbage recorded the least (4.38μg/ml) and Collard Green had the most amounts of chlorophyll-b (13.64μg/ml) and Cabbage had the least amount of chlorophyll-b(4.66μg/ml).

Methanol extraction determined Chlorophyll-a content in seven vegetable species the highest in Collard Green (17.21μg/ml) followed by Swiss Chard (16.35μg/ml) and the least value was in Tomato (3.89μg/ml) and also the content of chlorophyll-b in Collard Green showed the highest value (12.66μg/ml) and Cabbage showed the lowest value (4.25μg/ml).

In the third extracting solution ethanol the highest content of chlorophyll-a was found in Collard Green (10.88μg/ml) followed by Green

Pepper (9.54μg/ml) and the lowest content was in Cabbage (3.99μg/ml) and using ethanol extractantthe analysis of chlorophyll-b content of the vegetables used in the study revealed that it was highest in collard Green (13.45μg/ml) and least in Tomato(5.04μg/ml).
Highest extraction of chlorophylls (Ch-a andCh-b) is noted by using Methanol. Results also indicate Acetone as good extractant of chlorophylls after Methanol. For the studied vegetable species the Ethanol was lowest extraction.

Carotenoids Concentration in Vegetables
Carotenoids are located in chromoplast, contribution colour to vegetables/fruits; and also in chlorophylls, where together with chlorophylls involved in the two photosystems.Carotenoids group and their derivatives consist of about 70 compounds that are present in most vegetables and fruits (Costache et al., 2012).
Acetone solvent determined a different extraction of carotene, so at tomatoes the values 0.9349μg/ml in fresh product, in lettuce the values 2.2664 μg/ml,in Swiss Chard 2.6425 μg/ml,in Green Pepper 2.2579 μg/ml,in cabbage the value 1.1430 μg/ml, in collard green the value 3.2365 μg/ml, in carrot the value 2.8759 μg/ml, from these list collard green had the most amounts of total carotenoids and tomato had the least amount of total carotenoids were obtained (Table 3). Methanol extraction determined levels of carotene pigment in tomatoes obtained 0.5914μg/ml in fresh product, in pepper the concentrations of carotene were 1.6300 μg/ml in fresh product, in Swiss Chard the concentrations obtained 2.5414 μg/ml in fresh product, in lettuce the values 1.0028 μg/ml, in collard green2.3714 μg/ml, in carrot3.2096 μg/ml, in cabbage the values0.7633 μg/ml (Table 3). Due to this carrot had the most amounts of total carotenoids and tomato had the least amount of total-carotenoids. In the third extracting solution ethanol, the obtained carotene values in Swiss Chard, Lettuce, Cabbage, Collard Green, Tomato ,Green Pepper and Carrot were 1.0704,0.1454 ,0.5733 ,0.2107 ,0.5784 ,1.7274 and 0.6059 μg/ml respectively(Table 3), Collard Green had the most amounts of total carotenoids and lettuce had the least amount of total carotenoids.
Comparing the results of the three extractions of carotenoids, rapid extraction and highest value of carotenoids was observed by acetone, followed by methanol and the least extraction was by ethanol.

4. CONCLUSION

Results from this experiment clearly indicate that extraction of photosynthetic pigments by different solvents are depends on chemical nature of bio-molecules (cholorophyll-a, chlorophyll-b & carotenoids). The investigation reveals Ultraviolet-Visible spectrophotometry detection andPigments compound was extracted from seven vegetables with three different solvents in whichChlorophylls amount in collard green, Swiss chard, lettuce and Carotenoids content in carrot, Swiss chard, collard green were found the highest.Tomato and cabbage from seven vegetables were reported to have the least amount of chlorophylls and carotoinds. Moreover,the methanol was appeared an excellent extractant for chlorophylls and acetone was the best extractant for carotenoids from chosen vegetables for the study. The proposed method was rapid, simple, inexpensiveand easily performed. In addition, the method could be adopted by most quality control laboratories of food and drug industries since a spectrophotometer is a common instrument, which is generally available. However, there are some factors that must be taken into account, when comparing the content of natural pigments. For example climatic conditions, soil properties, environmental stresses and different types of vegetables carry great variability in the abundance of pigments; therefore further study in this context is recommended.

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Conflict of Interest Statement

The authors declare no conflict interest with regard to this publication.

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