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Enhanced Antioxidant Activity of *Capsicum annuum* L. and *Moringa oleifera* L. Extracts after Encapsulation in Microemulsions

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Received: 15 October 2017; Accepted: 13 November 2017; Published: 16 November 2017

Abstract: Carotenoids are powerful natural antioxidants that can easily degrade and are almost insoluble in water. Their incorporation into microemulsions (MEs) can solve these problems. In this study, ethanol extracts (prepared using different protocols) of *Capsicum annuum* L. (green and red), *Moringa oleifera* L. leaves, and their mixtures [Red Pepper/*M. oleifera* (50/50 *w*/*w*) and Green Pepper/*M. oleifera* (50/50 *w*/*w*)], were encapsulated in MEs for the first time. The encapsulation efficiency was determined and the physicochemical characteristics of the prepared MEs were assessed by particle size, turbidity, centrifugation, and thermal stress determination. The antioxidant activity of extracts and their MEs was determined by the DSC and DPPH methods. Prepared MEs did not present phase separation, creaming, sedimentation, presence of aggregates, or other unacceptable macroscopic drawbacks. Turbidity measurements showed that only small differences in optical density appeared. MEs' particle size dispersion was found to be around the average value and varied between 10 and 95 nm. The highest resistance to oxidation of crude extracts was observed by the *M. oleifera* leaf extract, followed by that of Red Pepper/*M. oleifera* (50/50 *w*/*w*) mixture, Green Pepper/*M. oleifera* (50/50 *w*/*w*) mixture, Red Pepper and, finally, Green Pepper. The results concerning MEs-encapsulated samples followed the same trend.

Keywords: microencapsulation; microemulsions; *Moringa oleifera*; *Capsicum annuum*; antioxidant activity; carotenoids

1. Introduction

The consumption of fruits and vegetables has been associated with the prevention of morbidity and mortality from degenerative diseases [1].

Capsicum annuum L. (pepper) is used worldwide, not only as a food, but also in traditional medicine against gastric ulcers, rheumatism, alopecia, and toothache [2]. Pepper fruit content in phenolic compounds, vitamin C, potassium, and provitamin A exemplifies its antioxidant capacity and nutritional importance. In addition, vitamin C and phenolic compounds, especially flavonoids, contribute to pepper fruit antioxidant activity [3]. Carotenoid pigments (especially β-carotene) and vitamins C and E are among the compounds that have been fairly well studied in pepper fruit. The green color of the pepper fruit is mainly due to the presence of chlorophyll and carotenoids typical of the chloroplast, such as oxygenated carotenoids or xanthophylls, violaxanthin, neoxanthin, and lutein, as well as α-carotene [4].

Moringa oleifera L. is widely known as a multi-purpose tree (also named "miracle tree"). It is used as source of food, medicine, cosmetic oil, forage for livestock, and water coagulant. *M. oleifera* leaves also have a high content of carotenoids such as lutein, zeaxanthin, and β-carotene [5].

Carotenoids are powerful natural antioxidants recognized as beneficial for preventing a broad range of cancers and cardiovascular diseases [3]. The role of carotenoids as antioxidants and regulators of the immune response system has been also reported [6]. The incorporation of carotenoids in functional foods has gained much interest, but problems such as solubility and stability limit their application in some foods since they can easily be degrade under high temperature, low pH, light, and reactive oxygen species. Additionally, their autoxidation and thermal degradation is promoted by a number of carotenoid oxidizing agents (i.e., acid, singlet oxygen, iron, and free radicals). Therefore, their degradation (isomerization and oxidation) is accelerated after they have been extracted from biological tissues [7].

Another important fact is that carotenoids are almost insoluble in water and only slightly oil-soluble at room temperature (about 0.2 g/L oil), but their solubility in oil greatly increases with increasing temperature. Their incorporation into micro- or nano-structures may influence their solubility and present a solution to their low solubility and rapid degradation [8]. Emulsions have several advantages as potential carotenoid delivery systems and can easily be engineered in order to enhance stability and decrease carotenoid degradation in functional food products. They are easy to incorporate into aqueous-based products at a relatively low cost and they can retain carotenoids' antioxidant properties once diluted into the food if their chemical and physical properties are retained. Emulsions can also contain a high amount of bioactives without having high lipid content [7].

A special kind of emulsion is microemulsion, which is usually formed from a surfactant, oil, water, and, sometimes, a co-surfactant or co-solvent. Microemulsions (MEs) are defined as thermodynamically stable, isotropic, and transparent emulsions of oil and water stabilized by an interfacial film of a suitable surfactant, and with a droplet size smaller than 100 nm [9]. They are food-grade colloidal delivery systems suitable for encapsulating, protecting, and delivering lipophilic components, such as carotenoids [10]. They have several advantages over conventional emulsions or nanoemulsions (Nes), because they can be prepared without involving high energy with the so called spontaneous emulsification method [11]. MEs also present transparency (which is important for clear beverages), a small droplet size (which plays a role in flavor release), increased solubilization and the bioavailability of bioactive constituents, the protection of solubilized components from degradative reactions, and high stability. These are important characteristics of MEs that find applications in industry, especially in the food industry [12].

As indicated above, peppers and *M. oleifera* are plants of exceptional nutritional interest, rich in carotenoids. In this study, to diminish any issues (such as solubility and stability) of carotenoids extracted using ethanol from *C. annuum* L. fruits and *M. oleifera* L. leaves, the extracts and their mixtures have been encapsulated in MEs. Initially, optimization of the extraction conditions in order to achieve the highest total carotenoid content had taken place. This optimization was carried out using different parameters such as extraction time, temperature, magnetic stirring, or ultrasounds. After the encapsulation of the extracts, total carotenoid content incorporated in MEs was determined. Next, preliminary stability tests, such as centrifugation and the thermal stress test, were carried out on MEs before studying their physicochemical characteristics by means of particle size and turbidity determination. Finally, the antioxidant activity of extracts was determined before using two different methods, namely, DPPH and Differential Scanning Calorimetry (DSC). After their encapsulation in MEs, their antioxidant activity was again tested using DSC.

To our knowledge, the encapsulation efficiency of carotenoids (extracted from pepper fruits and *M. oleifera* L. leaves) in MEs has been studied for the first time.

2. Materials and Methods

2.1. Plant Material

Green pepper fruits (*C. annuum* L., var Raico) were collected from plants cultivated in Nea Aghialos area (Magnesia county, Greece) (at 39°15′54.51″ N and 22°46′58.60″ E and elevation 19 m according to Google Earth version 7.1.7.2606, Google Inc., Mountain View, CA, USA). Red pepper fruits (*C. annuum* L., var 8108) were collected from plants cultivated in Platanidia area (Magnesia county, Greece) (at 39°19′02.26″ N and 23°02′19.69″ E and elevation 5 m according to Google Earth). Fresh *M. oleifera* leaves were collected from trees cultivated in Krya Vrisi area (Karditsa county, Central Greece) (at 39°19′6.97″ N and 21°52′39.16″ E and elevation 131 m according to Google Earth).

Fresh pepper fruits were collected during morning time on 15th of June in 2014 and delivered 2 h after collection to the lab. *M. oleifera* leaves were collected on 25th of September in 2014 and delivered 30 min after collection to the lab. All plant material was thoroughly washed with tap water and then with double distilled water. Then, they were dried using a Telstar Cryodos 80 freeze dryer (Telstar Industrial, S.A., Terrassa, Spain) for 12 h. The freeze-dried peppers and leaves were milled to particle sizes of about 0.5 mm using a blender.

2.2. Extraction of Plant Material and Preparation of MES

2.2.1. Selection of Extraction Protocol

For the selection of the most appropriate extraction protocol for carotenoids, three freeze-dried samples, namely Red Pepper fruit, Green Pepper fruit, and *M. oleifera* L. leaves, were used. Specifically, 2.5 g of each freeze-dried sample were extracted with 50 mL of absolute ethanol 99.8% v/v (Carlo Erba, Italy) using three different extraction techniques according to a modification of the method reported by Rutkowska and Stolyhwo [6]. The protocols tested were the following:

- Magnetic stirring (500 rpm) for 30 min at 45 °C.
- Ultrasonication using an Elmasonic S 100 H bath (Elma GmbH & Co. K.G., Singen, Germany) for 30 min at 45 °C.
- Magnetic stirring (500 rpm) for 5 h at 45 °C.

The extracts were filtered (the filtrates were usually less than 40 mL), and the residues were washed with ethanol several times until the filtrates were eventually decolorized (usually, additional 5–8 mL of alcohol was needed). The filtrates of each protocol were combined in a 50 mL amber volumetric flask. Each flask was then filled with the extraction solvent.

2.2.2. Determination of Carotenoid Content of the Extracts

The extracts' absorbance was measured using a UV/VIS spectrophotometer (UV-1700, Shimadzu Europa GmbH, Germany) set at 450 nm. The concentration of total carotenoids (*TCds*) was calculated according to the following equation [13]:

$$TCds(\mu g/g) = \frac{A \times V \times 10^4}{A^{1\%}_{1cm} \times W}$$

where: A = absorbance, V = the total extract volume (mL), $A^{1\%}_{1cm}$ = 2500, and W = the sample dry weight (g). A value of 2500 is often used to give an estimate of the total carotenoids content of an extract [14].

2.2.3. Preparation of the Final Samples Using the Selected Protocol

The final samples were prepared using the selected protocol (2nd protocol described in Section 2.2.1) were:

- Red Pepper fruit extract

- Green Pepper fruit extract
- *M. oleifera* L. leaf extract
- Red Pepper/*M. oleifera* L. leaf (50/50 *w*/*w*) extract
- Green Pepper/*M. oleifera* L. leaf (50/50 *w*/*w*) extract

These 50 mL extracts were used for all the determinations apart from Differential Scanning Calorimetry (DSC). Specifically, for the determination of the antioxidant activity using DSC, the ethanol was evaporated under vacuum using a rotary evaporator (at 30 °C) and the remaining solid extracts were put in Eppendorf tubes.

2.2.4. Preparation of MEs

An amount of 3 g of lecithin (Lecithin of Soya, >97%, Carlo Erba, Italy), 1 g of sunflower oil (Sol, Elais S.A., Athens, Greece) and 2 g of absolute ethanol were mixed with 50 mL of distilled water using a magnetic stirrer for approximately 30 min at 45 °C, until an emulsion was formed. This emulsion was used as the stock emulsion. A working emulsion (incorporating the carotenoids) was prepared from the stock emulsion by dilution with distilled water (1:10 *w*/*v*). An aliquot of 20 g of the working emulsion was mixed with 1 mL of extracts, and mixed at 400 rpm for 1 h at 45 °C. The entire procedure was executed under low light conditions in order to prevent carotenoid degradation. The MEs (incorporating extracts or mixtures of extracts) were then freeze-dried for 24 h apart from a small quantity that was used for the determination of centrifugation and thermal stress stability tests. A sample of "empty" MEs (not incorporating any extract) was prepared as blank.

2.3. Determination of Encapsulation Efficiency

The freeze-dried MEs were dissolved in *n*-hexane according to a modification of the method reported by Britton [15]. Briefly, an ultrasonic bath for 30 min at 45 °C was used to accelerate the extraction. Then, the samples were centrifuged for 20 min at 4500 rpm at 25 °C, and the absorbance of the supernatant was measured at 450 nm using a UV/VIS spectrophotometer (Shimadzu UV-1700). The amount of carotenoids (Encapsulated Carotenoids) present in the MEs was calculated using the following equation [14].

$$\text{Encapsulated Carotenoids} = \frac{A \times V}{A^{1\%}_{1\text{cm}} \times 100}$$

where Encapsulated Carotenoids is the mass of carotenoids (g), V the volume of the solution, A the measured absorbance, and $A^{1\%}_{1\text{cm}}$ = 2500 is the specific absorption coefficient of a solution of 1 g of total carotenoids in 100 mL of solution. The encapsulation efficiency is defined as the ratio between encapsulated carotenoids and total carotenoids added into the system. Encapsulation Efficiency was calculated using a modified equation of McNamee et al. [16] as follows:

$$\text{Encapsulation Efficiency} = \frac{\text{Total carotenoids} - \text{Encapsulated carotenoids}}{\text{Total carotenoids}} \times 100$$

2.4. Characterization of MEs

In order to gain knowledge about the particle size, turbidity measurements and stability tests (centrifugation and thermal stress stability) were carried out.

2.4.1. Particle Size Determination

The mean particle radius and particle size distribution (PSD) of MEs were measured by photon correlation spectroscopy using a Malvern Mastersizer 2000 Hydro (Malvern Instruments, Malvern, UK) according to a modification of the method described by Rao and McClements [17]. 0.125 gr of sample (lyophilized MEs) were dispersed in approximately 125 mL of distilled water in the sample chamber with agitation until approximately an 11–13% obscuration was obtained. Determinations

were carried out at an angle of 90° at 25 °C. Dispersions were diluted with double-distilled water to ensure that the light-scattering intensity was within the instrument's sensitivity range.

2.4.2. Turbidity Determination

Turbidity determination was carried out according to a modification of the method presented by Patel and San Martin-Gonzalez [18]. Briefly, MEs dispersions (in water) were diluted by mixing 3 mL of dispersion and 8 mL of distilled water using vortex for 1 min. Absorbance at 700 nm was read using UV/VIS spectrophotometer. Both dispersions remained at 4 °C and 35 °C and determinations were carried out after 0, 24, 48, and 72 h. Distilled water was used as blank.

2.4.3. Centrifugation and Thermal Stress Stability Tests

Preliminary stability tests, including centrifugation and thermal stress, were carried out, according to Oliveira et al. [19], on MEs samples before they were freeze-dried.

During centrifugation test (samples were prepared as indicated in Section 2.2.4), which took place 24 h after preparation; 5 g of ME samples were centrifuged (using a Digicen 20-R, Orto Alresa, Spain) by applying three different rotation speeds (1000, 2500, and 3500 rpm), with 15 min duration for each cycle and at 25 °C.

For the thermal stress test (samples were prepared as indicated in Section 2.2.4), samples were exposed to thermal stress in a thermostatic bath (Elmasonic S 100 H, Elma GmbH & Co. KG, Singen, Germany). The temperature during the test ranged from 40 up to 70 °C (±2 °C) with 5 °C increments. Initially the temperature was set at 40 °C and then was gradually increased 5 °C every 30 min up to 70 °C. Every 30 min an observation was made for any changes, namely phase separation and change of color or appearance of precipitate. "Empty" MEs were kept at the same conditions.

2.5. Antioxidant Activity

2.5.1. Determination of DPPH Radical Scavenging Activity

The antioxidant capacity of extracts was determined using DPPH as a free radical with a modified procedure proposed by Tsaknis and Lalas [20]. Briefly, 1.0 mL of 2,2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich, St. Louis, MO, USA) solution in methanol (0.01 mM) was added to 4.0 mL of sample solution (crude extracts and extract mixtures). The absorbance of this solution was measured at 517 nm against a control comprised of 4.0 mL of methanol and 1.0 mL of 0.5 mM methanolic DPPH solution, while α-tocopherol was used as the positive control.

2.5.2. Differential Scanning Calorimetry (DSC)

The antioxidant activity of the crude freeze-dried extracts and extract mixtures (after evaporation of the solvent using a rotary evaporator) and their freeze-dried MEs was estimated using DSC according to a modified procedure of Gortzi et al. [21]. Briefly, samples (pure extracts or extract mixtures or MEs) of 4 mg were placed in DSC aluminum crucibles closed with lids perforated with a hole (1 mm in diameter) in the center in order to allow the sample to be in contact with the oxygen stream. The purge gas used was oxygen. An empty crucible, hermetically sealed, was used as reference. The starting temperature of oxidation was determined as the onset temperature of the oxidation peak. The temperature program was: hold for 1 min at 40 °C, heat from 40 to 200 °C (40 °C/min), and, finally, heat from 200 to 580 °C (20 °C/min). A Perkin Elmer Diamond DSC (PerkinElmer Inc, Shelton, CT, USA) was used to evaluate the samples' oxidation stability.

2.6. Statistical Analysis

Results are presented as average ± standard deviation of three simultaneous assays. Statistical significance of the differences between mean values was assessed by ANOVA test. $p < 0.05$ was considered as statistically significant.

3. Results and Discussion

3.1. Carotenoids Extraction

The influence of various extraction parameters, i.e., their way of extraction and time, on the extraction of carotenoids was determined while bearing in mind the optimization of the procedure. During this study, ethanol was used as an environmentally friendly solvent with a low biological-hazard profile, since polar solvents (such as alcohols) are generally considered good extraction media for carotenoids which contain oxygenated groups at the end of the molecules [22]. Additionally, during this study, the techniques applied were chosen in order to reduce of the cost of the final products, and, at the same time, maintain high quality of extractables with potential applications in food industry.

Three different experimental procedures were carried out in order to estimate the impact of each one of the individual parameters on the concentration of carotenoids. In all cases, the selected temperature was 45 °C (Table 1), in order to avoid auto-oxidation and isomerization. It was proven that an increase in extraction time leads to an increase in total carotenoids extraction. Specifically, the third protocol (magnetic stirring for 5 h at 45 °C) induced the highest extraction percentage of total carotenoids, followed by the ultrasonic procedure protocol (30 min/45 °C). However, there were no significant differences in concentration, possibly because the long duration of the procedure led to a higher decomposition of carotenoids. Therefore, the ultrasonic procedure was finally chosen because of the much shorter extraction time needed, and it was then applied to the extraction of all samples (Green Pepper, Red Pepper and *M. oleifera*). Therefore, ultrasound-assisted extraction can accelerate the process and improve bioactive compound extraction and, additionally, reduce extraction time, save energy, increase yield, etc.

Table 1. Total carotenoids concentration (μg/g) after their extraction with three different protocols.

Sample	Magnetic Stirring (30 min/45 °C)	Ultrasound (30 min/45 °C)	Magnetic Stirring (5 h/45 °C)
Green Pepper	76.1 ± 2.6	102.7 ± 5.2	120.4 ± 5.5
Red Pepper	442.4 ± 13.3	484.9 ± 10.6	500.1 ± 6.4
Moringa oleifera	1442.1 ± 35.2	1482.5 ± 39.2	1502.6 ± 2.6

Results are means of triplicate determinations ± standard deviation.

During our study, the use of the ultrasonic extraction protocol showed that *M. oleifera* L. leaf extract presented the highest (significant at $p < 0.05$) carotenoid content, followed by that of Red Pepper extract and, finally, Green Pepper extract. The present results are in line with those presented by Raghu and Failla [23] who confirmed that *M. oleifera* L. leaves are rich in carotenoids (e.g., β-carotene). Red Pepper showed higher carotenoid content than Green Pepper, which is in line with the results of Zhang and Yasunori [24]. Various concentrations of carotenoids extracted from Red Pepper and *M. oleifera* L. have been reported in previous studies [25,26]. This could be due to the differences in genotype, maturity stage, and drying process of the plant material [25], post-harvest processing, and preparation procedure [27].

3.2. Encapsulation Efficiency of Carotenoids in MEs

Capsicum annuum L. fruit extract (Green and Red Pepper), *Moringa oleifera* leaf extract, and their mixtures (50/50 *w*/*w*) were incorporated into MEs prepared by mixing lecithin (emulsifier), sunflower oil (continuous oil phase), ethanol solution (co-surfactant), and water (dispersed phase). Lecithin-based MEs have been desirable as encapsulation carriers due to their tendency to mimic the phospholipid nature of cell membranes [28], and this delivery system could increase the bioavailability of incorporated carotenoids. The highest level (59.67% ± 1.01 encapsulation efficiency -EE) of carotenoids incorporated in MEs was observed by *M. oleifera* (significant at $p < 0.05$), followed by Red Pepper/*M. oleifera* (EE 57.77% ± 1.10), Red Pepper (EE 51.60% ± 1.70), and Green Pepper/*M. oleifera*

(EE 50.25% ± 0.92). The lowest level (EE 48.00% ± 1.31) was observed by Green Pepper MEs. The rate of encapsulation was likely to be proportional to the concentration of total carotenoids in the extracts. Total carotenoid content was the only (measured) parameter that varied between the various MEs, since all encapsulations were carried out under the same conditions (common encapsulation protocol).

3.3. Thermal and Centrifugation Stability Tests of MEs

No instability (indicated by phase separation, change of color, or appearance of precipitate) was observed in MEs after 24 h at room temperature. Centrifugation tests proved that MEs did not show any phase separation or color change or precipitation after being centrifuged at 1000, 2500 and 3000 rpm. MEs retained their transparent appearance.

Furthermore, the structure of MEs also remained stable during temperature increase (thermal test) from 40 to 70 °C (again without phase separation, change of color, or appearance of precipitate). Again, MEs retained their transparent appearance.

The above results confirmed that MEs are thermodynamically stable emulsions. Due to the presence of extremely small droplets (<100 nm) in MEs, the phase separation phenomenon does not occur even after long-time storage. Additionally, MEs do not require high inputs of energy or shear conditions for their formation. The formation and stability of MEs can be described by interface science or chemical solubility theories, or with the use of thermodynamics; thermodynamics explain that a net release of free energy is obtained when favorable entropic contributions from the mixing of small droplets in the continuous phase and the diffusion of surfactant in the inter-facial layer are larger than the unfavorable contribution of the reduction of surface tension [29], resulting in a thermodynamically stable dispersion [30].

3.4. Turbidity Determination

Temperature-scanning turbidity measurements were used to obtain information about potential changes in the microstructure of MEs during heating [17]. An increase in turbidity is interpreted as an increase in the aggregation of vesicles due to the decrease of their stability, and is tightly connected to an increase in turbidity and, therefore, can be used to monitor changes in the sample's stability [31].

In Figures 1 and 2 the change in optical density of MEs at 4 °C and 35 °C during the time intervals tested is presented. The results suggest that the encapsulation of extracts induced physicochemical changes in the MEs and improved the stability behavior of the system. The samples showed little change in optical density, which leads to the conclusion that they were stable in the tested conditions. Additionally, only small differences in optical density between the MEs were observed. The ME of *M. oleifera* L. leaf extract showed the lowest increase in optical density. However, there were no significant differences between the samples.

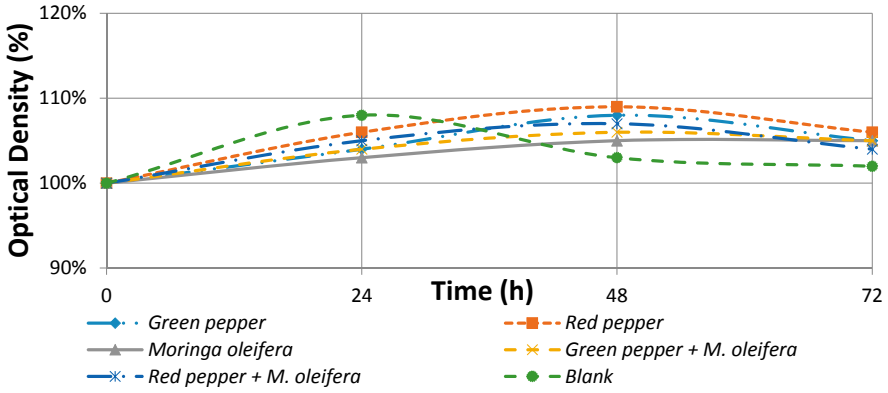

Figure 1. Changes in turbidity (optical density) of microemulsions (MEs) at 4 °C during 0, 24, 48, and 72 h.

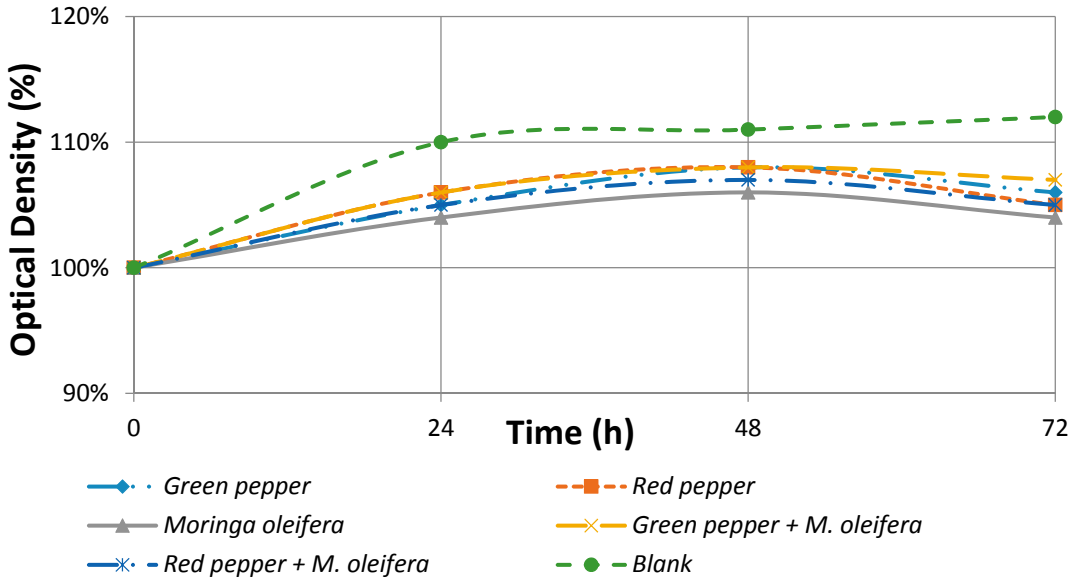

Figure 2. Changes in turbidity (optical density) of MEs at 35 °C during 0, 24, 48, and 72 h.

Results are in line with those of Cho [32] who also concluded that the turbidity of MEs did not change after accelerated stability tests, indicating that this microemulsion system was stable under the given harsh conditions. Turbidity determination results correlated well with those taken from the thermal and centrifugation stability tests, which also indicated the thermodynamic stability of the samples.

3.5. Particle Size of MEs

The particle size distribution is considered as the most important characteristic for the assessment of the nature and stability of the emulsions. The results (Figure 3) of the particle size of the prepared MEs showed that their average value varied between 10 and 95 nm. The mixture (50/50 *w*/*w*) of samples of Green or Red Pepper extracts with the extract from the leaves of *Moringa oleifera* L. tree showed that the distribution of values of the particle size has a narrower base (with a small variation of the sizes of these particles around the average value). *M. oleifera* MEs showed the narrowest base and the highest intensity among all samples tested, while Green Pepper MEs showed the widest base and the lowest intensity. It is concluded that all the prepared MEs have a highly uniform particle size (small size dispersion around the average value). Additionally, the absence of aggregates and stability of these structures was concluded. Finally, the distribution results showed that "empty" MEs (blank) exhibit a range around average value, which was larger compared to those MEs that incorporate extracts.

MEs formation process is generally a random stirring process. The resulting delivery system may end up in a polydispersed system, in which different droplet sizes can coexist [33]. In some cases, the loading of a lipophilic active compound could result in an increase in the droplet size and, eventually, could compromise system's physical stability. However, this was not the case in our study, where "empty" MEs seemed to be heterogeneous with wider particle size distribution than those of MEs encapsulating extracts. Smaller particle size and more homogeneous (narrow base) indicated that the extracts' molecules stabilize the MEs system. The surfactant used in our study possibly reduced the interfacial tension to a sufficiently low value, which helped to lower the particle size distribution with the addition of a carotenoid-rich extract [34].

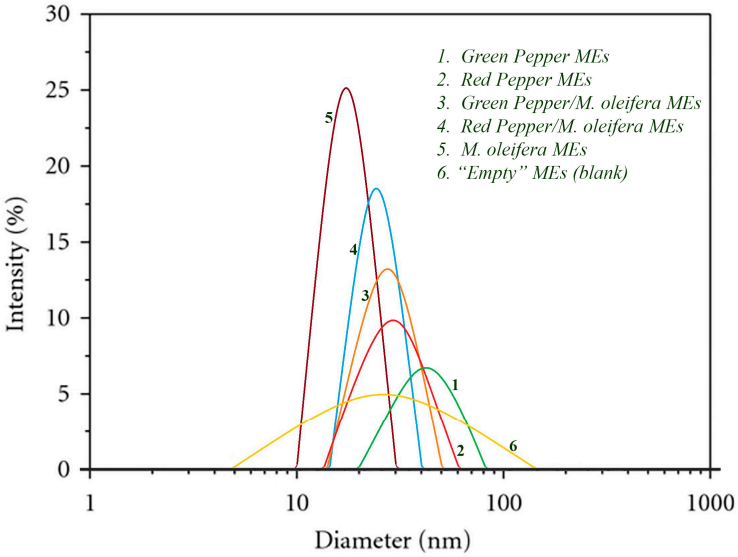

Figure 3. Particle size distribution of MEs.

3.6. Antioxidant Activity

3.6.1. DPPH Radical Scavenging Activity

The DPPH test is widely used to measure the antioxidant activity of hot and sweet peppers. Compounds with antioxidant activity are able to reduce the stable free radical DPPH to the yellow colored 2,2-diphenyl-1-picrylhydrazyl. However, sample interference has been reported for potential underestimation of the DPPH values measured at 515 nm [35]. The interferences may arise because of the presence of colored compounds, such as anthocyanins, carotenoids, etc.

During this work, the extracts and their mixtures were tested for their antioxidant activity using the DPPH method. However, something paradoxical was observed. A DPPH absorbance decrease occurred only for the samples with concentration ranging between 6.25–100 μg/mL. On the other hand, the DPPH absorbance decrease was not observed for samples with concentration higher than 100 μg/mL.

3.6.2. Differential Scanning Calorimetry (DSC) for Determination of Antioxidant Activity

DSC is a thermal analysis technique for estimating physical or chemical changes of materials as a function of temperature by detecting the heat changes associated with such processes. As reported by Gortzi et al. [36], antioxidant activity is evaluated by the extrapolated temperature at the beginning of the oxidation process based on the measurements of the incubation period. For that reason, the kinetic parameters of the non-inhibited and inhibited oxidation can be determined by the DSC technique. The method that was used in DSC analysis was based on experiments in which the temperature of the extrapolated onset of the thermo-oxidation process and the temperature of maximum heat flow were determined from the resulting measurement curves of the exothermic reaction [21].

Thermal oxidative degradation of pure *C. annuum* L. and *M. oleifera* L. extracts and their mixtures (evaporated), "empty" MEs (blank), as well as MEs encapsulating *C. annuum* L. and *M. oleifera* L. extracts and their mixtures, were studied (Figure 4). The differences in physicochemical characteristics of the system are responsible for differences in thermographs shape. Data indicated that the exothermic peaks of our samples were observed in the range of 45 °C to 580 °C related to their autoxidation process. In all cases, the encapsulation of extracts in MEs significantly ($p < 0.05$) increased antioxidant activity compared to the same extracts in pure form. It was also proven that encapsulation significantly ($p < 0.05$) increased the temperature of the initiation of their oxidation (at the same heating rate). The modified antioxidant action of extracts during their encapsulation

was expected, since the complexes (MEs + extracts) constitute a new system with new individual physicochemical characteristics.

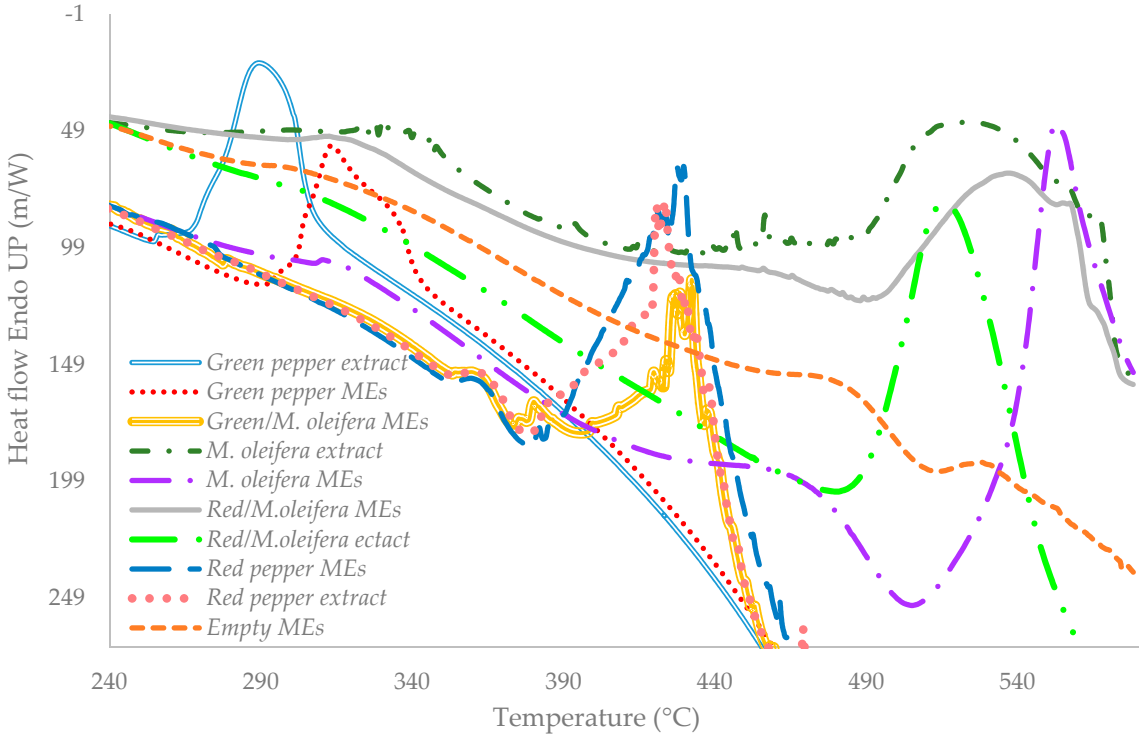

Figure 4. Differential Scanning Calorimetry (DSC) diagrams.

As is presented in Figure 4, the *M. oleifera* L. extract in pure and encapsulated form had the highest oxidation peaks (T_{max} = 521.06 °C and 553.90 °C, for pure and encapsulated extract, respectively), followed by the Red Pepper/*M. oleifera* L. mixture (T_{max} = 516.13 °C and 535.53 °C, for pure and encapsulated extract, respectively), Green Pepper/*M. oleifera* L. mixture (T_{max} = 390.17 °C and 432.64 °C, for pure and encapsulated extract, respectively), Red Pepper extract (T_{max} = 420.08 °C and 429.37 °C, for pure and encapsulated extract, respectively), and, finally, Green Pepper extract (T_{max} = 292.41 °C and 315.19 °C, for pure and encapsulated extract, respectively). The results appeared to be in line with those of the encapsulation efficiency of carotenoids in MEs. There was only one exception in the case of Red Pepper MEs. The possible explanation for this exception could be that antioxidant activity is related not only to the concentration of extracted carotenoids, but is also influenced by the chemical characteristics of the extracted compounds.

As indicated by the results, the unique structure of MEs improved the protection of lipophilic compounds and the scavenging reactive species generated in photooxidative process. Mixtures of extracts were more effective than single compounds.

As indicated in a previous study [21], the antioxidant activity of "empty" MEs (no extract added) of the same lipid composition appeared significantly ($p < 0.05$) lower than the extracts, which leads to the conclusion that the system extract + MEs is more stable.

4. Conclusions

Carotenoids are relatively unstable in food systems because they are susceptible to light, oxygen, and autooxidation, and their dispersion in pure form into ingredient systems can result in rapid degradation. The encapsulation of carotenoids improves their bioavailability, solubility, and protection in food systems. Specifically, microemulsions (MEs) may be a potential candidate to improve the solubility and increase the bioavailability of carotenoids, since they are stable delivery systems (due to

their structure and properties). In this study, ethanol extracts of pure *C. annuum* L. fruits (green and red), *M. oleifera* L. leaves, and their mixtures have been encapsulated in MEs. In order to study the physicochemical characteristics (particle size, stability, and turbidity determination) of prepared MEs, preliminary stability tests (the centrifugation test and the thermal stress test) were carried out. Based on the thermal stress and centrifugation tests, MEs did not show any sign of instability as phase separation, creaming, sedimentation, presence of aggregates, and other macroscopic drawbacks. Turbidity measurements showed that MEs were stable, as only small differences in optical density were observed. The MEs of *M. oleifera* L. leaves appeared to be more stable. The prepared MEs had a uniform particle size (small size dispersion around the average value), which varied between 10 and 95 nm. The results of the antioxidant activity of the extracts and their MEs showed that there was higher resistance in oxidation observed by *M. oleifera* leaf extract, followed by red pepper/*M. oleifera* mixture, green pepper/*M. oleifera* mixture, red pepper and, finally, green pepper extract. The results concerning MEs samples followed the same trend.

Author Contributions: Olga Gortzi, Stavros I. Lalas, and George D. Nanos conceived and designed the experiments; Georgia Batra, Anna Galidi, Angeliki Alibade performed the experiments; Georgia Batra, Stavros I. Lalas and Olga Gortzi and George D. Nanos analyzed the data; Georgia Batra, Stavros I. Lalas and Olga Gortzi wrote the paper.

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

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