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Effect of Temperature on the Chemical Quality of β-Carotene Extracted from *Azollafiliculoides* of Anzali Wetland

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Abstract:

Purpose: This study assesses the quality of β-Carotene from a natural source, *Azolla*, in comparison with synthetic β-Carotene, and looks at seasonal variation in composition.

Methods: Azolla collected from summer and winter was extracted using organic solvents. The synthetic β-Carotene used as a control was obtained from a pharmacy. All treatments were stored for one year at 5 °C. The samples were analyzed to measure purity and concentration, and then used for colorimetric and vitamin A analysis.

Main findings: Our results showed significant differences ($p< 0.05$) between experimental and control treatments. The winter *Azolla* samples contained larger amounts of the β-Carotene ($p <0.05$) than the β-Carotene samples from summer *Azolla* which contain β-Carotene but in lesser amounts than winter samples. Tetrahydrofuran provided the best β-Carotene solubility, and methanol and acetonitrile the lowest. Cyclohexanone provided the most degradation. After one year of storage, the experimental treatments did, however, retain a reasonable acceptable chemical quality.

Conclusion: Considering the health benefits of natural β-Carotene over synthetic β-Carotene, this study indicated β-Carotene extracted from *Azolla* could be a viable alternative to synthetic β-Carotene in the food industry.

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INTRODUCTION

Bioactive compounds are being extracted from plants in aquatic ecosystems because of increased interest inthe renewability of natural resources and the environmental benefits of harvesting value-added products from plants and algae. β-carotene is worth noting due to its high level of antioxidant activity and its role as the precursor of vitamin A, being an important product in the food, pharmaceutical, cosmetics, and health industries [1]. β-carotene is a carotenoid that is naturally occurring in the largest plant, algae, and cyanobacterial families.

Sustainable alternatives that are inexpensive are an important resource when market demand is increasing. An alternative is *Azolla*, an aquatic fern which is non-toxic and fast-growing and can be found in wetlands, rice paddies, and shallow ponds [2]. Different species of *Azolla* are notable for their nitrogen-fixing capabilities, protein content, as well as their utilization as animal feed, green manure, and for water purification. *Azolla*has caught interest as a source of bioactive compounds, such as β-carotene.

The Anzali Wetland, located in the Gilan Province (Bandar Anzali), is one of the most ecologically important wetland zones in Iran.The wetland's humid climate, large inputs of freshwater, and nutrient-rich sediment provides a good growing environment for many aquatic plants including species of *Azolla* [3].While the growth of *Azolla* is viewed as detrimental to the ecosystem when it proliferates, it provides an opportunity for sustainable development by harvesting the biomass for extraction of β-carotene. Such an approach provides a dual opportunity to economically utilize *Azolla* and manage the ecological integrity of the wetland [4].

Welive in a world where the alternatives to synthetic compounds are being sought worldwide. The extraction of β-carotene from *Azolla* fits very well with Sustainable Development Goals. Its attributes include a short growth period, no soil, can adapt to very shallow water, cheapperunit production, and access to the biomass is easy [4]. Environmental aspects impact the carotenoids amount in *Azolla*, such as light intensity, temperature, type of species, age (growth) of the plant, and water quality. For instance, aquatic green plants can produce greater amounts of carotenoids under specified stress conditions or controlled nutrient or light exposures, such as light exposure and/or nutrient limitation.

Assessing and analyzing environmental factors for the Anzali Wet land, as well as identifying the most suitable time of harvestis the next essential step. Beyond the ecological and economic benefits, this research contributes to the development of domestic expertise in bioactive compound extraction. It also holds the potential to stimulate small-scale industries and create sustainable employment opportunities in the Gilan region [5].

Emphasis on indigenous resources and sustainable eco system management will assist in tackling present-day environmental and economic issues. Using β-carotene extracted from A. filiculoides from the Anzali Wetland demonstrated the utilization of invasive or fast-growing species in terms of biodiversity conservation and value-added production. Thus, this can be seen as a model for sustain a blyin corporating the utilization of other aquatic plants in future management in other regions. The present study aims to investigate the quality and purity of β-carotene extracted from A. filiculoides from the Anzali Wetland, as well as compare it to synthetic β-carotene, and the seasonal effectsof β-carotene yield.

MATERIALS AND METHODS

Experimental Design and Treatments

The study emphasized two experimental treatments and a control. The experimental treatments included β-Carotene from wild A. filiculoides collected from the Anzali Wetland in two different astronomically different seasons, summer and winter. The control involvedthe use of β-Carotene which was a commercial standard synthetic (Sigma-Aldrich, USA).

Extraction of Pigment

Sampling took place in the summer and winter seasons and was always replicated three times. The organic solvent extraction method was used to extract β-Carotene from Azolla [11]. Theedibleportion was measured from one kilogram of Azolla. All samples were collected randomly from the Anzali Wetland, then washed with tap water, and dried in a hot air oven at 50 °C. Temp. A 5 g of the dried residue was mixed with 25 mL of 1 M sodium hydroxide and the mixture was homogenized with a heater. After cooling, 500 ppm of sodium ascorbate was added. A soxhlet apparatus was used to extract pigments with 100 mL of petroleum ether for 39 minutes. The extracted pigments were

Figure 1: *A filiculoides*on the Anzali wetland water during summer.

Figure 2: Cleaned and washed flowers of *A filiculoides* collected from the Anzali wetland.

purified with solidphase ODS (Octadecylsilane bonded silica) column. The sample was rinsed, and the solvent removed.

MATERIALS

All of the chemical materials were purchased from Merck

Chemical Analysis

Quality control was done by identifying vitamin composition, solubility, and stability in organic solvents, percentage of β-Carotene massvs. dried residue weight of Azolla (production efficiency), purity of the extract, and shelf life comparisons of the extract.

Determination of Vitamin A in Samples

The test was done using the Budowski and Bondi method (A colorimetric method), which required they converted vitamin A to anhydro vitamin A using anhydrous ethanolicHCl or p-toluenesulfonic acid, then measured absorbance near 480 nm. The reaction is very specific for vitamin A [12,13,14].

Purity

To determine the quality of the final extract, the purity percentage was determined by HPLC. The sample was injected using microliter syringes. Chromatographic analysis was conducted on a Perkin Elmer HPLC system, which comprised of an LC-1000 pump, polymeric C18 column, and an LC250 UV/VIS detector. Peak identification was done using CSW33 software.β-Carotene was measured by using a mobile phase of acetonitrile, methanol, and ethyl acetate in the ratio of 88:10:2,which included a flow rate of 1 mL/min with a detection wavelength of 250 nm. The chromatographic column was an Octadecylsilane colorless acetyl column with a particle size of 0.5 µm and an internal diameter of 4 mm [15,16,17].

Relative Solubility of β-Carotene in Organic Solvents

Solubility Analysis Method

To compare the solubility of β-carotene in different solvents, ~10 mg of β-carotene was mixed with 3 mL of different solvent like acetone, acetonitrile, benzene, chloroform, cyclohexane, cyclohexanone, dichloromethane, dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), absolute ethanol, ethyl acetate, ethyl ether, hexanes, 2-propanol, methanol, methyl tert butyl ether (MTBE), tetrahydrofuran (THF) with BHT, and toluene. All mixtures were then ultrasonicated for five minutes. If, following this treatment, a clear solution was obtained that contained no visible crystals, we added β-carotene incrementally until we visually identified undissolved crystals, which indicated that we reached saturation. The solutions were then filtered through a 0.2 µm membrane. The dilutions we prepared produced absorbance values that ranged between 0.5 - 1.0 at the maximum wavelength for that solvent. The background absorbance was subtracted from the solvent blank. β-carotene concentration was estimated using Beer's law. All measurements were made in triplicate only. We rounded the reported values to one significant figure because, ultimately, we were comparing relative solubility and not absolute solubility.

Workflow for β-Carotene Solubility Assessment

Adding β-carotene to 3 mL of solvent, ultrasonic for 5 minutes, adding β-carotene until reaching saturation, filtering through a 0.2 µm membrane, preparing dilutions (A = 0.5–1.0), subtracting blank absorbance, and calculating concentration using Beer's Law.

Stability

Stability was monitored for 10 days at room temperature by measuring absorbance changes at the wavelength maximum according to Table **3**.

Color Determination

β-Carotene was measured using a colorimeter (Hunter Lab) calibrated with a standard white ceramic plate. The luminosity (L*) describes the lightness of the sample, where 0 is black (absent reflectance) and 100 is white (full reflectance), such that higher values relate to lighter (whiter) color. The chromaticity value a* shows where the sample lands between green (negative values) and red (positive values), with the chromaticity value b* showing where the sample lands between blue (negative values) and yellow (positive values) based on the standards of the International Commission on Illumination (CIE) [19,20,21].

Nutritional Decomposition

Azollautilized for β-Carotene preparation was assessed for its nutritional quality: protein content (distillation method), fat content (acid hydrolysis method), moisture (ovendrying method), and ash (gravimetric). Anassay of the pigments in Azolla, including assessment of dried biomass weight before and after processing, was part of the nutritional evaluation process[22].

Statistical Analysis

All chemical and spectrometric analytical data were analyzed using SPSS statistics software package. One-way ANOVA was used for the assessment of significant differences between the experimental treatments and control, for parameters including β-Carotene purity, vitamin A content, solubility, stability, and colourimetric values. When statistically significant differences were identified ($P \leq 0.05$), Tukey's HSD post hoc test was used to notify specific groups differences. For storage-related differences, two-way ANOVA was performed with treatment type and duration as fixed factors. The data is reported as mean ± standard deviation (SD) and statistical significance was accepted at $P \leq 0.05$. Degrees of freedom (between-groups and within-groups) and F value were stated in the results.

RESULTS

The nutritional composition Table **1** of *A. filiculoides* collected in summer was generally higher for protein, fat, and mineral content than the winter samples. The differences in individual parameters were significant ($p < 0.05$).

Table **2** summarizes the colorimetric analysis of β-Carotene during the storage process. During the 12-month refrigeration period, we saw no significant change in brightness or chromatic parameters ($p>0.05$). The samples from the experimental period kept color stability similar to that of the control group ($p>0.05$). However, regarding brightness, summer samples rated brighter than winter treatments ($p<0.05$). The test samples were of lesser quality than the control samples ($p<0.05$).

As shown in Table **3**, β-Carotene was able to be solubilized most soluble in tetrahydrofuran (THF), the least in methanol and acetonitrile, mg/L. Most of the solvents during the testing maintained pigment stability for days, with cyclohexanone exhibiting the most degradation. These patterns of solubility were

Table 1: Nutritional Composition of A Filiculoides from Anzali Wetland during Summer and Winter Seasons (Mean ± SD)

Index Season	Protein (%)	Moisture (%)	Fat (%)	Ash (%)	Dry weight (%)
Summer	23.49 ± 2.13^{A}	32.84 ± 3.15^{A}	19.52 ± 2.18^{A}	24.89 ± 2.98^{A}	89.73 ± 3.15^{A}
Winter	21.89 ± 2.94^{B}	31.95 ± 2.16^{B}	18.25 ± 1.97^{B}	23.16 ± 2.40^{B}	88.13 ± 2.16^{B}

The different signs in the same column and row indicate significant differences ($p<0.05$).

Table 2: Beta-Carotene Colorimetric Results Extracted from A Filiculoides in Summer and Winter Seasons During Storage at Refrigeration (mean ± SD)

Treatment	Winter			Summer			Control		
Color spectrum Sampling time (Months)	Brightness	Redness green	Blueness	Brightness	Redness green	Blueness	Brightness	Redness green	Blueness
Zero	79.86 ± 2.67^{aC}	1.78 ± 1.11^{aA}	1.75 ± 1.13^{aA}	86.35 ± 3.36^{aB}	1.24 ± 1.12^{aA}	1.17 ± 0.97^{aA}	96.75 ± 3.24^{aA}	1.05 ± 0.78^{aA}	1.11 ± 0.86^{aA}
Three	79.73 ± 3.14^{aC}	1.92 ± 1.24^{aA}	1.89 ± 1.18^{aA}	86.35 ± 2.28^{aB}	1.36 ± 0.96^{aA}	1.25 ± 0.85^{aA}	96.63 ± 3.14^{aA}	1.16 ± 0.98^{aA}	1.15 ± 0.67^{aA}
Six	79.56 ± 4.12^{aC}	2.14 ± 1.43^{aA}	2.15 ± 1.22^{aA}	86.33 ± 3.16^{aB}	1.52 ± 0.99^{aA}	1.31 ± 1.12^{aA}	96.44 ± 2.98^{aA}	1.27 ± 0.93^{aA}	1.29 ± 1.13^{aA}
Nine	79.51 ± 3.89^{aC}	2.19 ± 1.34^{aA}	2.21 ± 1.35^{aA}	86.31 ± 4.11^{aB}	1.69 ± 1.27^{aA}	1.52 ± 0.99^{aA}	96.41 ± 2.64^{aA}	1.39 ± 0.91^{aA}	1.32 ± 0.94^{aA}
Twelve	$79.\ 48\pm3.25^{aC}$	2.22 ± 1.17^{aA}	2.23 ± 1.24^{aA}	86.29 ± 3.92^{aB}	1.71 ± 0.98^{aA}	1.56 ± 1.21^{aA}	96.35 ± 3.19^{aA}	1.45 ± 1.23^{aA}	1.38 ± 0.89^{aA}

The different signs in the same column and row indicate significant differences ($p<0.05$).

Table 3: Relative solubility and stability of β-Carotenein organic solventsin summer and winter seasonsduring storage at refrigeration

solvent	Sampling time Index Wavelength (nm)	Summer			Winter		
		Solubility (mg/l)	Absorptivity (E%, cm^{-1})	molar absorptivity (L mol^{-1} cm^{-1})	Solubility (mg/l)	Absorptivity (E%, cm^{-1})	molar absorptivity (L mol^{-1} cm^{-1})
Acetone	452	190.00^{A}	2516.00^{A}	137423.00^{A}	173.00^{B}	2417.00^{B}	136321.00^{B}
Acetonitrile	452	5.00^{A}	2505.00^{A}	136425.00^{A}	2.00^{B}	2401.00^{B}	135413.00^{B}
Benzene	462	3990.00^{A}	2001.00^{A}	124019.00^{A}	3889.00^{B}	1901.00^{B}	123011.00^{B}
Chloroform	462	1995.00^{A}	2292.00^{A}	125112.00^{A}	1895.00^{B}	2183.00^{B}	124002.00^{B}
Cyclohexane	454	1995.00^{A}	2106.00^{A}	134723.00^{A}	1893.00^{B}	2001.00^{B}	133613.00^{B}
Cyclohexanone	462	1995.00^{A}	2317.00^{A}	126723.00^{A}	1875.00^{B}	2207.00^{B}	125713.00^{B}
Dichloromethane	460	5900.00^{A}	2329.00^{A}	127213.00^{A}	5801.00^{B}	2219.00^{B}	126103.00^{B}
DMF	466	190.00^{A}	2348.00^{A}	128312.00^{A}	170.00^{B}	2237.00^{B}	127212.00^{B}
DMSO	466	20.00^{A}	2215.00^{A}	121315.00^{A}	11.00^{B}	2114.00^{B}	121206.00^{B}
Ethanol	450	20.00^{A}	2526.00^{A}	135811.00^{A}	11.00^{B}	2114.00^{B}	121206.00^{B}
ethyl acetate	452	490.00^{A}	2481.00^{A}	135310.00^{A}	390.00^{B}	2372.00^{B}	134200.00^{B}
ethyl ether	448	990.00^{A}	2617.00^{A}	142809.00^{A}	895.00^{B}	2507.00^{B}	143728.00^{B}
Hexane	448	580.00^{A}	2551.00^{A}	139234.00^{A}	475.00^{B}	2452.00^{B}	138215.00^{B}
2-propanol	450	30.00^{A}	2464.00^{A}	134721.00^{A}	19.00^{B}	2374.00^{B}	132621.00^{B}
Methanol	450	5.00^{A}	2500.00^{A}	136445.00^{A}	2.00^{B}	2401.00^{B}	135413.00^{B}
MTBE	450	990.00^{A}	2543.00^{A}	139067.00^{A}	882.00^{B}	2431.00^{B}	138054.00^{B}
THF	456	9970.00^{A}	2359.00^{A}	128856.00^{A}	9865.00^{B}	2247.00^{B}	128745.00^{B}
toluene	462	3990.00^{A}	2239.00^{A}	121942.00^{A}	3876.00^{B}	2126.00^{B}	121841.00^{B}

The different signs in the same column and row indicate significant differences ($p<0.05$).

extracts showed significantly lower purity compared to the synthetic control despite having similar durability and nutritional ability ($p<0.05$).

Degrees of Freedom Between Groups: $df_1 = 2$ Degrees of Freedom Within Groups: $df_2 = 6$ in most cases of one-way ANOVA (3 groups * 3 replicates = 9 total samples: 9 - 3 = 6 error df).

The statistical analysis detected substantial differences among treatment groups. For vitamin A content (IU), a one-way ANOVA (Analysis of variance) indicated a

Table 4: Results of Vitamin Composition, Purity, and Content of β-Carotene Produced of a Filiculoides from Anzali Wetland in Summer and Winter Seasons during Storage at Refrigeration (Mean ± SD)

Index	Vitamin composition (IU)			Purity (%)			B-Carotene content (mg/kg)		
Treatment Sampling time (months)	Summer	Winter	Control	Summer	Winter	Control	Summer	Winter	Control
Zero	9837±2.13^{aC}	10893±3.14^{aB}	12346±3.49^{aA}	89.3±4.17^{aB}	79.7±3.18^{aC}	99±3.23^{aA}	6648±3.48^{aC}	7539±3.54^{aB}	11863±4.12^{aA}
Three	9837±2.45^{aC}	10893±3.12^{aB}	12346±3.15^{aA}	89.3±4.13^{aB}	79.7±3.13^{aC}	99±2.34^{aA}	6648±2.78^{aC}	7539±2.97^{aB}	11863±4.31^{aA}
Six	9837±3.76^{aC}	10893±3.16^{aB}	12346±2.78^{aA}	89.3±4.71^{aB}	79.7±3.89^{aC}	99±1.89^{aA}	6648±2.96^{aC}	7539±1.98^{aB}	11863±3.26^{aA}
Nine	9837±4.12^{aC}	10893±3.65^{aB}	12346±4.17^{aA}	89.3±3.48^{aB}	79.7±3.67^{aC}	99±1.99^{aA}	6648±2.88^{aC}	7539±1.99^{aB}	11863±3.91^{aA}
Twelve	9837±3.98^{aC}	10893±3.49^{aB}	12346±3.99^{aA}	89.3±3.87^{aB}	79.7±2.54^{aC}	99±2.95^{aA}	6648±2.86^{aC}	7539±3.99^{aB}	11863±3.65^{aA}

The different signs in the same column and row indicate.

significant treatment group effect: F value (2,6) = 183.72, p< 0.001;in other words, the control group had significantly greater amounts of vitamin A than both seasonal Azolla extracts.

For β-Carotene content (mg/kg), a significant difference also existed: F value (2,6) = 289.51, p< 0.001, and the control group had the greatest concentration, andthe winter and then summer extracts.

In terms of purity (%), the ANOVA indicated significant treatment effects: F value (2,6) = 147.83, p< 0.001, where the synthetic β-Carotene had higher purity than both natural samples.

In terms of colorimetric data (Brightness only, L*) over time, additive effects were assessed as well. A one-way ANOVA did show that there were significant differences in brightness between treatments at baseline values: F value (2,6) = 42.16, p = 0.0004, however, repeated measures over time indicated here were no significant changes for a time duration, indicating the color remained stable during storage (p > 0.05).

Solubility values varied by solvent and season. For tetrahy drofuran (THF) which had the highest solubility values, a one-way ANOVA comparing summer and winter indicated: F value (1,4) = 22.74, p = 0.008 thus confirming that a difference between the seasons did exist. The two-way ANOVA examining the interaction between season (2 levels) and storage time (5 level) on β-Carotene retention displayed a significant main effect for season: F value (1,20) = 96.37, p< 0.001.There was no interaction effect between season and time: F value (4,20) = 1.22, p = 0.33 indicating that both seasons exhibited a similar pattern of retention across the 5 time points.

DISCUSSION

Nutritional Composition

There was no significant difference in the nutritional composition of *A. filiculoides* collected in summer versus winter (Table **1**). Samples collected in the summer had a higher nutritional value; however, this was likely due to a greater production of the herb during a more active time of growth. *Azolla* itself is high in protein, fat, and essential minerals [23].

β-Carotene Stability and Storage Conditions

As seen in Table **2**, β-Carotene stability during storage was observed, especially for samples stored in dark glass containers that received treatment with antioxidants. The average moisture in *Azolla* powder added to the protection against lipid oxidation, although this moisture contributed to optimized conditions for non-enzymatic browning. Storage using the low drying temperatures prevented isomerization of β-Carotene (e.g., 50 °C) [23]. A storage period of one year at 50 °C did not significantly affect the β-Carotene content and color attributes in either the experimental or control groups. Colorimetric results found no significant difference among test samples and a large difference between test and control groups. The breakdown of plant tissues and incomplete removal of cell walls during processing resulted in a decrease in spectrometric values. Although processing showed improvements in homogenization and carotenoid release, significant changes in the micro-nutrient stability of the foodstuff were not observed. The incomplete removal of cell walls, cell membranes, and destruction of plant tissues in the experimental samples led to a reduction of spectrometry percentage [24,25].

Solubility of β-Carotene by Solvents

The values for β-Carotene solubility indicated in Table **3** showed that β-Carotene exhibited the highest solubility in tetrahydrofuran (THF) and lowest solubility in methanol and acetonitrile, which is in agreement with results from Craft *et al*. The variance in solubility could be attributed to residual lipids, chlorophyll, sample purity, or physical state (crystalline vs amorphous) of β-Carotene. THF has been receiving more use in recent years despite being prone to peroxide formation per the page on solvents, due to its ability to dissolve an extensive amount of carotenoid compounds. Other common solvents include hexane, petroleum ether, and diethyl ether, but poor solubility of polar carotenoids in these solvents can result in extraction loss in some cases [18,20].

Variances in solubility values across different solvents are due to the differences in the physical state and purity of the β-Carotene sample, as well as limitations of the experimental method used. Lower than its melting point (456 K), β-Carotene may also have different solubilities when in a crystalline or amorphous physical state. Impurities can also affect solubility too, along with any degradation products, that act as co-solvents or anti-solvents affecting solubility [26].

Purity, β-Carotene Content, and Vitamin Compounds

The differences between β-Carotene and vitamin compounds in the test samples were significant (Table 4). Significant differences were also noted between the purity of test samples. Significant differences were like wise noted between purity, vitamin compounds, and content of β-Carotene solubility in the experimental treatments when compared to the control treatment. However, no significant differences were noted for the one-year retention period at 50 °C regarding any of the experimental or control groups. The maturation stage of plant leaves influences β-Carotene, with younger leaves having higher amounts [27,28]. The synthesis of lycopene, the precursor to β-Carotene, is more efficient and effective in young Azolla leaves for summer [29-31]. Therefore, β-Carotene in summer would be higher than that of winter.

The experimental groups demonstrated a lower purity than the control samples, which we attribute to the failure of removal of all the lipids and chlorophyll from the extraction liquid before quantifying β-Carotene [32-34].

According to Table **4**, the breakdown of cell walls and membranes enhanced the release of vitamin compounds and improved their detection through spectrometry. The β-Carotene concentration in these treatments was influenced by the degree of tissue disruption. Incomplete release of β-Carotene from plant tissues in the experimental samples led to a reduction in its measured content. Given that a single β-Carotene molecule can produce two molecules of vitamin A, and that β-Carotene is theoretically 100% convertible to vitamin A [35], the decrease in β-Carotene content in the experimental treatments resulted in a correspon-ding reduction in vitamin compound levels [36,37].

The thermal drying treatment of *Azolla* did not degrade carotenoids due to the low drying temperature; however, it did inactivate certain food enzymes, which contributed to solubility and release of carotenoids, and possibly assisted in bioavailability. In addition, homogenization during β-Carotene extraction improved its accessibility [38,39]. Despite processing, there were no changes in β-Carotene concentration or stability during storage at 50 °C (Table **4**).The findings of this study are consistent with those reported in previous research. Zarreh and Kianirad extracted β-Carotene using fermentation of the mold *Blakesleatrispora* [40], while Razavi *et al*. employed physicochemical methods involving fermentation with *Poralomycesruberrimus* H110 [41]. Baigan *et al*. used the algae *Donaliasalina* by modifying the culture medium [42], and Moghadasi *et al*. applied a saponification method using microalgae *Donaliasalina* [43,44]. Similar results were also reported by Lejeune *et al*. and Vennugopal *et al*., both of whom utilized solvent extraction, as well as by Mustafa *et al*., who employed the Tee method [45,46]. The present study's results support and align with these previous findings.

The β-Carotene contents from A filiculoides, showed clear differences compared to other Azolla species at different locations. Thevariationscould be due to differing genotype, the climatic factor, and the stages of growth and developmentof the plant [47].

The β-Carotene content of the samples was found to be highest for summer-collected samples, which may be due to the increased synthesis of lycopene, which is a β-Carotene precursor, that was most likely more evident in young and active leaves after the hot periods of summer were over. The seasonal changes in the content of leaf carotenoids indicate that leaf maturation components and environmental factors influence the carotenoid biosynthesis process as a whole.

CONCLUSION

Given the significant variations in the chemical properties and little to no differences in durability and shelf life among β-Carotene obtained from *Azolla* with the help of organic solvents and synthetic β-Carotene, these findings demonstrate that β-Carotene extracted from *A. filiculoides* in the summer season can be used as a green substitute for synthetic β-Carotene in the food industry. To support these results, more investigations are needed to examine the practicability of large-scale extraction, the retention of stability under varied storage conditions, and possible health benefits in food and nutraceutical applications.

REFERENCES

[1] MacDougall DB. Color in Food: Improving Quality. England: Woodhead Publishing; 2002: 392. https://doi.org/10.1201/9781439823194

[2] Stahl U, Donalies UEB, Nevoigt UEBU. Food Biotechnology. Germany: Springer; 2008: 269. https://doi.org/10.1007/978-3-540-70536-9

[3] Branen AL, Davidson M, Salminen SP, Thorngate J. Food Additives. Virginia: Taylor and Francis; 2001: 952-61. https://doi.org/10.1201/9780367800505

[4] Hopkins WG. Plant Biotechnology. United States: Infobase Publishing; 2006: 365.

[5] Rymbai H, Sharma RR, Srivastav M. Biocolorants and its implications in health and food industry: A review. Int J Pharm Technol Res 2001; 3: 2228-44.

[6] Caivano JL, Buera MDP. Color in Food: Technological and Psychophysical Aspects. Florida: CRC Press; 2012: 132. https://doi.org/10.1201/b11878

[7] Cosma P, Fini P, Rochira S, Catucci L, Castagnolo M, Agostiano A. Phototoxicity and cytotoxicity of chlorophyll a/cyclodextrins complexes on Jurkat cells. Bioelectrochem 2008; 74: 58-64. https://doi.org/10.1016/j.bioelechem.2008.04.020

[8] Schieber A, Carle R. Occurrence of carotenoid cis-isomers in food: Technological, analytical, and nutritional implications. Trends Food Sci Technol 2005; 16: 416-22. https://doi.org/10.1016/j.tifs.2005.03.018

[9] Hutchings JB. Food Color and Appearance. Gaithersburg, Md: Aspen Publishers; 2003. p. 273. https://doi.org/10.1007/978-1-4615-0707-9_1

[10] Agostiano A, Catucci L, Cosma P, Fini P. Aggregation processes and photophysical properties of chlorophyll a in aqueous solutions modulated by cyclodextrins. J Phys Chem 2003; 5: 2122-813. https://doi.org/10.1039/b211903j

[11] Hosotani K, Kitagawa M, Granado F, Olmedilla B, Gilmantines E, Blando I. A fast reliable and low cost saponification protocol for analysis of carotenoids in vegetables. J Food Compos Anal 2001; 14: 479-85. https://doi.org/10.1006/jfca.2001.0989

[12] Achikanu CE, Eze-Steven PE, Ude CM, Ugwuokolie OC. Determination of the vitamin and mineral composition of common leafy vegetables in southeastern Nigeria. Int J CurrMicrobiolAppl Sci 2013; 2: 347-53.

[13] Blake JA, Moran JJ. An improved colorimetric procedure for the analysis of vitamin A. Can J Chem. 1976; 54: 1757-64. https://doi.org/10.1139/v76-250

[14] Budowski P, Bondi A. Determination of vitamin A by conversion to anhydrovitamin A. Analyst. 1957; 82: 751-9. https://doi.org/10.1039/an9578200751

[15] Muller H. Determination of the carotenoid content in selected vegetables and fruit by HPLC and photodiode array detection. Z LebensmUntersForsch. 1997; 204: 88-94. https://doi.org/10.1007/s002170050042

[16] Schierle J, Pietsch B, Ceresa A, Fizet C. Method for the determination of carotene in supplements and raw materials by reversed-phase liquid chromatography: Single laboratory validation. J AOAC Int 2004; 87: 1070-82. https://doi.org/10.1093/jaoac/87.5.1070

[17] Khalil IA, Varananis FR. Carotenoid extraction and analysis by reversed phase HPLC system. Sarhad J Agric. 1996; 105: 15-21.

[18] Craft NE. Relative solubility, stability, and absorptivity of lutein and β-carotene in organic solvents. J Agric Food Chem. 1992; 40: 431-4. https://doi.org/10.1021/jf00015a013

[19] Vieira DAP, Caliari M, Souza ERB, Junior MSS. Methods for pigment extraction and determination of color in tomato processing cultivars. Food Sci Technol 2018; 3: 1-7.

[20] Davies BH. Carotenoids. In: Goodwin TW, editor. Chemistry and Biochemistry of Plant Pigments. England: Academic Press; 1976. p. 155.

[21] International Commission on Illumination (CIE). The Effect of Spectral Power Distribution on Lighting for Urban and Pedestrian Areas. Austria: CIE; 2014. p. 78.

[22] AOAC. Official Methods of Analysis Manual. 18th ed. United States: AOAC; 2005. p. 123.

[23] Amaya R. The effect of processing and storage on carotenoids content of vegetables. J Food Sci 2001; 7: 20055802.

[24] Anjum F, Barkat AK, Noreen N, Tariq M, Faisal S. Effect of boiling and storage on beta-carotene content of different vegetables. Pak J Life Soc Sci 2008; 6: 63-7.

[25] Çinar I. Carotenoid pigment losses of freeze-dried plant samples under different storage conditions. Food Sci Technol 2004; 37: 363-7. https://doi.org/10.1016/j.lwt.2003.10.006

[26] Araus KA, Canales RI, Valle JM, Fuente JC. Solubility of β-carotene in ethanol- and triolein-modified CO_2. J ChemThermodyn 2011; 43: 1991-2001. https://doi.org/10.1016/j.jct.2011.07.013

[27] Venugopal V, Prasanna P, Sood A, Jaiswal P, Kaushik BD. Stimulation of pigment accumulation in Anabaena-*Azolla* strains: effect of light intensity and sugars. Folia Microbiol 2006; 51: 50-6. https://doi.org/10.1007/BF02931450

[28] Qiu D, Chen ZR, Li HR. Qualitative analysis of β-carotene isomers. Food Sci 2008; 29: 50-3.

[29] Hackett M, Lee J, Schwartz S. Thermal stability and isomerization of lycopene in tomato oleoresins from different varieties. J Food Sci 2002; 69: 536-41. https://doi.org/10.1111/j.1365-2621.2004.tb13647.x

[30] Khanipour E, Keramat J, HosseiniParvar SH, Motamedzadegan A, Ghorbani A, Shahidi SA. Application of extracted tomato carotenoids in heated and unheated foods: Study of color stability during storage. Iran J Food Sci Technol 2006; 2: 13-22.

[31] Prasanna R, Pabby A, Singh PK. Effect of glucose and light/dark environment on pigmentation profiles in Calothrixelenkeni. Folia Microbiol 2004; 49: 26-30. https://doi.org/10.1007/BF02931641

[32] Bendich A, Higdon GS. Biological actions of beta-carotene. Am J Nutr 2004; 32: 225-30. https://doi.org/10.1093/jn/134.1.225S

[33] Alcaíno A, Barahona S, Carmona M, Lozano C, Marcoleta A, Iklitschek M, *et al*. Cloning of the cytochrome P450 reductase (crtR) gene and its involvement in the astaxanthin biosynthesis of *Xanthophyllomycesdendrorhous*. BMC Microbiol 2008; 8: 1-13. https://doi.org/10.1186/1471-2180-8-169

[34] Dentuto PL, Catucci L, Cosma P, Fini P, Agostiano A, Hackbarth S. Cyclodextrin/chlorophyll a complexes as supramolecular photosensitizers. Bioelectrochem 2007; 70: 39-43. https://doi.org/10.1016/j.bioelechem.2006.03.026

[35] Mercandate A, Amaya R. Carotenoid composition and vitamin A value of some native Brazilian green leafy vegetables. J Food Sci. 1990; 25: 213-9. https://doi.org/10.1111/j.1365-2621.1990.tb01077.x

[36] Agte VV, Tarwadi KV, Mengale S, Chiplonkar SA. Potential of traditionally green leafy vegetables as natural sources for supplementation of eight micronutrients in vegetarian diets. J Chromatogr 2000; 13: 885-91. https://doi.org/10.1006/jfca.2000.0942

[37] Chromatogr BJ. Improved simultaneous determination method of beta-carotene and retinol with saponification in human serum and rat liver. Anal Technol Biomed Life Sci 2003; 1: 305-13. https://doi.org/10.1016/S1570-0232(03)00233-2

[38] Negi P, Roy S. Effect of drying condition on quality of green leaves during long-term storage. Food Res Int 2000; 34: 283-7. https://doi.org/10.1016/S0963-9969(00)00165-4

[39] Yamini C, Ranjana N, Chaturvedi Y, Nagar R. Levels of beta-carotene and effects of processing on selected fruits and vegetables of the arid zone of India. J Food Sci 2001; 56: 7-32.

[40] Zareh D, Kiani Rad M. Comparison of beta-carotene production by *Blakesleatrispora* fungi in fifteen liter fermenter and seventy-five-liter airlift. Res Const 2004; 64: 2-7.

[41] Razavi H, Rezaei K, Mark I. Comparison of pigment extraction (carotenoids) methods from Sporobolomycesruberrimus H110 yeast. Res Food SciTechnol Iran 2005; 1: 33-42.

[42] Baigan E, Rasekh F, Rasekhi N. Production and extraction of carotenoids from living organisms. In: National Conference on New Findings of Chemistry in Industry and Medicine; Iran: Islamic Azad Univ. Ray Branch; 2009. p. 156.

[43] Moghadassi Z, Emtiazjoo M, Rabanie M, Emtiazjoo M, Azargashb E, Mosaff N. Potential β-carotene production from *Dunaliellasalina* of Shahe Lake under salinity stress. J Fish Islam Azad UnivAzadshahr Br 2011; 5: 90-101.

[44] Moghadassi Z, Emtyazjoo M, Rabanie M, Emtyazjoo M, Labibie F, Azarghashb E, *et al*. Study of anti-cancer ethanol extract of *Dunaliellasalina* isolated from Hoz-Soltan against squamous cell carcinoma *in vitro*. Iran J Med Aromat Plants 2011; 27: 306-15.

[45] Lejeune A, Peng J, Boulenge EL, Larondelle Y, Hove CV. Carotene content of *Azolla* and its variations during drying and storage treatments. Anim Feed Sci Technol 2000; 84: 293-301. https://doi.org/10.1016/S0377-8401(00)00129-2

[46] Mustafa EM, Ibrahim MM. HPLC analysis of non-enzymatic antioxidants in *Azollacaroliniana* (Pteridopsida) subjected to UV-B. Biol Sci 2012; 3: 19-30. https://doi.org/10.21608/eajbsh.2012.17003

[47] Anguelova T, Warthesen Y. Degradation of lycopene, α-carotene and β-carotene during lipid peroxidation. J Food Sci 2000; 65: 71-5. https://doi.org/10.1111/j.1365-2621.2000.tb15958.x