agronomy

MDPI

Article

The Efficient Extraction of β-Carotene from Sea Buckthorn Berries Using a Novel Solvent, Fatty Acid Ethyl Esters, and a Combination of Ultrasound and Microwave

Vasile Staicu [1], Ioan Calinescu [1], Mircea Vinatoru [1], Daniela Ghimpeteanu [1], Ioana Popa [1,*] and Timothy J. Mason [2]

[1] Faculty of Chemical Engineering and Biotechnologies, National University of Science and Technology POLITEHNICA Bucharest, 011061 Bucharest, Romania; vasile.staicu@yahoo.ro (V.S.); ioan.calinescu@gmail.com (I.C.); mircea.vinatoru@gmail.com (M.V.); ghimpeteanu_daniela@yahoo.com (D.G.)

[2] Faculty of Health and Life Sciences, Coventry University, Coventry CV1 5FB, UK; sonochemistry@hotmail.com

* Correspondence: asofiei.ioana@yahoo.com

Abstract: The move towards environmentally friendly processing has been a driving force for research into green methods for the extraction of bioactive compounds from plant materials. In this study, a green and efficient process for the extraction of β-carotene from sea buckthorn berries through the simultaneous use of ultrasound and microwave, using a novel green solvent, fatty acid ethyl esters (FAEE), is described. For the same extraction time (45 min), the β-carotene content in the extract was significantly increased (according to ANOVA analysis—$p < 0.05$) by the simultaneous use of ultrasound and microwave, compared with the separate use of these technologies—an increase of 15 and 89% compared with ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE), respectively. The resulting extract can be used directly, without further purification, as a food supplement because the solvent itself is safe for consumption. Furthermore, FAEE contains omega-3 and omega-6 fatty acids which add to the health benefits of the extract. When β-carotene is extracted from the plant matrix, it is subjected to degradation due to oxidation, but the addition of the antioxidant vitamin E (13 mg/mL of extract) to the extract extends its stability to more than 90 days at room temperature even when exposed to light. The addition of vitamin E also enhances the health benefits of the extract.

Keywords: β-carotene; fatty acid ethyl esters; ultrasound; microwave; sea buckthorn berries; nutraceuticals

Citation: Staicu, V.; Calinescu, I.; Vinatoru, M.; Ghimpeteanu, D.; Popa, I.; Mason, T.J. The Efficient Extraction of β-Carotene from Sea Buckthorn Berries Using a Novel Solvent, Fatty Acid Ethyl Esters, and a Combination of Ultrasound and Microwave. *Agronomy* **2024**, *14*, 416. https://doi.org/10.3390/agronomy14030416

Academic Editors: Laura Siracusa and Luana Pulvirenti

Received: 14 January 2024
Revised: 6 February 2024
Accepted: 19 February 2024
Published: 21 February 2024

1. Introduction

Plants contain a wide range of chemical constituents which are beneficial to human health and contribute to overall well-being. These compounds can be obtained either by the consumption of nutrient-rich foods or through the appropriate use of food supplements. A study performed in 21 European countries in 2017 revealed that less than 40% of the recommendations for nutrient consumption had been met [1]. Thus, the development of complex food supplements which ensure the recommended daily intake for several nutrients simultaneously is of great interest.

Carotenoids are a family of more than 600 organic fat-soluble pigments. They occur naturally in many yellow-orange vegetables, fruits, and fungi, providing the orange color to carrots and sea buckthorn berries and the red color to tomato and peppers, etc. [2,3]. The most well-known of the carotenoids is carotene, which has three isomers: alfa, beta, and gamma. The beta isomer is important for the production of vitamin A (retinol) in the body, which aids vision, and protects against macular degeneration, which is the principal cause of irreversible blindness in adults [4,5]. The European Food Safety Authority has

recommended a Population Reference Intake (PRI) of 750 µg retinol equivalents/day for men and 650 µg retinol equivalents/day for women. The conversion factor for β-carotene to retinol has been calculated to be 6:1, which means that approximately 5 mg of β-carotene is needed to meet the daily requirement of vitamin A [6].

In addition to its role in eyesight protection, β-carotene in food supplements has beneficial antioxidant properties [6]. It can reduce the risk of cardiovascular diseases [7], different types of cancer [8–10], and strengthen the immune system [11].

An important source of β-carotene and, consequently, of retinol is sea buckthorn. This feedstock draw attention to many groups of researchers, leading to the setup of the International Sea Buckthorn Association (ISA) [12,13]. Sea buckthorn (*Hippophae rhamnoides* L.) is a shrub which belongs to the Elaegnaceae family which grows in different regions of Europe and Asia. Every part of the plant contains bioactive compounds which are beneficial to the human body [14].

There are many methods for extracting active principles from various fruits and plants described in the literature [15]. A recent trend has been towards the concept of green extraction, which involves techniques that minimize the consumption of both energy and solvent and, where possible, allow the use of green solvents [16]. The choice of solvent is a particularly important factor because many extraction methods use organic solvents which may be toxic, e.g., hexane [17], ethyl acetate [18] and petroleum ether [19]. As a result, their use is not desirable, particularly in the context of food and phytopharmaceutical applications. Fatty acid ethyl esters (FAEE) can be more suitable for these industries. Their capacity to extract liposoluble bioactive compounds has already been proven by Diacon et al. [20]. FAEE are derived from renewable materials, and are environmentally friendly and non-toxic [21].

In recent years, more efficient extraction processes such as pulsed electric field extraction [22], pressurized and supercritical fluid extraction [23,24], enzyme-assisted extraction [25], and ultrasound (UAE)- and microwave (MAE)-assisted extractions [26] have been developed. However, a promising method used recently for the extraction of bioactive compounds from plants is simultaneous ultrasound- and microwave-assisted extraction (UMAE) [27]. This strategy combines the advantages of both ultrasound and microwave extractions. Ultrasonically assisted extraction enhances the mass transfer rate through cavitation. The latter is characterized by the formation of cavities or bubbles that collapse upon reaching a maximum size. When bubbles collapse in close proximity to a solid surface, it can induce an asymmetric collapse. Consequently, cavitation can disrupt the cell wall, facilitating the penetration of solvent into the plant matrix and, therefore, enhancing the mass transfer rate of extraction [27]. Microwaves, on the other hand, generally heat the whole volume of the extraction mixture; however, in some cases, because of their non-uniform structure, the vegetal material can be heated selectively, and mass transfer is limited [28]. However, this limitation can be overcome by combining microwave technology with ultrasound.

The aim of this study was to develop a green and efficient extraction procedure for β-carotene from sea buckthorn berries using an innovative apparatus that applies simultaneous ultrasound and microwave conditions. In addition, the use of a green solvent (FAEE) means that the total extract obtained from sea buckthorn berries can be used directly as a food supplement without the need to remove the solvent. The total extract has added health benefits because it contains not only β-carotene but also omega-3 and omega-6 fatty acids from the FAEE. A successful method of prolonging the shelf-life of the β-carotene extract through the addition of vitamin E is also reported.

2. Materials and Methods

2.1. Materials

The sea buckthorn berries (*Hippophae rhamnoides* L.) were provided by Hofigal S.A., Bucharest, Romania. The freshly harvested berries underwent drying using an air flow-heating oven at 40 °C until a constant weight was achieved, resulting in a final water

content of 6%). Following this process, the berries were granulated with an electric grinder, and screened to attain a particle size below 1 mm, producing the dried material (DM). The milled berries were then portioned into 25 g samples, enclosed in plastic vessels, and kept in the refrigerator until their use for the extraction of β-carotene.

β-Carotene standard was purchased from Sigma-Aldrich Co., Bucharest, Romania. Vitamin E, manufactured by MAYAM (Elemental SRL, Oradea, Romania) in liquid form, in bottles of 10 mL, was purchased from a local store (Bucharest, Romania). This product is recommended for pharmaceutical and cosmetic uses.

The FAEE used as extraction solvent was obtained by enzymatic transesterification using hemp oil (provided by Hofigal S.A., Bucharest, Romania), absolute ethanol (Sigma-Aldrich Co., Bucharest, Romania), and the candida antarctica lipase enzyme (purchased from Sigma-Aldrich Co., Bucharest, Romania). The synthesis of FAEE has been reported [20] and is the subject of a Provisional Patent application [21].

2.2. Simultaneous Ultrasound and Microwave Equipment

The innovative equipment used for the UMAE of β-carotene from sea buckthorn berries was described by Calinescu et al. [29] and it is shown in Figure 1. It uses a Bandelin Sonopuls HD 4200 ultrasound generator (BANDELIN electronic GmbH & Co. KG, Berlin, Germany) and a Miniflow 200SS microwave mono-mode applicator (SAIREM, Décines-Charpieu, France).

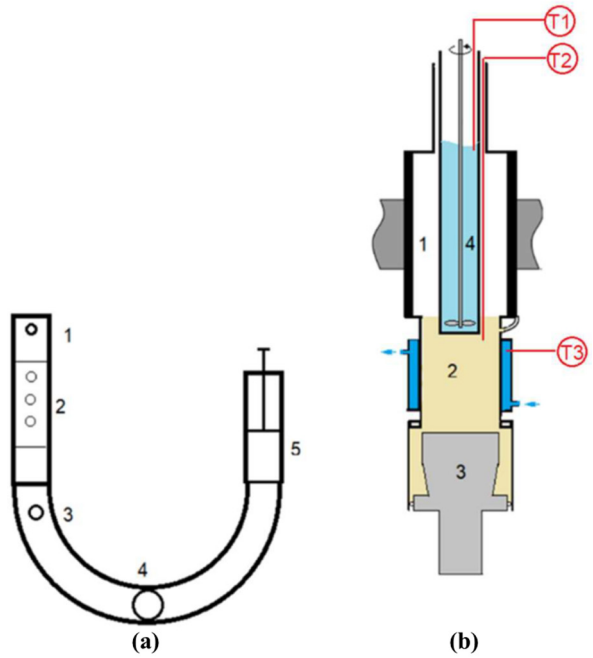

Figure 1. Innovative equipment for the extraction of β-carotene from sea buckthorn berries by simultaneous microwave and ultrasound treatment: (**a**) microwave mono-mode applicator: 1—transition WR340 to coaxial adaptor, 2—stub tuner, 3—adjustable iris, 4—mono-mode applicator, 5—sliding short circuit; (**b**) extraction reactor (microwave and ultrasound): 1—microwave mono-mode applicator, 2—thermostatic ultrasonic bath of coupling fluid, 3—ultrasound cup–horn, 4—extraction reactor, T1, T2, T3—thermocouples.

The extraction reactor, equipped with mechanical stirring, is introduced into the microwave cavity. An ultrasonic cup horn is placed below the microwave cavity to form an ultrasonic bath with its energy transferred into the base of the reactor through thermostatted dodecane, which acts as coupling liquid [29].

2.3. β-Carotene Extraction Procedure

The extraction of β-carotene from sea buckthorn berries was performed using four different methods: conventional extraction (CONV), UAE, MAE, and UMAE, using FAEE derived from hemp oil as the extraction solvent.

1. The CONV method was conducted in a jacketed vessel made of glass. To ensure the required temperature for the extraction process, water was circulated through an external jacket during all CONVs. These experiments were performed at a stirring rate of 800 rpm (using a magnetic stirrer) and at different temperatures (50, 60, and 70 °C).
2. The UAE method was carried out using the innovative equipment described in Figure 1, without using microwave. The sonication was applied continuously using different amplitudes of the cup–horn system (40, 50, and 70%) corresponding to a power introduced into the system of 45.2, 50.7, and 56.9 W, respectively (determined directly from the ultrasonic power supplied). To maintain a constant extraction temperature of 50 °C, a cooling agent at a temperature of 20–30 °C was circulated through the bath mantle of coupling fluid.
3. The MAE method was carried out using the same equipment (Figure 1) without ultrasound. The microwave power applied was 15 W for all experiments. To maintain the extraction temperature at 50 °C, the temperature of the circulating cooling agent was 15 °C.
4. The UMAE method was performed using continuous sonication at an amplitude of 40% and a microwave power of 15 W. The cooling agent temperature was 8 °C to maintain the extraction at 50 °C.

For all non-conventional extractions using the equipment shown in Figure 1, the extraction mixture was stirred using a mechanical stirrer to ensure that all particles of plant materials are equally subjected to ultrasound and microwave energies. The reactor was placed in the coupling liquid (dodecane) at a depth of 2 cm below the liquid surface. The position of the reactor in the ultrasonic bath was chosen based on the position of maximum transfer of ultrasonic energy into the reactor. To ensure that the same conditions were used for all three non-conventional methods, the reactor was also placed in this position for MAE (where ultrasound was not used). The plant material was added to the reactor only after the extraction solvent had reached the steady-state temperature of 50 °C. During all ultrasonic and/or microwave extractions, the temperature of extraction mixture T1, coupling liquid T2, and cooling agent T3 (see Figure 1) were recorded using thermocouples.

For all four methods (conventional and non-conventional), the extraction solvent was FAEE with a purity of 91% [20,21]. All experiments were carried out for 15, 30, 45, and 60 min with a ratio of 1/20 (w/v) between ground dried berries and solvent.

The fatty acid profile of FAEE is presented in Table 1.

Table 1. Fatty acid profile.

Fatty Acid	Composition (%)
Palmitic acid	6.88
Stearic acid	3.16
Arahic acid	0.81
Behenic acid	0.26
Palmitoleic acid	0.12
Oleic acid	14.06
Eicosanoic acid	0.47
Linoleic acid	58.17
Eicosadienoic acid	0.13
Linolenic acid	15.18
γ-Linolenic acid	0.76

2.4. Determination of β-Carotene Content

After each extraction, the extract samples were diluted 10 to 15 times with FAEE and then analyzed spectrophotometrically to determine the β-carotene content of sea buckthorn berries. The dilution was performed in order to fit within the absorbance range required. The absorbance was measured at 453 nm using a Jasco V-550 UV/VIS Scanning Spectrophotometer (JASCO International Co., Ltd., Tokyo, Japan). The content of β-carotene was represented as milligrams of β-carotene per 1 g of dry material (mg/g DM). A standard curve, constructed using a 2–40 mg/L β-carotene solution in FAEE, was employed to quantify the β-carotene in the extracts. All the analyses were performed in triplicate. All measurements include standard deviation values being presented as the mean value (represented as error bars on graphs).

2.5. Assessment of total β-Carotene Content

To assess the efficiency of these methods, the total amount of extractable β-carotene found in sea buckthorn berries was measured. This determination was achieved by a repeated extraction using the UMAE method (under the same conditions as described above). After a first extraction, the resulting mixture was centrifuged at 3000 rpm for 10 min at room temperature. The supernatant was analyzed in order to determine the content of β-carotene. A fresh portion of solvent (FAEE) was added to the residual plant material and the mixture was subjected to another UMAE. This procedure was repeated until the β-carotene content became insignificantly low compared to the values obtained in the present study using either extraction method. Thus, when only 0.17 mg β-carotene/g DM remained in the plant material, the repeated extraction was considered complete, and the raw material exhausted. After four successive extractions, a total amount of 11.26 mg β-carotene/g DM was achieved.

2.6. Determination of β-Carotene Stability over Time

The stability of β-carotene over time was examined using the UMAE extract obtained after 60 min of extraction. This determination was performed in the absence and presence of vitamin E. Without adding vitamin E, three 6 mL samples were taken and treated as follows:

- Kept at room temperature and exposed to light.
- Kept at room temperature in the dark.
- Kept in the refrigerator in the dark.

Vitamin E was added to three additional 6 mL samples, and they were treated as described above. The concentration of β-carotene for each sample was measured after 1, 3, 7, 15, 30, 60, and 90 days.

Vitamin E (recognized as a good liposoluble antioxidant) was added to the extracts in order to reduce the rate of β-carotene degradation. The amount of vitamin E added was calculated from the daily dose recommended by the European Food Safety Authority (approximatively 13 mg for each 1 mL of extract). The UV–VIS spectra of vitamin E did not interfere with that of β-carotene.

2.7. Statistical Analysis

Data were reported as the mean value ± SD (standard deviation) based on triplicate measurements (n = 3). The dissimilarities of the data obtained were evaluated through univariate one-way ANOVA analysis. To identify the significant statistical variations between the averages of β-carotene content of two or more independent groups, statistical analysis of the data by Duncan's new multiple comparison test was carried out. The differences were considered statistically significant at a p-value below 0.05. The XLSTAT Version 2019.1 (Addinsoft, New York, NY, USA) was used for statistical analysis.

3. Results and Discussion

3.1. Conventional Extraction of β-Carotene from Sea Buckthorn Berries

Generally, active principles from plant materials are thermolabile compounds. For example, Umair et al. showed that the extraction of β-carotene by UAE at high temperatures can lead to its degradation [30]. In general, at elevated temperatures the solubility of bioactive compounds increases, improving the mass transfer rate between the matrix of the plant material and the solvent. Higher temperatures can result in swift ruptures of cell walls, facilitating the release of targeted compounds. Nonetheless, prolonged extraction time and elevated temperatures may induce oxidation of bioactive compounds or alter the conformation of the extracted compounds. Additionally, to preserve heat-sensitive compounds, it is beneficial to maintain a low temperature, preventing the degradation of bioactive compounds [31].

Umair et al. showed that the optimal UAE temperature for β-carotene was 32 °C [30], but the purpose of the present study was the development of an extraction procedure for β-carotene under simultaneous ultrasound and microwave conditions. For MAE to be effective, the temperatures must be greater than 45 °C, and so the three higher temperatures of 50, 60, and 70 °C were chosen. During MAE, when temperatures surpass 45 °C, the plant material is heated selectively. Microwave heating specifically focuses on the water traces within the cells of dried plant material, initiating evaporation. Evaporation increases the pressure created inside the cells, causing the cell walls to stretch and eventually rupture. This leads to an easier release of the targeted compounds [28,31].

The influence of temperature on the β-carotene content was first investigated by conventional extraction (CONV). As shown in Figure 2, ANOVA analysis indicated that the β-carotene content increases significantly with increases in the temperature from 50 to 70 °C ($p < 0.05$) and from 50 to 60 °C ($p < 0.05$) for the extraction times of 15 and 30 min, respectively. The highest β-carotene content was achieved after 45 min of extraction. Prolonged extraction (beyond 45 min) led to some degradation of β-carotene, particularly at 60 and 70 °C. ANOVA analysis showed no significant ($p > 0.05$) increase in the 50–70 °C range, for 45 min of extraction, and since only a slight degradation occurs after 60 min, the best extraction temperature was judged to be 50 °C. With CONV, only 24% of the total available β-carotene content found in sea buckthorn berries was achieved.

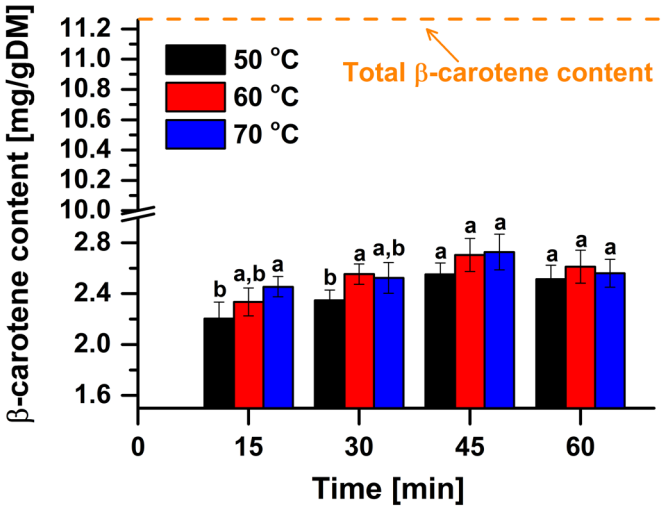

Figure 2. Influence of temperature on the β-carotene content, extracted by CONV (conventional method)—ground dried berries to solvent ratio of 1/20. The letters a and b within graph show the significant difference between groups analyzed by ANOVA ($p < 0.05$) and Duncan's new multiple comparison test.

3.2. Non-Conventional Extraction of β-Carotene from Sea Buckthorn Berries

The results of β-carotene extraction via non-conventional methods (UAE, MAE, and UMAE) are shown in Figures 3 and 4.

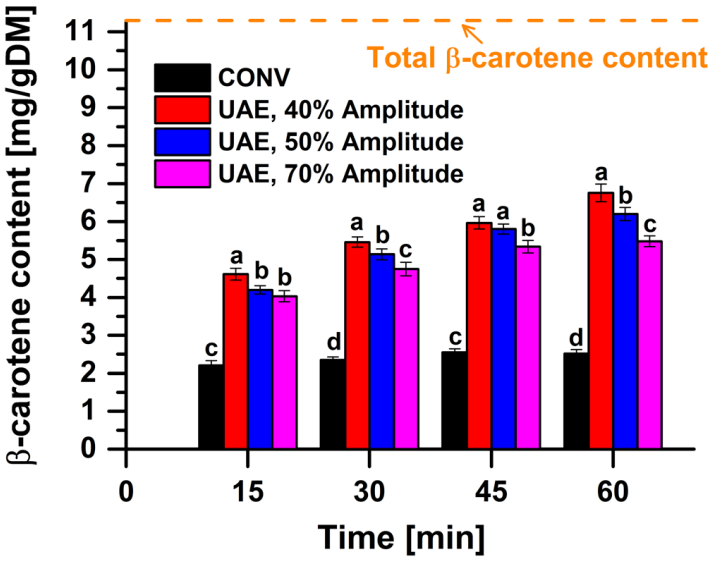

Figure 3. Influence of ultrasound amplitude on the β-carotene content for the ultrasound-assisted extraction (UAE) method (ground dried berries to solvent ratio of 1/20, temperature of 50 °C). The letters a–d on the graph show the significant difference between samples analyzed by ANOVA ($p < 0.05$) and Duncan's new multiple comparison test.

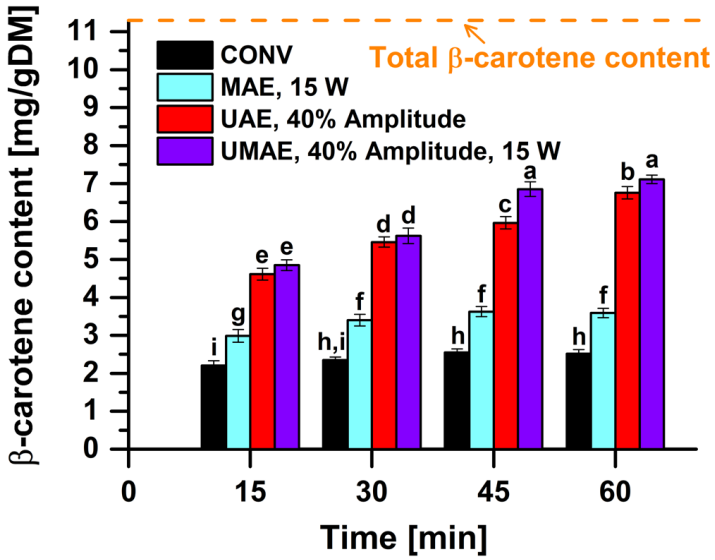

Figure 4. Influence of extraction method on the β-carotene content (ground dried berries to solvent ratio of 1/20, a temperature of 50 °C). The letters a–i on the graph show the significant difference between samples analyzed by ANOVA ($p < 0.05$) and Duncan's new multiple comparison test. Microwave-assisted extraction (MAE), simultaneous ultrasound- and microwave-assisted extraction (UMAE).

In the investigation of UAE, the power of ultrasound (ultrasound amplitude) is a crucial parameter because it is an indicator of the energy transmitted to the solvent. The literature data indicate that there is also the possibility of ultrasonically induced degradation of the targeted compound at higher ultrasonic powers [32]. The extent of degradation depends on the stability of the extracted material. Hence, the effect of ultrasound amplitude

on the extraction efficiency was investigated to determine the optimal amplitude value yielding the highest β-carotene content.

Sonication can enhance the dissipation of mechanical energy, generating a "heating effect" at the surface of the vegetal material and consequently increasing diffusivity effects. Likewise, UAE involves cavitation of the solvent which can promotes disruption of the stationary solvent layer surrounding the vegetal matter and breaking of cell tissue which together will facilitate solvent access into the plant matrix, leading to an increase in the mass transfer rate [27,33].

The results of applying three different ultrasonic amplitudes (40, 50, and 70%) used for UAE are shown in Figure 3. Statistical analysis showed that all three amplitudes (40, 50, and 70%) used for UAE lead to significant better results compared with the CONV method, irrespective of the extraction time ($p < 0.05$).

The UAE experiments reveal that better results are achieved for 40% ultrasonic amplitude and that increasing the amplitude beyond 40% leads to β-carotene degradation. Using ultrasound alone for the β-carotene extraction, approximately 53% of the total β-carotene content found in sea buckthorn berries can be achieved in 45 min of extraction. The significant influence of the ultrasound amplitude on β-carotene content was confirmed by ANOVA test and Duncan's post hoc *t*-tests (see Figure 3).

The results of all four extractions with conventional and non-conventional methods are shown in Figure 4. In the case of the MAE method, the highest β-carotene content was achieved after 45 min, showing a 42% increase in yield compared with CONV. Better yields were obtained using UAE (at an amplitude of 40%) and UMAE with increases of 134 and 167%, respectively. This shows that the use of standalone microwave technology is not as efficient a method for extracting β-carotene from sea buckthorn berries as UAE, and that UMAE is the best. Using microwave alone for the β-carotene extraction, approximately 32% of the total β-carotene content found in sea buckthorn berries can be achieved in 45 min of extraction.

For the same extraction time, UAE (at an amplitude of 40%) and UMAE resulted in a significant ($p < 0.05$) increase (65 and 89% for UAE and UMAE, respectively) compared with MAE (see Figure 4).

As depicted in Figure 4, statistical analysis showed that the increase between 45 and 60 min of extraction for UMAE is non-significant ($p > 0.05$). Thus, the best extraction time was considered to be 45 min. In the same time interval (45 min), UMAE yielded 6.85 mg of β-carotene/g DM which is 15% better than that obtained using UAE (5.96 mg/g DM). This increase was confirmed by ANOVA test and Duncan's post hoc *t*-tests. This suggests that although microwave technology on its own yielded only modest results, it can improve yields when combined with ultrasound. The yield of 61% of the total β-carotene content found in sea buckthorn berries using UMAE was obtained at an ultrasonic amplitude of 40%, a microwave power of 15 W, and an extraction time of 45 min.

The β-carotene yield of 6.85 mg of β-carotene/g DM is higher compared with other studies in the literature. For example, Aaby et al., using conventional extraction, obtained a total carotenoid content ranging from 0.83 to 0.95 mg/g DM using a mixture of hexane, acetone, and ethanol as a solvent [34]. Bhimjiyani et al. conducted UAE of β-carotene from sea buckthorn pomace using flaxseed oil as solvent in order to valorize the oil by introducing carotenoids. The extraction process yielded 0.28 mg/g DM of β-carotene after 75 min of extraction at an ultrasound amplitude of 80% and a temperature of 20 °C [35]. In that study it was also shown that an increase in amplitude leads to degradation of the target material. It is interesting to note that a longer extraction time (75 min) was necessary to attain a high β-carotene content at an ultrasound amplitude of 80%. This is significantly different from the conditions that were used in the present study, where 45 min of extraction were used at an amplitude of only 40%. This difference could be due to the use of different ultrasound equipment for extraction and the difference in the solvent characteristics between flaxseed oil and FAEE.

3.3. Determination of β-Carotene Stability over Time

An important parameter of this extract aimed to be used as food supplement is the stability of β-carotene during storage. This was evaluated using the UMAE extract obtained after 60 min of extraction. The content of β-carotene for each sample was measured after 1, 3, 7, 15, 30, 60, and 90 days (Table 2). In each case, the β-carotene content decreases drastically even over one day. After 90 days, the amount of β-carotene remaining was insignificant. This behavior was confirmed by ANOVA and Duncan's post hoc *t*-tests.

Table 2. Stability of β-carotene over time (in the absence of vitamin E). The letters a–h on the table show the significant difference between samples analyzed by ANOVA ($p < 0.05$) and Duncan's new multiple comparison test.

Days	0	1	3	7	15	30	60	90
Room temperature, exposed to light, 1st vial	7.10 ^{a} ±0.12 mg/g DM	3.66 ^{b} ±0.09	2.65 ^{c} ±0.06	2.22 ^{d} ±0.04	1.75 ^{e} ±0.03	0.35 ^{f} ±0.01	0.29 ^{f} ±0.01	0.25 ^{f} ±0.01
Room temperature, kept in the dark, 2nd vial		5.38 ^{b} ±0.10	4.52 ^{c} ±0.15	4.34 ^{d} ±0.10	3.93 ^{e} ±0.05	2.03 ^{f} ±0.05	1.67 ^{g} ±0.03	0.94 ^{h} ±0.01
Refrigerator, kept in the dark, 3rd vial		6.17 ^{b} ±0.19	5.16 ^{c} ±0.12	4.87 ^{d} ±0.13	4.27 ^{e} ±0.07	3.10 ^{f} ±0.05	2.59 ^{g} ±0.04	1.90 ^{h} ±0.05

To reduce the rate of degradation, Vitamin E (a good antioxidant) was added to the extracts and another study of the stability of β-carotene was carried out (Table 3). The addition of vitamin E greatly improved the stability of β-carotene in the extract. ANOVA analysis indicated non-significant degradation after 30 days of storage in a refrigerator ($p > 0.05$).

Table 3. Stability of β-carotene over time (with added vitamin E). The letters a–e on the table show the significant difference between samples analyzed by ANOVA ($p < 0.05$) and Duncan's new multiple comparison test.

Days	0	1	3	7	15	30	60	90
Room temperature, exposed to light, 4th vial	7.10 ^{a} ±0.12 mg/g DM	6.72 ^{b} ±0.11	6.60 ^{b} ±0.09	6.27 ^{c} ±0.20	5.99 ^{d} ±0.20	5.88 ^{d,e} ±0.16	5.82 ^{d,e} ±0.15	5.67 ^{e} ±0.09
Room temperature, kept in the dark, 5th vial		6.90 ^{a} ±0.21	6.80 ^{a} ±0.17	6.35 ^{b} ±0.09	6.16 ^{b,c} ±0.15	6.14 ^{b,c} ±0.22	5.94 ^{c,d} ±0.20	5.79 ^{d} ±0.16
Refrigerator, kept in the dark, 6th vial		7.05 ^{a,b} ±0.16	6.97 ^{a,b} ±0.25	6.90 ^{a,b,c} ±0.19	6.87 ^{a,b,c} ±0.12	6.86 ^{a,b,c} ±0.10	6.77 ^{b,c} ±0.19	6.59 ^{c} ±0.12

A distinct advantage of using FAEE as the extraction solvent is that the total extract can be used directly as a food supplement because FAEE contains omega-3 and omega-6 fatty acids [21]. This conforms with food additives allowed to be added directly to food (Code of Federal Regulations, 2023—Title 21: 21CFR172.515) [36]. The use of Vitamin E as a stabilizer is also allowed as a food additive.

4. Conclusions

In this study, a novel green solvent, FAEE, obtained from hemp oil, was employed for the extraction of β-carotene from sea buckthorn berries. The resulting extract could be used directly as a food supplement, providing a supply of omega-3 and omega-6 fatty acids from the FAEE solvent. To enhance the extraction yield, an innovative apparatus that allowed the simultaneous application of ultrasound and microwave was used. This combination proved to be more efficient than conventional extraction or the use of either ultrasound or microwave separately.

Under conventional extraction conditions at 50 °C after 45 min, only 24% of the total available β-carotene content was extracted. There was no significant difference when the temperature was increased to 70 °C. After 45 min of extraction, UAE (at an amplitude of

40%) and UMAE led to an increase of 65 and 89%, respectively, compared with the use of microwaves alone. Thus, UMAE enhanced the β-carotene content by 15% compared with UAE. This increase was confirmed as significant by ANOVA test and Duncan's post hoc *t*-tests. Thus, the most efficient extraction method was the UMAE using an ultrasonic amplitude of 40%, a microwave power of 15 W, a 50 °C temperature, and a 45 min extraction time, which produced approximatively 61% of the total amount of β-carotene available from dried sea buckthorn berries.

The addition of vitamin E (which has strong antioxidant activity) reduced the degradation of β-carotene in the extract. Degradation over a period of 90 days was reduced from 73% to 7% when the extract was stored in the dark, in a refrigerator. Even more significant was the result at room temperature, in the light, when the degradation was reduced from 96% to 20%. It should be noted that the benefits of FAEE containing β-carotene as a food supplement are improved by the addition of vitamin E.

The FAEE solvent could potentially find applications in the phytopharmaceutical field as an alternative carrier for various liposoluble bioactive compounds.

5. Patents

Calinescu: I.: Vinatoru, M.; Diacon, A.; Chipurici, P. Provisional Patent Application, Biodegradable and non-toxic solvent for the extraction of liposoluble active principles. Provisional Patent Application RO133507-A0, "Biodegradable and non-toxic solvent for the extraction of liposoluble active principles", 2018.

Author Contributions: Conceptualization, I.C., M.V. and T.J.M.; methodology, I.C., M.V. and I.P.; software, I.P.; validation, I.C., M.V. and T.J.M.; formal analysis, I.P., V.S. and D.G.; investigation, V.S. and D.G.; resources, I.C. and I.P.; data curation, I.P. and V.S.; writing—original draft preparation, I.P.; writing—review and editing, I.C. and M.V.; visualization, I.C., M.V. and I.P.; supervision, T.J.M.; project administration, I.C. and I.P.; funding acquisition, I.P. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by a grant from the National Program for Research of the National Association of Technical Universities—GNAC ARUT 2023.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Rippin, H.L.; Hutchinson, J.; Jewell, J.; Breda, J.J.; Cade, J.E. Adult Nutrient Intakes from Current National Dietary Surveys of European Populations. *Nutrients* **2017**, *9*, 1288. [CrossRef] [PubMed]
2. Krinsky, N.I.; Johnson, E.J. Carotenoid actions and their relation to health and disease. *Mol. Asp. Med.* **2005**, *26*, 459–516. [CrossRef] [PubMed]
3. Maiani, G.; Caston, M.J.; Catasta, G.; Toti, E.; Cambrodon, I.G.; Bysted, A.; Granado-Lorencio, F.; Olmedilla-Alonso, B.; Knuthsen, P.; Valoti, M.; et al. Carotenoids: Actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans. *Mol. Nutr. Food Res.* **2009**, *53*, S194–S218. [CrossRef] [PubMed]
4. Clark, S. Beta Carotene. In *xPharm: The Comprehensive Pharmacology Reference*; Enna, S.J., Bylund, D.B., Eds.; Elsevier: Cardiff, UK, 2007; pp. 1–3.
5. Gul, K.; Tak, A.; Singh, A.K.; Singh, P.; Yousuf, B.; Wani, A.A.; Yildiz, F. Chemistry, encapsulation, and health benefits of β-carotene—A review. *Cogent Food Agric.* **2015**, *1*, 1018696. [CrossRef]
6. EFSA Panel on Dietetic Products, Nutrition and Allergies. Scientific Opinion on Dietary Reference Values for vitamin A. *EFSA J.* **2015**, *13*, 4028. [CrossRef]
7. Yang, J.; Zhang, Y.; Na, X.; Zhao, A. β-Carotene Supplementation and Risk of Cardiovascular Disease: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Nutrients* **2022**, *14*, 1284. [CrossRef] [PubMed]
8. Vance, T.M.; Azabdaftari, G.; Pop, E.A.; Lee, S.G.; Su, L.J.; Fontham, E.T.; Bensen, J.T.; Steck, S.E.; Arab, L.; Mohler, J.L.; et al. Intake of dietary antioxidants is inversely associated with biomarkers of oxidative stress among men with prostate cancer. *Br. J. Nutr.* **2016**, *115*, 68–74. [CrossRef]
9. Huang, J.; Lu, M.-S.; Fang, Y.-J.; Xu, M.; Huang, W.-Q.; Pan, Z.-Z.; Chen, Y.-M.; Zhang, C.-X. Serum carotenoids and colorectal cancer risk: A case-control study in Guangdong, China. *Mol. Nutr. Food Res.* **2017**, *61*, 1700267. [CrossRef]

10. Sowmya Shree, G.; Yogendra Prasad, K.; Arpitha, H.S.; Deepika, U.R.; Nawneet Kumar, K.; Mondal, P.; Ganesan, P. Beta-carotene at physiologically attainable concentration induces apoptosis and down-regulates cell survival and antioxidant markers in human breast cancer (MCF-7) cells. *Mol. Cell Biochem.* **2017**, *436*, 1–12. [CrossRef]
11. Hughes, D. Effects of carotenoids on human immune function. *Proc. Nutr. Soc.* **1999**, *58*, 713–718. [CrossRef]
12. Chen, A.; Feng, X.; Dorjsuren, B.; Chimedtseren, C.; Damda, T.-A.; Zhang, C. Traditional food, modern food and nutritional value of Sea buckthorn (*Hippophae rhamnoides* L.): A review. *J. Future Foods* **2023**, *3*, 191–205. [CrossRef]
13. International Seabuckthorn Association. Available online: https://uia.org/s/or/en/1100066556 (accessed on 2 February 2024).
14. Parvu, C. Enciclopedia plantelor. In *Enciclopedia Plantelor. Plante din Flora Romaniei*; Editura Tehnică: Bucuresti, Romania, 2003.
15. Jha, A.K.; Sit, N. Extraction of bioactive compounds from plant materials using combination of various novel methods: A review. *Trends Food Sci. Technol.* **2022**, *119*, 579–591. [CrossRef]
16. Armenta, S.; Garrigues, S.; de la Guardia, M. The role of green extraction techniques in Green Analytical Chemistry. *TrAC Trends Anal. Chem.* **2015**, *71*, 2–8. [CrossRef]
17. Aprodu, I.; Ursache, F.M.; Turturica, M.; Rapeanu, G.; Stanciuc, N. Thermal stability of the complex formed between carotenoids from sea buckthorn (*Hippophae rhamnoides* L.) and bovine beta-lactoglobulin. *Spectrochim. Acta Part A* **2017**, *173*, 562–571. [CrossRef] [PubMed]
18. Pop, R.M.; Weesepoel, Y.; Socaciu, C.; Pintea, A.; Vincken, J.P.; Gruppen, H. Carotenoid composition of berries and leaves from six Romanian sea buckthorn (*Hippophae rhamnoides* L.) varieties. *Food Chem.* **2014**, *147*, 1–9. [CrossRef] [PubMed]
19. Giuffrida, D.; Pintea, A.; Dugo, P.; Torre, G.; Pop, R.M.; Mondello, L. Determination of carotenoids and their esters in fruits of sea buckthorn (*Hippophae rhamnoides* L.) by HPLC-DAD-APCI-MS. *Phytochem. Anal.* **2012**, *23*, 267–273. [CrossRef] [PubMed]
20. Diacon, A.; Călinescu, I.; Vinatoru, M.; Chipurici, P.; Vlaicu, A.; Boscornea, A.C.; Mason, T.J. Fatty Acid Ethyl Esters (FAEE): A New, Green and Renewable Solvent for the Extraction of Carotenoids from Tomato Waste Products. *Molecules* **2021**, *26*, 4388. [CrossRef]
21. Calinescu, I.; Vinatoru, M.; Diacon, A.; Chipurici, P. Biodegradable and Non-Toxic Solvent for the Extraction of Liposoluble Active Principles. Provisional Patent Application, RO133507-A0p 10, Romania. 2018. Available online: https://worldwide.espacenet.com/patent/search/family/067386041/publication/RO133507A0?q=RO133507&queryLang=en:de:fr (accessed on 12 January 2024).
22. Lopez-Gamez, G.; Elez-Martinez, P.; Martin-Belloso, O.; Soliva-Fortuny, R. Pulsed electric field treatment strategies to increase bioaccessibility of phenolic and carotenoid compounds in oil-added carrot purees. *Food Chem.* **2021**, *364*, 130377. [CrossRef] [PubMed]
23. Patel, A.S.; Kar, A.; Dash, S.; Dash, S.K. Supercritical fluid extraction of beta-carotene from ripe bitter melon pericarp. *Sci. Rep.* **2019**, *9*, 19266. [CrossRef]
24. Tumbas Šaponjac, V.; Kovačević, S.; Šeregelj, V.; Šovljanski, O.; Mandić, A.; Ćetković, G.; Vulić, J.; Podunavac-Kuzmanović, S.; Čanadanović-Brunet, J. Improvement of Carrot Accelerated Solvent Extraction Efficacy Using Experimental Design and Chemometric Techniques. *Processes* **2021**, *9*, 1652. [CrossRef]
25. Jalali-Jivan, M.; Fathi-Achachlouei, B.; Ahmadi-Gavlighi, H.; Jafari, S.M. Improving the extraction efficiency and stability of β-carotene from carrot by enzyme-assisted green nanoemulsification. *Innov. Food Sci. Emerg. Technol.* **2021**, *74*, 102836. [CrossRef]
26. Hladnik, L.; Vicente, F.A.; Košir, A.; Grilc, M.; Likozar, B. Stirred, ultrasound-assisted and microwave-assisted extraction process of β-carotene from Rhodotorula glutinis in biorefinery downstream. *Sep. Purif. Technol.* **2023**, *311*, 123293. [CrossRef]
27. Khadhraoui, B.; Ummat, V.; Tiwari, B.K.; Fabiano-Tixier, A.S.; Chemat, F. Review of ultrasound combinations with hybrid and innovative techniques for extraction and processing of food and natural products. *Ultrason. Sonochem.* **2021**, *76*, 105625. [CrossRef]
28. Lee, C.S.; Binner, E.; Winkworth-Smith, C.; John, R.; Gomes, R.; Robinson, J. Enhancing natural product extraction and mass transfer using selective microwave heating. *Chem. Eng. Sci.* **2016**, *149*, 97–103. [CrossRef]
29. Calinescu, I.; Vinatoru, M.; Ghimpeteanu, D.; Lavric, V.; Mason, T.J. A new reactor for process intensification involving the simultaneous application of adjustable ultrasound and microwave radiation. *Ultrason. Sonochem.* **2021**, *77*, 105701. [CrossRef] [PubMed]
30. Umair, M.; Jabbar, S.; Nasiru, M.M.; Lu, Z.; Zhang, J.; Abid, M.; Murtaza, M.A.; Kieliszek, M.; Zhao, L. Ultrasound-Assisted Extraction of Carotenoids from Carrot Pomace and Their Optimization through Response Surface Methodology. *Molecules* **2021**, *26*, 6763. [CrossRef]
31. Bitwell, C.; Indra, S.S.; Luke, C.; Kakoma, M.K. A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. *Sci. Afr.* **2023**, *19*, e01585. [CrossRef]
32. Shen, L.; Pang, S.; Zhong, M.; Sun, Y.; Qayum, A.; Liu, Y.; Rashid, A.; Xu, B.; Liang, Q.; Ma, H.; et al. A comprehensive review of ultrasonic assisted extraction (UAE) for bioactive components: Principles, advantages, equipment, and combined technologies. *Ultrason. Sonochem.* **2023**, *101*, 106646. [CrossRef] [PubMed]
33. Ozuna, C.; Puig, A.; Garcia-Perez, J.V.; Cárcel, J.A. Ultrasonically enhanced desalting of cod (*Gadus morhua*). Mass transport kinetics and structural changes. *LWT—Food Sci. Technol.* **2014**, *59*, 130–137. [CrossRef]
34. Aaby, K.; Martinsen, B.K.; Borge, G.I.A.; Røen, D. Bioactive compounds and color of sea buckthorn (*Hippophae rhamnoides* L.) purees as affected by heat treatment and high-pressure homogenization. *Int. J. Food Prop.* **2020**, *23*, 651–664. [CrossRef]

35. Bhimjiyani, V.H.; Borugadda, V.B.; Naik, S.; Dalai, A.K. Enrichment of flaxseed (*Linum usitatissimum*) oil with carotenoids of sea buckthorn pomace via ultrasound-assisted extraction technique: Enrichment of flaxseed oil with sea buckthorn. *Curr. Res. Food Sci.* **2021**, *4*, 478–488. [CrossRef] [PubMed]
36. *21CFR172.515*; Code of Federal Regulations, Synthetic Flavoring Substances and Adjuvants. Food and Drug Administration: Silver Spring, MD, USA, 2023.