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Determination of β-carotene in five commonly used Ethiopian vegetables using UV-Vis spectrophotometric method

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ABSTRACT

In this study, the amount of β-carotene in five commonly used Ethiopian vegetables and their parts including the flesh, peel, and seeds of pumpkin and butternut squash, the flesh and peel of eggplant and zucchini, and the carrot was determined and compared using UV-Vis spectrophotometry. Using the UV-Vis technique, β-carotene was determined at 450 nm. β-Carotene content was found to be 632 μg/g in carrots, 17-445 μg/g and 70.9-188 μg/g in flesh, peel and seed parts of pumpkin and butternut squash, respectively. 70.4-437 μg/g and 33-344 μg/g found in flesh and peel parts of zucchini and eggplant, respectively. In this study content of β-carotene was obtained higher in the carrot followed by the peel parts of pumpkin, zucchini and eggplants. β-Carotene in the seed parts of pumpkin and butternut squash were determined and found lower than the peel and flesh parts. The significant variance in β-carotene content from different vegetables was noted in this study. This variance may depend on the type of vegetables as well as the parts of the vegetables. According to the study's findings, all the five types of vegetables (pumpkin, butternut squash, eggplant, zucchini, and carrot) are rich sources of β-carotene.

Capsule Summary: The β-carotene content in five commonly used Ethiopian vegetables and their parts including the flesh, peel, and seeds of pumpkin and butternut squash, the flesh and peel of eggplant and zucchini, and the carrot was determined and compared using UV-Vis spectrophotometry.

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INTRODUCTION

Carotenoids are yellow, orange, and red lipophilic compounds found in plants, fruits and vegetables. Carotenoids can also be used as dyes in food, pharmaceutical products, cosmetic products, and can reduce the risk of various diseases such as eye diseases, degenerative diseases, and antioxidants (El-Qudah, 2009; Putri et al., 2021). Carotenoids are fat soluble compounds that are associated with the lipidic fractions. From a chemical point of view, carotenoids are polyisoprenoid compounds and can be divided into two main groups: (a) carotenes or hydrocarbon carotenoids only composed of carbon and hydrogen atoms and (b) xanthophylls that are oxygenated hydrocarbon derivatives that contain at least one oxygen function such as hydroxyl, keto, epoxy, methoxy or carboxylic acid groups. Their structural characteristic is a conjugated double bond system, which influences their chemical, biochemical and physical properties (Dincel et al., 2019). Among all carotenoids, β-carotene (Figure 1) is widely distributed in fruits and vegetables (Biswas et al., 2011). β-Carotene has the structural formula $C_{40}H_{56}$ which contains 40 carbons with 11 conjugated double bonds and two β-ionone rings at the end of the molecule. This structure makes β-carotene highly hydrophobic and non-polar in nature (El-Qudah, 2009). It is a fat-soluble pigment (Putri et al., 2021).

β-Carotene is the carotenoid compound present in abundance in the human diet and subsequently found in all human tissues including blood. Due to its high bioactivity, it is also widely used in medicine. Among the numerous functions of β-carotene in the human body, the important one is related to pro-vitamin A supply, further affecting embryonic development, correct growth, and sight (Berman et al., 2014; Harasym and Oledzki, 2014). β-Carotene in the body is converted into retinol (vitamin A). Retinol is very beneficial for the retina of the eyes, skin, and mucous membranes. β-Carotene also has properties such as reducing the risk of cancer, infectious diseases, antioxidants (El-Qudah, 2009; Eroglu and Harrison, 2013). β-Carotene can lower the risk of a number of diseases, including eye disorders, degenerative diseases, and antioxidants. It is utilized as a color in food, pharmaceutical items, and cosmetic products. It is advised that we eat these vegetables for a healthy lifestyle.

Not only bioactivity makes β-carotene a valuable food additive but the industrial importance also results from its coloring properties. In food industry, β-carotene is used as an orange-red pigment in many products, including non-thermally treated non-alcoholic beverages with a taste of tropical fruits, edible fats, cheese, pastry, and ice cream. In the pharmaceutical industry, it acts as a coloring agent for tablets, and in cosmetics industry, it is used as a bioactive ingredient of creams, which protects skin lesions against oxidation and exposure to UV radiation (Meléndez-Martínez et al., 2010; Fratianni et al., 2010). The recommended daily intake of vitamin A is 900 mcg for men, 700 mcg for women, and 300-600 mcg for children (Nisa and Walanda, 2021).

β-Carotene is a substance found in various food ingredients, especially in many green, yellow, orange, and red vegetables and fruits (Derradji-Benmeziane et al., 2014). However, the levels of β-carotene are influenced by several factors such as, type of the vegetables and fruits, level of maturity, climatic conditions, geographical location, part of the vegetables and fruits utilized, storage conditions, and types of solvent used for the extraction (Derradji-Benmeziane et al., 2014; Hagos et al., 2022). The β-carotene content in pumpkin, summer squash, tomato, red spinach, red chili, moringa, cherry, pineapple, banana, cabbage, sweet potato, and carrot (Biswas et al., 2011; Naid et al., 2012; De Carvalho et al., 2012; Sukmawati and Flanng, 2013; Octaviani et al., 2014; Tahir et al., 2016; Chandra et al., 2017; Zarnila et al., 2018; Makahity et al., 2019; Putri et al., 2021) cultivated in different countries has been determined. However, to the best of our knowledge, there is only, one reported data on β-carotene content in pumpkin (flesh, peel, and seeds) grown in Ethiopia (Hagos et al, 2022). In this study five vegetables such as pumpkin, butternut squash, zucchini, eggplant, and carrot were selected to determine and compare the content of β-carotene.

Pumpkin (*Cucurbita maxima*) is considered as an important vegetable due to its nutritional values. It is widely cultivated and consumed throughout the world (Hagos et al, 2022). It is low in calories but rich in vitamins and minerals, all of which are also in its seeds, leaves, and juices. It has a range of fantastic benefits, including being one of the best-known sources of β-carotene. Many studies have suggested that eating more plant foods, such as pumpkin, decreases the risk of obesity and mortality (Zuhanis, 2014).

Butternut squash (*Cucurbita moschata* Duchesne) is in the same family and genus as pumpkin with higher β-carotene content. Butternut squash is vegetable harvested in stages during the summer season and preserved for 3–6 months. “Butternut squash” varieties consumed in worldwide have orange colored peel and flesh. The change in peel color from green to orange is used as an indicator of fruit maturity and harvesting time. The yellow and orange color, on peel and pulp, are attributed to carotenoids contents (Zaccari and Galietta, 2015).

Eggplant (*Solanum melongena*) is among the nutritionally important and valuable crop in the Solanaceae family and grown worldwide for its edible vegetables (Mibei et al., 2016). Eggplant is from the nightshade family (which also includes tomatoes, okra, and zucchini) that grows in a variety of shapes, sizes, and colors, and thrives in hot climates and during the summer months. They range from small globes about two inches in diameter to more oblong and tubular varietals 12 inches long or larger. Eggplant vegetable is rich source of carbohydrate, fibers, minerals, vitamins (B_1, B_2, B_6, C, K, thiamine, niacin, and ascorbic acid and β-carotene) (Hornal et al., 2007; Shukla and Kumar, 2012; (Mibei et al., 2016).

Zucchini (*Cucurbita pepo* subsp. *pepo*) is a monoecious species grown as a vegetable, most parts are smooth and cylindrical; they are in general uniformly dark or light green but some cultivars are variegated. They have soft and edible skin (Río-Celestino et al., 2012). Like pumpkins, melons and cucumbers, zucchini belongs to the family of Cucurbitaceae (*Cucurbita*). Zucchini is a seasonal vegetable with high nutritional and medicinal values (Martínez-Valdivieso et al., 2017). Zucchini is rich in several vitamins, minerals, carotenoids, and other beneficial compounds (Blanco-Díaz et al., 2014).

The carrot (*Caucus Carlota* L.) belongs to the family *Apiaceae,* which is widely cultivated throughout the world. Edible part of carrot is root, which contains carotenoids, flavonoids, polyacetylenes, vitamins, and minerals. It possess numerous nutritional and health benefits. Besides lending truth to the old adage that carrots are good for eyes, carotenoids, polyphenols and vitamins present in carrot act as antioxidants, anticarcinogens and immunoenhancers (Aremu and Nweze, 2017).

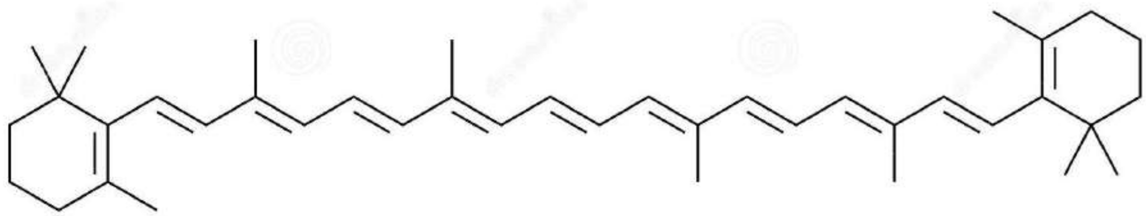


Fig. 1: Structure of β-carotene

Different analytical methods have been reported in the literature for the determination of β-carotene in fruits and vegetables such as HPLC (Kreck et al., 2006; Norshazila et al., 2014; Pritwani and Mathur, 2017), NMR (Moh et al., 1999; Norshazila et al., 2014; Quijano-ortega et al., 2020) and FTIR (Moh et al., 1999; Norshazila et al., 2014). UV-Visible spectrophotometry is a useful tool in identifying major carotenoids because the long conjugated polyene chain absorbs light in the range of 400 to 500 nm. It is important to note that the absorption spectra only allow for distinguishing different chromophore of the carotenoids. For quantitative analysis of carotenoids, UV-Vis spectrophotometry is the most convenient method (Moh et al., 1999; Barba, 2006; Carvalho et al., 2014; Zahra et al., 2016; Aremu and Nweze, 2017; Nisa and Walanda, 2021), by measuring the absorbance at different wavelengths (Moh et al., 1999; Norshazila et al., 2014; Zahra et al., 2016; Nisa and Walanda, 2021) UV-Vis spectrophotometry requires little sample amounts and preparation, and rapidly provides valuable and robust information about the presence of particular classes of metabolites, such as carotenoids (Moresco et al., 2015; Hagos et al., 2022).

Hence, the objective of the present study was to determine and compare the β-carotene content in five different selected Ethiopian vegetables and their different parts such as pumpkin (flesh, peel, and seeds), butternut squash (flesh, peel, and seeds), eggplant (flesh and peel), zucchini (flesh and peel) and carrot using UV-Vis spectrophotometric method.

MATERIAL AND METHODS

Instruments and apparatus

Samples were weighed using electronic balance (ARA520, Ohaus Corp, China). Blending device mortar and pestle was used to ground the dried samples. Centrifuge machine (800D, China), double-beam spectrophotometer (Lambda 950-UV-Vis-NIR, Perkin Elmer, UK) interfaced with a computer using 2 nm resolution in a 1 cm path length quartz cell were used for determination of β-carotene.

Chemicals and reagents

Acetone (Acsiso-Reagent, European Pharmacopoeia, 99.8%) was used for β-carotene extraction. β-Carotene standard (Sigma-Aldrich, 99%) were obtained from Ethiopian Public Health Institute.

Preparation of β-carotene standard solutions

The standard stock solution (15 μg/mL) of β-carotene was prepared by dissolving 750 μg of the standard β-carotene in 50 mL volumetric flask in acetone. From this stock solution, serial dilutions were made to obtain 0.1, 0.5, 1.5, 3, 6, 9, and 12 μg/mL of β-carotene. The working standard solutions were scanned in the spectral range (350–600 nm) selected for this study. The absorption spectral data were collected from their typical absorption peak maximum obtained at 453 nm for plotting the calibration curves.

Sample collection and preparation

The five different ripen vegetables were purchased from local market in Addis Ababa, Ethiopia. The vegetables included pumpkin (*Cucurbita maxima*), butternut squash (*Cucurbita moschata Duchesne*), zucchini (*Cucurbita pepo*), eggplant (*Solanum aethiopicum*), and carrot (*Daucus carota*). The pumpkin (*Cucurbita maxima*) and butternut squash (*Cucurbita moschata Duchesne*) have three parts (peel, flesh and seed), zucchini (*Cucurbita pepo*) and eggplant (*Solanum aethiopicum*) have two parts (peel and flesh) while carrot (*Daucus carota*) has only one part. Each of the five selected vegetables samples were washed with fresh running water and then with distilled water to remove any foreign materials attached. The separation of the three parts (peel, flesh, and seed) of the pumpkin and butter squash and the two parts (peel and flesh) of eggplant and zucchini was done manually with the help of a knife. The samples were cut in to small pieces and dried at room temperature for one week. The dried samples were ground with mortar and pestle. Finally, the powdered samples were used for the extraction of β-carotene.

Extractions of β-carotene

β-Carotene extraction was done according to the method reported by Hagos et al. (2022) with some modifications. The β-carotene was extracted by soaking 0.5 g of samples in 10 mL of acetone at room temperature under dark condition in order to get a complete extraction. The mixture was magnetically stirred for 30 min.

The extracts were centrifuged to separate the supernatant, and these operations were repeated until the pulp was completely colorless. The volume was made up to 30 mL with the extracting solvent.

Statistical analysis of data

All measurements were done in triplicates and results were presented as mean±SD (standard deviation) (n = 3). Statistical analyses were conducted using Origin 6.0 (Microcal Software, Inc., Northampton, USA).

RESULTS AND DISCUSSION

Identification of β-carotene in vegetable samples

Acetone was used as the extraction solvent for the UV-Vis spectroscopic methods (Hagos et al. (2022). The UV-Vis spectrum of standard β-carotene in acetone was scanned from 350 to 600 nm, and maximum absorption was obtained at 450 nm. This was in good agreement with that reported in literature (Moh, et al., 1999; Barba et al., 2006; Norshazila et al., 2014; Zahra et al., 2016; Nisa and Walanda, 2021; Hagos et al. 2022). The UV-Vis spectra of all vegetables extracts showed the maximum UV-Vis absorption at the same wavelength (Figure 2). This confirmed that the extraction procedure was valid and the extract contained β-carotene.

Method validation

The method was evaluated in terms of linearity, accuracy, precision, limits of detection (LOD), and quantitation (LOQ). Linearity of the method was performed in a range of 0.1 to 12 µg/mL, and the method showed good linearity with a regression equation: y = 0.25549x - 0.00344 (R^2 = 0.999) (Table 1 and Figure 3). In addition, both the detection limit and quantification limit were calculated to be 0.034 µg/mL and 0.10 µg/mL, respectively. Method precision showed RSD% of 1.5% to 11%. Method accuracy was evaluated by standard addition to the sample, and the results obtained showed good percent recovery (%R) of 83% to 95% (Table 1). The analytical parameters obtained are better than results in the literature reported (Karnjanawipagu et al., 2010; Biswas et al., 2011) (Table 1).

Quantification β-carotene

The concentration of β-carotene in the acetone extract of the samples of pumpkin (peel, flesh, and seeds), butternut squash (peel, flesh, and seeds), eggplant (peel and flesh), zucchini (peel and flesh), and carrot (flesh) was determined using the UV-Vis method. β-Carotene content in the samples (µg/g) were calculated using calibration curve. The levels of β-carotene were determined in the five-flesh part of pumpkin, butternut squash, eggplant, zucchini and carrot.

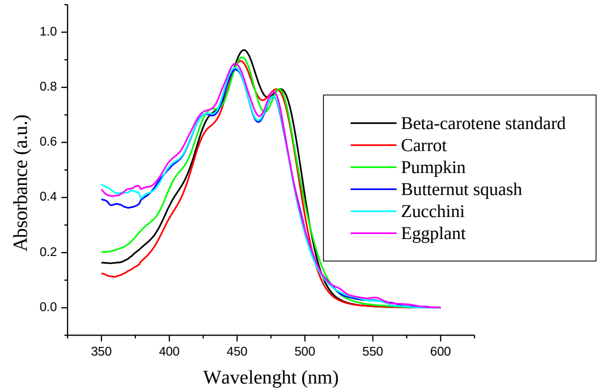


Fig. 2: UV–Vis spectra of standard β-carotene and β-carotene in the extract from pumpkin, butternut squash, eggplant, carrot and zucchini samples

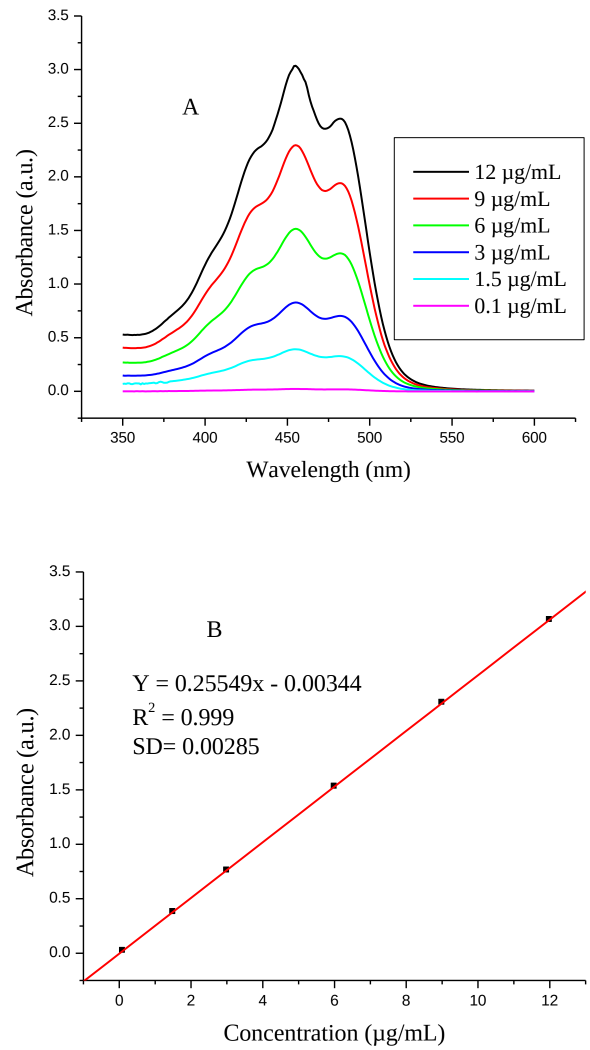


Fig. 3: UV–Vis spectra of different concentration of standard β-carotene in acetone (A) and calibration curve (B)

Table 1: Analytical parameters of this (UV-Vis) method such as R^2, LODs, LOQs, RSDs, and recoveries compared with the previously published methods

Linearity range (μg/mL)	R^2	LOD (μg/mL)	LOQ (μg/mL)	RSD %	Recovery %	Reference
0.1–12	0.999	0.034	0.10	1.5-11	83-95	This study
1-8	0.999	0.04	0.11	6.4	100	(Karnjanawipagu et al., 2010)
0.015–8	0.994	NR	NR	3.4–8.9	67.8–98.8	(Biswas et al., 2011)

Table 2: β-Carotene content (μg/g) of the flesh, parts of the five different vegetables

Flesh parts of different vegetables	β-Carotene (μg/g)
Pumpkin	341±0.12
Butternut squash	188±0.11
Zucchini	70.4±0.09
Eggplants	33.0±0.05
Carrot	632±0.22

Table 3: β-Carotene content (μg/g) of the peel, parts of the four different vegetables

Peel parts of different vegetables	β-Carotene (μg/g)
Pumpkin	445±0.13
Butternut squash	70.9±0.08
Zucchini	437±0.12
Eggplants	344±0.15

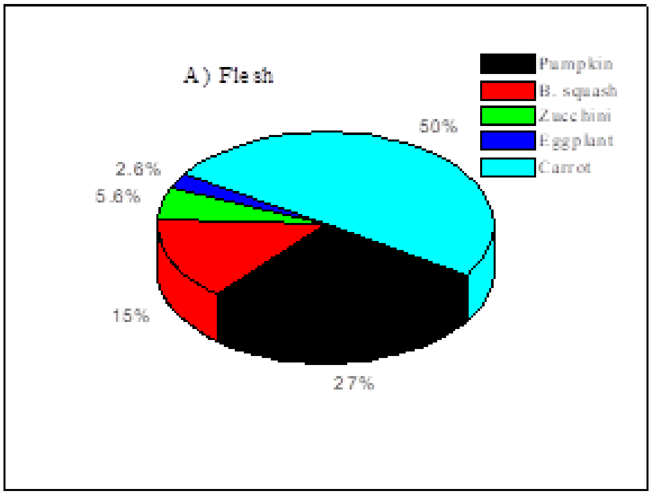
Table 4: Comparison of the β-carotene content (μg/g) of five different Ethiopian vegetables and their parts (peel, flesh and seed)

Vegetables types	Content of β-carotene (μg/g)		
	Peel	Flesh	Seed
Pumpkin	445±0.13	341±0.12	17.0±0.02
Butternut squash	70.9±0.08	188±0.11	120±0.21
Zucchini	437±0.12	70.4±0.09	ND
Eggplants	344±0.15	33.0±0.05	ND
Carrot	ND	632±0.22	ND

ND = not determined

The β-carotene content was found 33 μg/g in the eggplant flesh, 70.4 μg/g in the zucchini flesh, 188 μg/g in the butternut squash flesh, 341 μg/g in the pumpkin flesh and 632 μg/g in the carrot flesh. Content of β-carotene was obtained higher amount in the carrot followed by pumpkin, butternut squash, zucchini and eggplant. There was statistically significant difference ($p<0.005$) among the flesh parts in β-carotene content (Table 2).

Due to the absence of a peel on the carrot, β-carotene levels were also determined in the four vegetables with peels of pumpkin, butternut squash, eggplant, and zucchini. The β-carotene concentration was determined to be 70.9 μg/g in the peel of the butternut squash, 344 μg/g in the peel of the eggplant, 437 μg/g in the peel of the zucchini and 445 μg/g in the peel of the pumpkin. Pumpkin peels had the highest concentration of β-carotene, followed by those from zucchini, eggplant, and butternut squash. In terms of β-carotene content, there was a statistically significant difference ($p<0.005$) between the peel components (Table 3). Since zucchini, eggplant, and carrot do not contain seeds, β-carotene was only determined in pumpkin and butternut squash seeds. β-Carotene was found 17 μg/g and 120 μg/g in pumpkin and butternut squash seeds, respectively (Table 4). A comparison was made between the amounts of β-carotene found in the flesh, peel, and seeds of various vegetables, including pumpkin, butternut squash, zucchini, eggplants, and carrots (Table 4 and Figure 4).



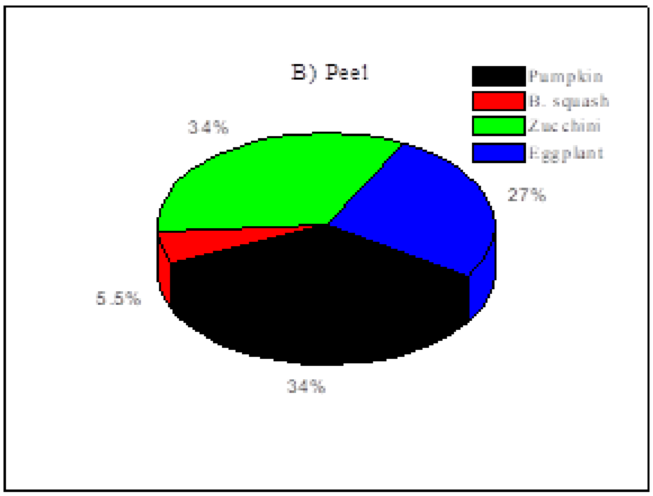


Fig. 4: Comparison of the β-carotene content (μg/g) A) flesh parts of pumpkin, butternut squash, eggplant, zucchini and carrot, B) peel parts of pumpkin, butternut squash, eggplant, and zucchini

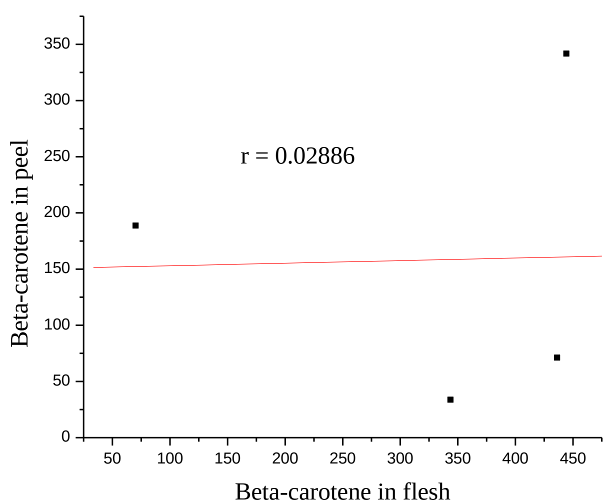


Fig. 5: Correlation of β-carotene content in the peel and flesh parts of the pumpkin, butternut squash, eggplant, and zucchini

β-Carotene in the vegetable parts were found in the range of 70.4 to 632 μg/g in the flesh, 70.9 to 445 μg/g in the peel, and 17 to 120 μg/g in the seeds. In this study, carrots' β-carotene content was shown to be higher than that of the other vegetables. When β-carotene content in the three parts of a pumpkin were compared, it was found that the pumpkin peel contained higher β-carotene than the flesh and seed. However, β-carotene in the pumpkin flesh was found higher than in the peel and seed when the three parts of a butternut squash were compared. The β-carotene content of the flesh and peel of zucchini and eggplants were compared, it was found that the peel had the highest levels, followed by the flesh (Table 4 and Figure 4). In terms of β-carotene content, there was a statistically significant difference ($p<0.005$) between the flesh, peel and seed parts of vegetables. In this study, the variation in β-carotene content was noted. This variation in β-carotene content may depend on the type of vegetables as well as the parts of the vegetables.

The amount of β-carotene found was compared to previously published research. In one study, between 156 and 2137 μg/g of β-carotene were found in pumpkin flesh (Quijano-ortega et al., 2020). The amount of β-carotene in pumpkin (*C. moschata*) flesh ranged in a study from 168 to 202 μg/g (Carvalho et al., 2014). In a research utilizing the HPLC-DAD method on pumpkin (*C. maxima*) flesh and peel, the amount of β-carotene varied from 17 to 263 μg/g in the flesh and 10 to 403 μg/g in the peel (Pritwani and Mathur, 2017). The concentration of β-carotene was found to be 17 μg/g in flesh, 123 μg/g in peel, and 31 μg/g in seed powder samples, according to Kim et al. (2012).

Comparison of the β-carotene content in the fresh pumpkin and pumpkin powder was done in order to use it in various baking goods (Pongjanta et al., 2006). Fresh pumpkin and powdered pumpkin both contained 24 and 73 μg/g of β-carotene, respectively. In three different types of pumpkin flesh samples, Nakazibwe et al. (2020) also showed β-carotene contents ranging from 27 to 1215 μg/g. Utilizing HPLC-UV-Vis, the presence of β-carotene in pumpkin (*Cucurbita moschatav* D.) was examined. β-Carotene content was also determined, with a result of 244 μg/g (Carvalho et al., 2014). The results of the β-carotene study on carrots (*Daucus carota*) were found 41 μg/g (Dai and Row, 2019). In carrot, β-carotene levels were measured, and the results ranged from 61.9-145.9 μg/g (Karnjanawipagu et al., 2010). In addition, when β-carotene levels in carrots were assessed, the findings ranged from 47.5 to 1030 μg/g (El-Qudah, 2009). In butternut squash, β-carotene levels were determined, and the results ranged from 75.6 to 119.8 μg/g (Mahmoud and Mehder, 2022). In zucchini, β-carotene levels were determined, and the results ranged from 0.1 to 24 μg/g (Dai and Row, 2019) in flesh and 7.43-157 μg/g in peel (Xu et al., 2021). β-Carotene levels in zucchini and eggplant flesh were also assessed, the results revealed 1.46 μg/g and 0.18 μg/g respectively (El-Qudah, 2009) (Table 5). In this study, the β-carotene concentration of pumpkin seed, flesh, and peel was found to be 17, 344, and 445 μg/g, respectively. β-Carotene levels were found 120 μg/g, 188 μg/g and 70.9

µg/g, in seed, flesh, and peel parts of butternut squash respectively. β-Carotene levels were found 437 µg/g, 70.4 µg/g in zucchini peel and flesh. 344 µg/g and 33 µg/g of β-carotene were found in peel and flesh parts of eggplant, respectively (Table 5).

peel and flesh. The levels of β-carotene among the five vegetables and their distinct parts varied significantly in this study. Varied amounts of β-carotene in vegetables were due to several environmental factors like types of vegetables and parts of vegetables.

Table 5: Comparison of β-carotene content (µg/g) in the five selective Ethiopian vegetables with previously reported researches

Sample	β-Carotene content (µg/g)			Reference
	Peel	Flesh	Seed	
Pumpkin	445	341	17	This study
Pumpkin	ND	156 - 2137	ND	(Quijano-ortega et al., 2020).
Pumpkin	123	17	31	(Kim et al., 2012)
Pumpkin	ND	24-73	ND	(Pongjanta et al., 2006).
Pumpkin	ND	27-1215	ND	Nakazibwe et al. (2020)
Pumpkin	450	771.1	1393.7	(Hussain et al., 2021)
Butternut squash	70.9	188	120	This study
Butternut squash	ND	75.6-119.8	ND	(Mahmoud and Mehder, 2022)
Carrot	ND	632	ND	This study
Carrot	ND	61.9-145.9	ND	(Karnjanawipagu et al., 2010)
Carrot	ND	47.5-1030	ND	(El-Qudah, 2009)
Zucchini	437	70.4	ND	This study
Zucchini	ND	0.1-24	ND	(Dai and Row, 2019)
Zucchini	7.43-157	ND	ND	(Xu et al., 2021)
Zucchini	ND	1.46	ND	(El-Qudah, 2009)
Eggplant	344	33.0	ND	This study
Eggplant	ND	0.18	ND	(El-Qudah, 2009)

Correlation of the β-carotene contents

The relationship between β-carotene content in the two parts of the pumpkin, butternut squash, eggplant, and zucchini were investigated. The correlation between the β-carotene content of flesh and peel ($r = 0.02886$) was determined using the Pearson correlation and the result found positive weak correlation (Figure 5).

CONCLUSION

β-Carotene was determined in five different selective Ethiopian vegetables using UV-Vis spectrophotometric method. Acetone was used as extraction solvent in this study. The method is applicable to determine β-carotene content in pumpkin, butternut squash, eggplant, carrot and zucchini with good linearity, precision, accuracy, and sensitivity. β-Carotene content was found to be 632 µg/g in carrots, 17-445 µg/g in pumpkin and butternut squash flesh, and 70.9 µg/g in their peels and seeds, respectively. β-Carotene is present in the flesh and peel of the zucchini and eggplant and the amounts found 70.4-437 µg/g and 33-344 µg/g, respectively. In this study, the carrot had the highest concentration of β-carotene, followed by the peel parts of pumpkin, zucchini, and eggplant. β-Carotene was determined only in the seed of pumpkin and butternut squash and the result were found lower than the

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