Improvement for Oxidative Stability and Sensory Properties of Sunflower Oil Flavored by Huai Chrysanthemum × morifolium Ramat. Essential Oil during Accelerated Storage

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Abstract: Flavored oils, as one of the most important condiments in cuisine, are widely used in vegetable oils all over the world. The oxidative stability and sensory qualities of sunflower oil, flavored by essential oil obtained from Chrysanthemum × morifolium Ramat. (HCEO) extraction, were studied. After the accelerated storage at 65 °C for 30 days, HCEO (1600 mg/kg) was able to markedly inhibit the increase in some important indicators of lipid alteration, among which acidity, peroxide, ρ-anisidine and total oxidation values, together with other parameters (thiobarbituric acid reactive substances, conjugated dienes and trienes). Finally, it was observed that the sunflower oil flavored by HCEO (1600 mg/kg) restrain the modifications of fatty acid compositions and showed improved sensory properties in respect to non-added oil. Consequently, HCEO can be considered a valid additive for flavored vegetable oils with antioxidant effects.

Keywords: Chrysanthemum × morifolium Ramat.; essential oil; sunflower oil; oxidative stability; sensory property

1. Introduction

All over the world, flavored vegetable oils are seasonings widely used in cooking [1,2]. In Mediterranean countries, flavored olive oils are interesting products that have increasingly attracted the interest of consumers for their improved sensory and nutritional properties [3]. Additionally, essential oils obtained from spices and herbs are a rich source of bioactives for preparing flavored oils. They play a vital part in the flavoring process due to their extraordinary flavor and functional characteristics [4].

Recently, essential oils derived from herbs and spices had been demonstrated to not only provide pleasing flavors and acceptable odors but also ameliorate the oxidative stability and sensory properties of vegetable oils [5]. For instance, Chandran et al. reported that coconut oils (CCO) showed improved oxidative stability after flavoring with black pepper and ginger essential oil (1%). These authors found that, during the accelerated storage, CCO had antioxidant effects similar to CCO supplemented with synthetic antioxidants (tert-buty1 hydroquinone, TBHQ) at a concentration of 200 mg/kg [6]. Furthermore, the panelists in the sensory evaluation study preferred the salad added with CCO after the flavoring process with essential oil. In a previous study, sunflower oil flavored with Punica granatum cv. Heyinshiliu peel essential oil (800 mg/kg) showed better sensory properties and oxidative stability during accelerated storage [7]. Therefore, the flavored oils, prepared by essential oils obtained from herbs and spice extraction, are considered as a prominent approach to enhance the overall qualities of vegetable oils.

Lipid oxidation is a spontaneous chemical reaction that leads to lipid rancidity and degradation, particularly in some vegetable oils characterized by the high content of

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polyunsaturated fatty acids (PUFA) [8]. Sunflower oil is a kind of nutritious vegetable oil that contains lots of PUFA (more than 85%), especially represented by linoleic acid (more than 60% of fatty acids) [9]. It has been confirmed that linoleic acid, known to be essential for humans, could be used for the medical treatment of arteriosclerosis and hyperlipidemia [10]. Moreover, the reduction of overall quality takes place in sunflower oil and the linoleic acid might decrease since sunflower oil suffering oxidative deterioration, even produced some harmful effects on consumers’ health [11]. In order to prevent degradation and rancidity of vegetable oils, synthetic antioxidants (TBHQ, butylated hydroxyanisole, butylated hydroxytoluene) have generally been used to restrain the [12]. Despite the effective antioxidant activity of synthetic antioxidants, safety concerns are increasingly alarming among consumers [13]. Currently, to reduce the negative effects of these effective antioxidants, the permissible upper safety limit of 200 ppm has been already set in several countries. Moreover, in many countries, the use of butylated hydroxytoluene and butylated hydroxyanisole has been also restricted [14]. Therefore, it is quite meaningful to explore natural antioxidants such as essential oils from herbs and spices, which provide good antioxidant activity and improved sensory properties [15–17].

Huai Chrysanthemum × morifolium Ramat. (Asteraceae family) is a special kind of local Chinese herbs of Henan province. Due to its anti-inflammatory, antifungal, analgesic and antipyretic effects, it is used for medical applications [18–20]. It has been reported that the leaves of C. morifolium cv. Hang-ju (a cultivar of C. morifolium Ramat.) were an abundant source of preservatives due to its antibacterial activity [21]. Furthermore, several extracts of C. morifolium were identified to possess anti-oxidant effects, including flavonoids, polysaccharides, polyphenols and essential oils due to its high content of alkenes and terpenes [22,23]. To the best of our knowledge, the Huai C. morifolium Ramat. essential oil (HCEO) has never been investigated and used as flavored oil in sunflower oil until now. As a consequence, for the first time, this research investigated the oxidative stability and sensory properties of sunflower oil flavored with HCEO during the accelerated storage at 65 °C for 30 days.

2. Materials and Methods

2.1. Material and Chemicals

The flowers of Huai Chrysanthemum × morifolium Ramat. harvested in Jiaozuo City, China, were purchased from Henan Zhangzhongjing Pharmacy Co., Ltd., Zhengzhou, China. Sunflower oil produced by China Oil and Foodstuffs Corporation (COFCO) was obtained from Jingdong Mall (Beijing, China) with the sunflower seeds harvested in Ukraine. Vitamin C (Vit. C), TBHQ, 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonate) diammonium salt (ABTS) and potassium ferricyanide [K₃Fe(CN)₆] were from Sigma-Aldrich (Milan, Italy). n-hexane was purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Additionally, all the other analytical-grade chemicals were obtained from Tianjin Kemiou Chemical Reagent Co., Ltd. (Tianjin, China).

2.2. Extraction of HCEO

HCEO was extracted using steam distillation, using Wang et al.’s method [24] with a little modification. In brief, the flowers of Huai C. morifolium (800.0 g) were crushed using a multifunctional grinder (HC-2500Y304, Haina Co., Ltd., Wuyi, China) into small particles (diameter < 1.0 mm). Subsequently, the particles were divided into four parts (each 200.0 g). For each part, the particles were hydro-distilled by a steam distillation apparatus (XH-1000, Xinhu Co., Ltd., Shanghai, China) for 6.0 h with water (1000 mL) in a flask (2000 mL). For hydro-distillation, 1.0 mL of n-hexane was placed on the top of the distillate layer to obtain the organic phase. After hydro-distillation, the organic phase was collected and dried over anhydrous Na₂SO₄ to obtain the essential oil, HCEO. Immediately, HCEO was stored at −4 °C in a dark brown bottle for the following experiments.
2.3. Chemical Analysis of HCEO

The chemical analysis of HCEO was carried out by gas chromatography–mass spectrometry (GC–MS) by using Wang et al.’s method [25]. Briefly, GC–MS (Agilent 6890-5973N, Santa Clara, CA, USA) was operated at 70 eV ionization energy. The chromatographic separation was carried out using an HP-5MS capillary column (30.0 m × 0.25 mm × 0.25 µm, Agilent Technologies, Santa Clara, CA, USA). The temperatures of the injector and detector were maintained at 200 °C and 290 °C, respectively. The program of oven temperature was: from 50 °C to 70 °C at 10 °C/min, held for 2 min; heated to 125 °C at 2 °C/min, held for 2 min; heated to 210 °C at 4 °C/min, held for 2 min; finally, heated to 280 °C at 20 °C/min, held for 5 min. Then, 1 µL of the sample was injected (1:20 split ratio), and helium was employed as carrier gas (1.0 mL/min flow rate). MS spectra were recorded in the 30–500 m/z mass range. The chemical compounds were identified by comparison with the NIST database 2017 (http://webbook.nist.gov, accessed on 5 November 2019) and quantified by using the normalization method.

2.4. Antioxidant Activity of HCEO

The antiradical activity of HCEO was studied by ABTS radical (ABTS••) and hydroxyl radical (HO•) assays, while the reducing power of HCEO was investigated by ferric ion reducing antioxidant power (FRAP) and total reducing power [K₃Fe(CN)₆], following the methods reported in previous papers [26,27].

2.5. Preparation of Sunflower Oil Flavored by HCEO

Table 1 shows the samples prepared and analyzed in the following. The control sample was non-added, while the others were added with TBHQ (200 mg/kg) or HCEO (200–1600 mg/kg) after the direct addition to sunflower oil. It has been reported that storage at 65 °C for 24 h is equivalent to one month of storage at room temperature [28]. On this base, the sunflower oil samples (added and non-added) were stored in dark brown bottles in an oven at 65 °C for 30 days in order to study the effects of accelerated storage. The sunflower oils were analyzed every 6 days for exploring the antioxidant effect of HCEO in sunflower oil.

Table 1. The preparation of sunflower oil samples flavored by HCEO.

| Groups     | Addition for Sunflower Oil Sample        |
|------------|------------------------------------------|
| Control    | Nothing (TBHQ-0 + HCEO-0)                |
| TBHQ       | TBHQ at 200 mg/kg                        |
| 200 mg/kg  | HCEO at 200 mg/kg                        |
| 400 mg/kg  | HCEO at 400 mg/kg                        |
| 800 mg/kg  | HCEO at 800 mg/kg                        |
| 1600 mg/kg | HCEO at 1600 mg/kg                       |

2.6. Determination of Acidity, Peroxide, p-Anisidine, and Total Oxidation Values

The values of acidity (AV), peroxide (PV), and p-anisidine (pAnV) were evaluated according to the Chinese National Standard (CNS) by using GB 5009.229-2016, GB 5009.227-2016 and GB 24304-2009/ISO 6885-2006 procedures, respectively. The total oxidation value (TOTOX) was determined applying the following formula:

\[ \text{TOTOX} = 2 \times \text{PV} + \rho\text{AnV} \]  

where PV is the peroxide value, and pAnV is the acidity value.

2.7. Determination of TBARS, K232 and K268 Values

Thiobarbituric acid reactive substances (TBARS) were determined by Takeungwongtrakul and Benjakul’s method [29], with a little modification. Briefly, oil (1.0 g) was mixed with TBA reagent (3.5 mL) containing 0.25 M HCl, 0.375% thiobarbituric acid (w/v), and
15% trichloroacetic acid (w/v). The reaction solution was heated for 15 min at 95 °C. After cooling with running tap water and centrifugation (4000 r/min at room temperature for 15 min) by an LD5-10 centrifuge (Beijing Jingli centrifuge Co., Ltd., Beijing, China), the water phase has been obtained. Finally, the absorbance at 532 nm of supernatant was evaluated by using a UV-6000PC spectrophotometer (Shanghai Metash instruments Co., Ltd., Shanghai, China). TBARS were calculated according to a malonaldehyde (MDA) standard curve, and the values were expressed as mg MDA/kg.

Conjugated diene (K232) and conjugated triene (K268) were determined by evaluating the absorbance at 232 and 268 nm, respectively, in accordance with CNS GB/T 22500-2008 method.

2.8. Chemical Analysis of Fatty Acid Composition

Fatty acid methyl esters of sunflower oil samples were obtained by esterification through boron trifluoride, in accordance with CNS GB 5009.168-2016 method, and then analyzed by GC instrument coupled with a flame ionization detector (FID). The chromatographic separation was carried out using a HP-88 capillary column (100 m × 0.252 mm × 0.2 µm). The program of oven temperature was: from 170 °C to 220 °C at 4 °C/min, and heated to 235 °C at 1 °C/min; heated to 240 °C at 1 °C/min (the whole system operated for 32.5 min). The temperatures of the injector and FID were maintained at 250 °C and 280 °C, respectively. Then, 1 µL of the sample was injected (1:50 split ratio), and hydrogen, provided by a high purity generator (SGH-300, Beijing Oriental essence Technology Co., Ltd., Beijing, China), was employed at 30 mL/min. Nitrogen was used as carrier gas (1 mL/min, 1:50 split ratio), and the air was generated by a generator (QY-3, Jinan Qingchuan Instrument Co., Ltd., Jinan, China) and used at 400 mL/min.

2.9. Sensory Evaluation of Sunflower Oil Samples Treated with HCEO

The sensory properties of sunflower oil samples flavored by HCEO were evaluated every 6 days, during the accelerated storage, by 100 semi-trained panelists from shopper representatives of sunflower oil in Guangzhou Xinyuan Wholesale Food Market (Guangzhou, China). To be sure of the accuracy of sensory evaluation, all panelists were demanded to carefully read the information on the sensory sheet and understand the meaning of the attribute. After that, adequate time was provided to the panelists to familiarize themselves with the procedures of sensory assessment. In order to optimize the sensory analysis, all the sunflower oil samples were randomly showed with a 3-digit code number. In order to evaluate the sensory properties of flavor, taste, appearance and acceptability of the samples (extremely dislike—2, moderately dislike—4, neither like nor dislike—6, moderately like—8, extremely like—10), a 10-point hedonic scale was applied.

2.10. Statistical Analysis

With the exception of the chemical analysis of HCEO, the other experiments were performed in triplicate. Unless otherwise indicated, the experimental results were reported as mean value, while presenting in Tables and Figures were indicated as mean value ± standard deviation (SD). The data analysis was elaborated by GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA) and IBM SPSS Statistics 20.0 by using a one-way analysis of variance test (ANOVA). The significant standards of probability levels of 95% (p < 0.05) and 99% (p < 0.01) were statistically significant or highly significant, respectively.

3. Results and Discussion

3.1. Chemical Composition of HCEO

As a Chinese traditional herb, a large number of cultivars of C. morifolium were gradually evolved, and Huai C. morifolium is one of them, which was widely cultivated in Jiaozuo, Henan province [30]. In the present study, the essential oil was extracted (yield = 0.53% w/w) from Huai C. morifolium flowers. Table 2 shows the chemical composition of HCEO determined by GC–MS analysis. Sixty-two compounds, representing 99.00% of the essen-
tial oil, were identified. Among them, the major HCEO constituents were: methyl esters, monoterpenes and sesquiterpenes, including methyl linoleate (13.2%), methyl oleate (13.0%), (±)-camphor (11.8%), cineole (10.4%), methyl stearate (5.7%), β-sesquiphellandrene (3.4%), methyl stearate (3.1%), trans-β-farnesene (3.1%) and chamazulene (3.1%). Furthermore, the chemical composition of C. morifolium essential oil from Sichuan province had been found to mainly contain: monoterpenes and sesquiterpenes, including α-curcumene (12.6%), nonadecane (4.2%), α-farnesene (3.5%) and n-heptadecane (3.2%) [31]. Obviously, the chemical compounds of HCEO were quite different from the study. It has been reported that the chemical components of essential oils may be affected by lots of factors, such as the growing environment and extraction methods [24]. However, although the specific chemical compounds of C. morifolium essential oil from Jiaozuo, Henan province were different from Sichuan province, monoterpenes and sesquiterpenes were the main components. Consequently, the chemical components of HCEO may be influenced by its place of origin and extraction approach [19]. However, excluding the fatty acid methyl ester influenced by the extraction method, monoterpenes and sesquiterpenes were the main components and expected bioactive compounds.

Table 2. Chemical composition of HCEO.

| No. | RT a | Compound Name | Molecular Formula | RA b   |
|-----|------|---------------|-------------------|--------|
| 1   | 51.63| Methyl linoleate | C_{19}H_{34}O_2   | 13.16% |
| 2   | 51.80| Methyl oleate   | C_{19}H_{36}O_2   | 12.96% |
| 3   | 14.38| (±)-Camphor     | C_{10}H_{16}O     | 11.81% |
| 4   | 9.07 | Cineole         | C_{10}H_{18}O     | 10.40% |
| 5   | 48.15| Methyl palmitate| C_{17}H_{34}O_2   | 5.68%  |
| 6   | 2.12 | Cyclohexane     | C_6H_{12}         | 4.38%  |
| 7   | 36.50| β-Sesquiphellandrene | C_{15}H_{24}   | 3.41%  |
| 8   | 52.27| Methyl stearate | C_{19}H_{38}O_2   | 3.09%  |
| 9   | 32.78| trans-β-Farnesene| C_{15}H_{24}     | 3.07%  |
| 10  | 43.46| Chamazulene     | C_{14}H_{16}      | 2.89%  |
| 11  | 26.24| α-Terpinyl acetate| C_{12}H_{20}O_2  | 2.44%  |
| 12  | 16.15| Terpinen-4-ol   | C_{10}H_{18}O     | 2.34%  |
| 13  | 34.00| cis-β-Copaene   | C_{15}H_{24}      | 2.15%  |
| 14  | 16.90| (+)-α-Terpineol | C_{10}H_{18}O     | 1.93%  |
| 15  | 41.33| (−)-α-Cadinol   | C_{15}H_{26}O_2   | 1.53%  |
| 16  | 22.40| (+)-Borneol acid| C_{12}H_{20}O_2   | 1.45%  |
| 17  | 15.47| Borneol         | C_{10}H_{18}O     | 1.23%  |
| 18  | 6.94 | Sabineine       | C_{10}H_{16}      | 1.20%  |
| 19  | 56.26| Pentacosane     | C_{25}H_{52}      | 1.15%  |
| 20  | 10.20| γ-Terpinene     | C_{10}H_{16}      | 1.04%  |
| 21  | 30.18| β-Caryophyllene | C_{15}H_{24}      | 1.03%  |
| 22  | 7.05 | β-pinene       | C_{10}H_{16}      | 0.93%  |
| 23  | 42.25| α-Bisabolol     | C_{15}H_{26}O_2   | 0.85%  |
| 24  | 5.76 | α-Pinene        | C_{10}H_{16}      | 0.80%  |
| 25  | 6.19 | (+)-Camphene    | C_{10}H_{16}      | 0.78%  |
| 26  | 8.77 | p-Cymene        | C_{10}H_{14}      | 0.53%  |
| 27  | 35.05| Zingiberene     | C_{15}H_{24}      | 0.52%  |
| 28  | 32.16| α-Caryophyllene | C_{15}H_{24}      | 0.48%  |
| 29  | 12.75| 1-Octen-3-yl-acetate | C_{10}H_{16}O_2   | 0.43%  |
| 30  | 8.45 | Terpinolene     | C_{10}H_{16}      | 0.52%  |
| 31  | 38.79| Caryophyllene Oxide | C_{15}H_{26}O_2  | 0.38%  |
| 32  | 24.29| (-)-α-terpinyl acetate | C_{15}H_{26}O_2 | 0.33%  |
| 33  | 42.37| Shyobunol       | C_{15}H_{26}O_2   | 0.28%  |
| 34  | 7.49 | β-myrcene       | C_{10}H_{16}      | 0.27%  |
| 35  | 40.46| 1,3,4,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S,4S,4aS,8aR)- | C_{15}H_{26}O_2 | 0.25%  |
Table 2. Cont.

| No. | RT a  | Compound Name                | Molecular Formula | RA b |
|-----|-------|------------------------------|-------------------|------|
| 36  | 34.59 | cis-Muurola-4(15),5-diene     | C_{15}H_{24}      | 0.24%|
| 37  | 57.66 | Heptacosane                   | C_{27}H_{36}      | 0.23%|
| 38  | 34.59 | Bicyclosesquiphellandrene     | C_{15}H_{24}      | 0.22%|
| 39  | 44.63 | 11-Isopropylidene-cis-tricyclo[6,2,1,02,7]undeca-2,4,6,9-tetraene | C_{14}H_{14} | 0.20%|
| 40  | 35.75 | (S)-β-Bisabolene              | C_{15}H_{24}      | 0.19%|
| 41  | 46.36 | Perhydrofarnesyl acetone      | C_{16}H_{36}O     | 0.19%|
| 42  | 41.07 | Heptane                       | C_{7}H_{16}       | 0.14%|
| 43  | 2.28  | (−)−α-Cubebene                | C_{15}H_{24}      | 0.13%|
| 44  | 13.34 | 4,6,6-Trimethylbicyclo[3.1.1]hept-3-en-7-one | C_{10}H_{14}O | 0.13%|
| 45  | 12.12 | Linalool                      | C_{10}H_{18}O     | 0.12%|
| 46  | 55.02 | Methyl isocosoate             | C_{2}H_{42}O      | 0.12%|
| 47  | 55.78 | 2,2′-Methylenebis(6-tert-butyl-4-methylphenol) | C_{15}H_{24}O | 0.11%|
| 48  | 27.66 | α-Copaene                     | C_{10}H_{14}O     | 0.11%|
| 49  | 27.66 | Phenylacetaldehyde            | C_{9}H_{16}O      | 0.10%|
| 50  | 9.53  | Cubenene                      | C_{10}H_{18}O     | 0.10%|
| 51  | 36.78 | Ylangenol                     | C_{10}H_{18}O     | 0.10%|
| 52  | 41.15 | cis-α-Bergamotene             | C_{15}H_{24}      | 0.10%|
| 53  | 31.28 | trans-α-Bergamotene           | C_{15}H_{24}      | 0.10%|
| 54  | 31.28 | 6-Methylhept-5-en-2-one       | C_{15}H_{24}O     | 0.09%|
| 55  | 7.33  | cis-α-Phellandrene            | C_{10}H_{16}      | 0.08%|
| 56  | 40.70 | 1R,5R,9S-11,11-Dimethyl-4,8-bismethylenecyclo[7.2.0]undecan-5-ol | C_{15}H_{24}O | 0.09%|
| 57  | 18.49 | 1R,5R,9S-11,11-Dimethyl-4,8-bismethylenecyclo[7.2.0]undecan-5-ol | C_{15}H_{24}O | 0.08%|
| 58  | 39.13 | (+/−)-cis-Cardamomone         | C_{10}H_{16}      | 0.08%|
| 59  | 5.56  | α-Phellandrene                | C_{10}H_{16}      | 0.07%|
| 60  | 5.57  | (−)-α-Thujene                 | C_{10}H_{16}      | 0.07%|
| 61  | 27.07 | (−)-cis-Cardamomone acetate   | C_{10}H_{18}O     | 0.07%|
| 62  | 25.39 | (2-methyl-5-prop-1-en-2-ylcyclohex-2-en-1-yl) Acetate | C_{12}H_{18}O | 0.06%|

Total 99.00%

3.2. Antioxidant Activity of HCEO

The antioxidant properties of essential oils obtained from herbs and spices have aroused great interest around the world, and their free radical scavenging activity is widely studied [16]. In the measurement for antioxidant activity of HCEO, it demonstrated a dose-dependent scavenging capacity against ABTS+ (Figure 1A) and OH• radicals (Figure 1B). The free radical scavenging percentages, with the concentration of HCEO at 2, 4, 6, 8 and 10 mg/mL, were 59.3%, 83.0%, 94.5%, 95.8% and 97.4% against ABTS+ and 88.0%, 89.2%, 89.3%, 89.7% and 91.1% against OH•, respectively. Meanwhile, FRAP (Figure 1C) and total reducing power (Figure 1D) were determined in order to found the HCEO reducing power. Interestingly, the reducing power of HCEO was similar to its radical scavenging ability. The FRAP and optical density (OD) at 700 nm values were 1.2, 2.1, 2.9, 3.6 and 4.6 mmol/L and 0.7, 1.0, 1.2, 1.6 and 1.8, respectively, taking into consideration the HCEO concentration (2, 4, 6, 8 and 10 mg/mL, respectively). The results indicated that HCEO has an effective antioxidant capacity and could be used as one natural antioxidant [21]. Although the antioxidant effects were demonstrated by the determination of antioxidant activity, the performance for the antioxidant ability of HCEO in sunflower oil needs further investigation.
During the storage of sunflower oil, AV is employed to determine the amount of free fatty acids developed by lipid oxidation because of the hydrolysis of triglycerides [32]. Furthermore, the primary and secondary hydrolysis products, including hydroperoxides, carbonyls and aldehydes, are manufactured during the oxidation of vegetable oils, and PV and ρ-AnV are employed as indicators of primary and secondary products [33]. Consequently, AV, PV and ρ-AnV were applied to monitor the alteration of sunflower oils during the accelerated storage at 65 °C. As revealed in Figure 2, after preserved for 30 days, AV, PV and ρ-AnV values of the control group were significantly increased (p < 0.01). Apparently, after the addition of HCEO at 1600 mg/kg, the values for AV, PV and ρ-AnV were significantly restricted to 0.4 mg KOH/kg, 128.2 meq O$_2$/kg and 18.8 (p < 0.01) at the end of the accelerated storage, respectively. In addition, the TOTOX value was measured to support a better evaluation of progressive oxidative degradation of sunflower oil due to its ability to directly reflecting the primary and secondary oxidation product contents [16]. As shown in Figure 2, at the end of the accelerated storage, the increased the TOTOX value of the sunflower oil samples added by HCEO at 200, 400, 800 and 1600 mg/kg were distinctly decreased to 506.3, 411.8, 390.1 and 275.3 (p < 0.01), respectively. Therefore, according to the results of these four parameters, the flavoring process of HCEO was able to reduce the development of AV, PV, ρ-AnV and TOTOX of sunflower oil samples during the accelerated storage, which was in agreement with the results in antioxidant capacity.

3.3. Effects of HCEO on AV, PV, ρ-AnV and TOTOX

As revealed in Figure 2, after preserved for 30 days, AV, PV and ρ-AnV values of the control group were significantly increased (p < 0.01). Apparently, after the addition of HCEO at 1600 mg/kg, the values for AV, PV and ρ-AnV were significantly restricted to 0.4 mg KOH/kg, 128.2 meq O$_2$/kg and 18.8 (p < 0.01) at the end of the accelerated storage, respectively. In addition, the TOTOX value was measured to support a better evaluation of progressive oxidative degradation of sunflower oil due to its ability to directly reflecting the primary and secondary oxidation product contents [16]. As shown in Figure 2, at the end of the accelerated storage, the increased the TOTOX value of the sunflower oil samples added by HCEO at 200, 400, 800 and 1600 mg/kg were distinctly decreased to 506.3, 411.8, 390.1 and 275.3 (p < 0.01), respectively. Therefore, according to the results of these four parameters, the flavoring process of HCEO was able to reduce the development of AV, PV, ρ-AnV and TOTOX of sunflower oil samples during the accelerated storage, which was in agreement with the results in antioxidant capacity.
Processes 2021, 9, x FOR PEER REVIEW ... ameliorate the oxidative stability of sunflower oil samples, which was in agreement with our previous study results [15].

3.4. Effects of HCEO on TBARS, K232 and K268

As the standard biomarker of lipid peroxidation of vegetable oils, malondialdehyde (MDA) is generated from the hydroperoxides and determined using the TBARS method [14]. As displayed in Figure 3, the TBARS values of all groups increased after heating for 30 days. After the addition of HCEO at 200, 400, 800 and 1600 mg/kg, the increased TBARS values of the control group were significantly decreased to 2.3, 2.8, 2.2 and 2.0 mg/kg (p < 0.01), respectively, which indicated that HCEO was able to restrain the development of secondary oxidation products.

Conjugated diene and conjugated triene could be generated during vegetable oil oxidation, and they can be evaluated by the OD determined at 232 nm (K232) and 268 nm (K268), respectively [34]. As exhibited in Figure 3, during the storage at 65 °C, the values for K232 and K268 of the control group sharply rose in the control sample. HCEO at 1600 mg/kg could obviously inhibit the increase in K232 and K268 to 22.5 and 3.3 on the 30th day (p < 0.01). The results in TBARS, K232 and K268 described that HCEO at 1600 mg/kg was able to restrict the generation of MDA, conjugated diene and conjugated triene in sunflower oil during the accelerated storage. In consequence, HCEO could effectively retard the lipid degradation and ameliorate the oxidative stability of sunflower oil samples, which was in agreement with our previous study results [15].

Figure 2. The influences of HCEO on AV (A), PV (B), ρ-AnV (C) and TOTOX (D) of sunflower oil samples during the accelerated storage. Values are expressed as means ± SD (n = 10). (Control) sunflower oil added with nothing, (TBHQ) sunflower oil added with TBHQ at 200 mg/kg, (200 mg/kg) sunflower oil added with HCEO at 200 mg/kg, (400 mg/kg) sunflower oil added with HCEO at 400 mg/kg, (800 mg/kg) sunflower oil added with HCEO at 800 mg/kg, (1600 mg/kg) sunflower oil added with HCEO at 1600 mg/kg. AV, acid value, PV, peroxide value, ρ-AnV, ρ-anisidine value, TOTOX, Total oxidation value.
FIGURE 3. The influences of HCEO on TBRAS (A), K₂₃₂ (B) and K₂₆₈ (C) of sunflower oil samples during the accelerated storage. Values are expressed as means ± SD (n = 10). (Control) sunflower oil added with nothing. (TBHQ) sunflower oil added with TBHQ at 200 mg/kg, (200 mg/kg) sunflower oil added with HCEO at 200 mg/kg, (400 mg/kg) sunflower oil added with HCEO at 400 mg/kg, (800 mg/kg) sunflower oil added with HCEO at 800 mg/kg, (1600 mg/kg) sunflower oil added with HCEO at 1600 mg/kg. TBRAS, Thiobarbituric acid reactive substance, K₂₃₂, conjugated dienes, K₂₆₈, conjugated trienes.

3.5. Effects of HCEO on Fatty Acid Composition

During the accelerated storage process, the fatty acid composition would be affected by the oxidation reaction in sunflower oil [24]. As displayed in Table 3, the fatty acid composition of the sunflower oil sample was confirmed by a GC-FID. From day 0 to day 30, for the sunflower oil sample of the control group, the amount of linoleic acid was evidently decreased by 10% (p < 0.01). However, the saturated fatty acid percentage, including palmitic, stearic and behenic acids, was markedly increased (p < 0.05 or p < 0.01). Quite interestingly, the result in oleic acid content, which elevated approximately 5% after accelerated storage, was different from our previous study, and the variances may be caused by the origin of sunflower seeds [7]. After the addition of TBHQ, compared with the control group, the transformations of percentages for all fatty acids were memorably restricted (p < 0.01). In the meantime, after the flavoring process of HCEO at 1600 mg/kg, the changes of percentages for saturated fatty acids and unsaturated fatty acids were prominently inhibited as well (p < 0.01). As a result, the addition of HCEO at 1600 mg/kg was demonstrated to improve the transformations for the fatty acid composition of sunflower oil during the accelerated lipid oxidation period.
Table 3. The influence of HCEO on the fatty acid composition of the sunflower oil samples at 65 °C for 30 days a.

| Days | 200 mg/kg | 400 mg/kg | 800 mg/kg | 1600 mg/kg |
|------|-----------|-----------|-----------|------------|
|      | C14:0 b   | C16:0     | C18:1     | C18:2      | C20:0      | C18:3     | C22:0     |
| 0    | 0.07 ± 0.01 | 6.42 ± 0.02 | 0.10 ± 0.02 | 3.74 ± 0.01 | 25.73 ± 0.01 | 62.61 ± 0.12 | 0.25 ± 0.01 | 0.30 ± 0.02 | 0.78 ± 0.02 |
| 0    | 0.07 ± 0.01 | 6.42 ± 0.02 | 0.10 ± 0.02 | 3.74 ± 0.01 | 25.73 ± 0.01 | 62.61 ± 0.12 | 0.25 ± 0.01 | 0.30 ± 0.02 | 0.78 ± 0.02 |
| 5    | 0.07 ± 0.01 | 6.42 ± 0.02 | 0.10 ± 0.02 | 3.74 ± 0.01 | 25.73 ± 0.01 | 62.61 ± 0.12 | 0.25 ± 0.01 | 0.30 ± 0.02 | 0.78 ± 0.02 |
| 10   | 0.07 ± 0.01 | 6.42 ± 0.02 | 0.10 ± 0.02 | 3.74 ± 0.01 | 25.73 ± 0.01 | 62.61 ± 0.12 | 0.25 ± 0.01 | 0.30 ± 0.02 | 0.78 ± 0.02 |
| 15   | 0.07 ± 0.01 | 6.42 ± 0.02 | 0.10 ± 0.02 | 3.74 ± 0.01 | 25.73 ± 0.01 | 62.61 ± 0.12 | 0.25 ± 0.01 | 0.30 ± 0.02 | 0.78 ± 0.02 |
| 20   | 0.07 ± 0.01 | 6.42 ± 0.02 | 0.10 ± 0.02 | 3.74 ± 0.01 | 25.73 ± 0.01 | 62.61 ± 0.12 | 0.25 ± 0.01 | 0.30 ± 0.02 | 0.78 ± 0.02 |
| 25   | 0.07 ± 0.01 | 6.42 ± 0.02 | 0.10 ± 0.02 | 3.74 ± 0.01 | 25.73 ± 0.01 | 62.61 ± 0.12 | 0.25 ± 0.01 | 0.30 ± 0.02 | 0.78 ± 0.02 |
| 30   | 0.07 ± 0.01 | 6.42 ± 0.02 | 0.10 ± 0.02 | 3.74 ± 0.01 | 25.73 ± 0.01 | 62.61 ± 0.12 | 0.25 ± 0.01 | 0.30 ± 0.02 | 0.78 ± 0.02 |

a Values are expressed as means ± SD (n = 10); b C14:0, myristic acid; C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, stearic acid; C18:1n9, oleic acid; C18:2n6, linoleic acid; C20:0, arachidic acid; C18:3n3, α-linolenic acid; C22:0, behenic acid; c As compared to the same group on day 0: p < 0.05; d As compared to the same group on day 0: p < 0.01; e As compared to the control group on the same day: p < 0.05; f As compared to the control group on the same day: p < 0.01.
In accordance with the antioxidant activity, the flavoring process of HCEO at 1600 mg/kg was confirmed to not only restrain the increases of values for AV, PV, p-AnV, TOTOX, TBARS, K$_{232}$ and K$_{268}$ of sunflower oil but also inhibit the fatty acid composition modifications during high-temperature storage. As a Chinese traditional herb, the essential oil of Huai C. morifolium was used as a flavor oil to improve the oxidative stability of sunflower oil. Taking the results of the present study into consideration, the essential oil extracted from Huai C. morifolium flowers was demonstrated to be effective in the accelerated storage of sunflower oil and the production of sunflower oil flavored with HCEO was regarded as an available way to enhance its oxidative stability.

3.6. Sensory Analysis of Sunflower Oil Flavored by HCEO

Over the years, herbs and spices have been used in the culinary tradition as preservatives for flavor and aroma, and essential oil is one of the main components among its extracts [17]. Therefore, the sensory analysis was spontaneously evaluated because of the hypothesis that the sensory properties of the sunflower oil sample would be ameliorated by HCEO during the storage for 30 days at 65 °C. Table 4 shows the values of flavor, taste, appearance and the overall acceptability of the control group and of the other samples (Table 1). It can be noted that the parameters of the control group were gradually decreased during the entire period, while, after HCEO addiction, the values for flavor, taste, appearance and overall acceptability of sunflower oil samples were increased in different degrees. Moreover, after the addition of HCEO at 1600 mg/kg, the values for flavor, taste, appearance and overall acceptability of sunflower oil samples were obviously increased (p < 0.01) to 7.3, 7.0, 7.1 and 7.4, at the end day of the storage, respectively. The results herein favorably verified the hypothesis above, and the sensory evaluation manifested that the sunflower oil flavored by essential oil of Huai C. morifolium was confirmed to possess better sensory attributes. As a consequence, HCEO could be used as flavored oils in sunflower oil and improve its sensory properties and oxidative stability, which was in agreement with previous reports [6,7].

Table 4. The influences of HCEO on flavor, taste, appearance and overall acceptability of sunflower oil.

| Items          | Days | Control | TBHQ 200 mg/kg | 400 mg/kg | 800 mg/kg | 1600 mg/kg |
|----------------|------|---------|----------------|-----------|-----------|------------|
| Flavor         | 0    | 8.44 ± 0.76 | 8.44 ± 0.76 | 8.44 ± 0.76 | 8.44 ± 0.76 | 8.44 ± 0.76 |
|                | 6    | 7.65 ± 0.83 | 7.89 ± 0.59 | 7.52 ± 0.62 | 7.58 ± 0.62 | 7.49 ± 0.71 | 8.12 ± 0.62 |
|                | 12   | 6.56 ± 0.71 | 6.61 ± 0.58 | 6.65 ± 0.60 | 6.52 ± 0.58 | 6.56 ± 0.72 | 7.94 ± 0.66 |
|                | 18   | 6.09 ± 0.77 | 6.12 ± 0.61 | 6.18 ± 0.58 | 6.10 ± 0.57 | 6.02 ± 0.65 | 7.70 ± 0.71 |
|                | 24   | 5.61 ± 0.64 | 5.66 ± 0.64 | 5.72 ± 0.48 | 5.56 ± 0.68 | 5.68 ± 0.60 | 7.49 ± 0.66 |
|                | 30   | 5.02 ± 0.56 | 5.09 ± 0.51 | 5.11 ± 0.52 | 4.99 ± 0.51 | 5.05 ± 0.55 | 7.33 ± 0.59 |
| Taste          | 0    | 8.26 ± 0.69 | 8.26 ± 0.69 | 8.26 ± 0.69 | 8.26 ± 0.69 | 8.26 ± 0.69 |
|                | 6    | 7.55 ± 0.66 | 7.41 ± 0.61 | 7.62 ± 0.69 | 7.59 ± 0.51 | 7.46 ± 0.59 | 7.98 ± 0.95 |
|                | 12   | 6.61 ± 0.54 | 6.66 ± 0.52 | 6.69 ± 0.47 | 6.74 ± 0.56 | 6.60 ± 0.62 | 7.71 ± 0.82 |
|                | 18   | 5.90 ± 0.56 | 5.82 ± 0.58 | 5.85 ± 0.50 | 5.95 ± 0.62 | 5.84 ± 0.67 | 7.44 ± 0.74 |
|                | 24   | 5.22 ± 0.48 | 5.31 ± 0.48 | 5.35 ± 0.50 | 5.39 ± 0.59 | 5.14 ± 0.49 | 7.26 ± 0.71 |
|                | 30   | 4.81 ± 0.52 | 4.72 ± 0.52 | 4.85 ± 0.58 | 4.75 ± 0.47 | 4.90 ± 0.49 | 7.03 ± 0.66 |
| Appearance     | 0    | 8.08 ± 0.72 | 8.08 ± 0.72 | 8.08 ± 0.72 | 8.08 ± 0.72 | 8.08 ± 0.72 |
|                | 6    | 7.56 ± 0.77 | 7.62 ± 0.65 | 7.55 ± 0.68 | 7.48 ± 0.74 | 7.59 ± 0.70 | 7.88 ± 0.59 |
|                | 12   | 7.18 ± 0.81 | 7.15 ± 0.68 | 7.25 ± 0.60 | 7.04 ± 0.74 | 7.19 ± 0.72 | 7.78 ± 0.75 |
|                | 18   | 6.71 ± 0.62 | 6.61 ± 0.66 | 6.52 ± 0.52 | 6.57 ± 0.71 | 6.78 ± 0.59 | 7.49 ± 0.62 |
|                | 24   | 6.20 ± 0.65 | 6.31 ± 0.49 | 6.18 ± 0.54 | 6.14 ± 0.48 | 6.25 ± 0.58 | 7.27 ± 0.59 |
|                | 30   | 5.81 ± 0.52 | 5.77 ± 0.51 | 5.70 ± 0.62 | 5.82 ± 0.59 | 5.89 ± 0.47 | 7.09 ± 0.69 |
| Overall acceptability | 0    | 8.65 ± 0.82 | 8.65 ± 0.82 | 8.65 ± 0.82 | 8.65 ± 0.82 | 8.65 ± 0.82 |
|                | 6    | 7.85 ± 0.71 | 7.77 ± 0.61 | 7.95 ± 0.55 | 7.69 ± 0.41 | 7.58 ± 0.55 | 8.36 ± 0.59 |
|                | 12   | 7.16 ± 0.74 | 7.01 ± 0.77 | 7.25 ± 0.47 | 7.18 ± 0.55 | 7.29 ± 0.62 | 8.08 ± 0.87 |
|                | 18   | 6.52 ± 0.66 | 6.44 ± 0.52 | 6.65 ± 0.61 | 6.59 ± 0.49 | 6.41 ± 0.77 | 7.78 ± 0.78 |
|                | 24   | 6.00 ± 0.54 | 6.11 ± 0.49 | 6.05 ± 0.52 | 5.89 ± 0.74 | 5.78 ± 0.58 | 7.52 ± 0.65 |
|                | 30   | 5.44 ± 0.52 | 5.61 ± 0.55 | 5.58 ± 0.57 | 5.31 ± 0.62 | 5.26 ± 0.49 | 7.39 ± 0.77 |

* Values are expressed as means ± SD (n = 100); b As compared to control group on the same day: p < 0.05; c As compared to control group on the same day: p < 0.01.
4. Conclusions

In this paper, value-added sunflower oil was produced using an essential oil extracted from flowers of Huai C. morifolium. During the accelerated storage (65 °C, 30 days), the addition of HCEO to sunflower oil, at the highest concentration (1600 mg/kg), restrains the increasing of some important indicators of lipid alteration (AV, PV, p-AnV, TOTOX, TBARS, K232 and K260), as well as inhibits the transformation of fatty acids. Additionally, it has been demonstrated that the sunflower oil flavored by HCEO at 1600 mg/kg is able to improve the sensory attributes, including flavor, taste, appearance and overall acceptability. As a result, it can be affirmed that HCEO is a valid additive to produce flavored vegetable oils with antioxidant effects as an alternative to synthetic preservatives. As a further study, it will be necessary to isolate and characterize the bioactive compounds to explore the action mechanism of the Huai C. morifolium essential oil.

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