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Sunflower Oil Flavored by Spearmint through Conventional and Ultrasound-Assisted Maceration: Differences in Oxidative Stability, Microbial Contamination and Sensory Properties

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Abstract: The preparation of flavored vegetable oils using spice and herb plants is considered to be an indispensable opportunity for the application of these plants. In the present exploration, sunflower oil (SFO) samples flavored by *Mentha spicata* L. (spearmint) were prepared using the maceration method (MM) and ultrasound-assisted maceration (UM). The antioxidant effects, sensory evaluation and the oxidative stability during accelerated storage; the physicochemical properties including the levels for acid value (AV), peroxide value (PV) and *p*-anisidine value (AnV); and the specific extinction values at 232 nm (K_{232}) and 268 nm (K_{268}) of the SFO samples were measured. The contents of beneficial ingredients including chlorophylls, carotenoids, polyphenols and tocopherols, and the micro-organism colonies for yeasts, molds, *Listeria monocytogenes* and Enterobacteriaceae bacteria were determined. The results show that the physicochemical properties, beneficial ingredients, antioxidant effects, sensory attributes and the oxidative stability in accelerated storage were greater in the flavored SFO sample than the control. Therefore, the SFO flavored by spearmint can be developed as flavored vegetable oils, and the ultrasound-assisted maceration can be widely employed in the preparation of flavored vegetable oils in the future.

Keywords: flavored sunflower oil; ultrasound-assisted maceration; oxidative stability; microbial survival; sensory attributes

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1. Introduction

As the fourth largest source of vegetable oils after soybean oil, rapeseed oil and cottonseed oil in China, sunflower oil (SFO) is becoming increasingly popular in urban and rural populations because of its delicious mouth-taste, attractive primrose yellow color and abundant nutritional ingredients [1]. According to the industrial information collected and released by the National Grain and Oil Information Center of China (NGOICC), the annual consumption amount for SFO in China was approximately 5.0 million tons in 2020 (domestic amount: about 3.0 million tons; overseas import amount: about 2.0 million tons). At the 2021 Summit Forum on Processing and Nutrition for Sunflower Oil (SFPNSO) in Xiamen, China, the Chief Expert of the China Cereals and Oils Association (CCOA), Prof. Ruiyuan Wang, recommended SFO for diabetes mellitus and heart disease patients. As reported by him, the high content of unsaturated fatty acids (UFAs) of SFO, especially linoleic acid, could sweep the low-density lipoprotein–cholesterol (LDL-C) away from the arteria coronaria of patients [2]. In terms of its application, SFO has long been employed in the preparation of Chinese dishes due to the balanced amounts of saturated fatty acids (SFAs, about 5.0%), monounsaturated fatty acids (MUFAs, about 20.0%) and polyunsaturated fatty acids (PUFAs, about 68.0–72.0%) [3]. For example, during the traditional deep-frying procedures of wheat flour and poultry meat products, SFO not only attached the attractive primrose yellow color to these fried products but also supplied them with satisfying nutritional ingredients and gratifying sensorial attributes [4]. Furthermore, except for the routine applications in China mentioned above, by means of the flavored vegetable

oils, SFO has been employed in the preparation of flavored fried products in the past few years [5,6]. In our previous explorations, the application of the flavored SFO prepared by the direct addition of essential oils from spice and herb plants in the deep-frying procedures of flavored fried products including *Maye*, *Matuan*, *Caijiao* and *Youmotou* was demonstrated to be an innovative approach to guarantee the high physicochemical quality of SFO and the high overall acceptability of the fried products [7–10]. Therefore, the preparation of flavored SFO using spice and herb plants is considered a production trend for the SFO industry.

As reported, there are three traditional methods for the preparation of flavored vegetable oils using spice and herb plants [11]. (1) Maceration/Infusion Method (MM): The dried spice and herb plants are uniformly smashed, naturally macerated in vegetable oils and successively homogenized at room temperature for about 7 days or 14 days, until no changes occur in the external surface of vegetable oils. After one filtration procedure to remove the residual turbidity and flavoring materials, the ready-to-use flavored vegetable oils are acquired [12]. (2) Co-pressing/Malaxation Method (CM): The smashed spice and herb plants are added to the vegetable oilseeds during the cold-pressing or malaxation processes of vegetable oils, and the processes are continued for 4 h or 8 h until vegetable oils no longer flow out. After the residual turbidity and flavoring materials are removed by high-pressure and high-speed centrifugation, the flavored vegetable oils from the cold-pressing machine or malaxation mash are obtained [13]. (3) Extraction/Enrichment Method (EM): The flavoring materials of spice and herb plants are extracted by liquid–solid extraction or liquid–liquid extraction using organic solvents and then added directly to the vegetable oils [14]. After successively homogenizing at room temperature for about 3 days or 6 days, until no changes occur in the external surface of vegetable oils, the flavored vegetable oils are obtained. Comparing these methods, in the CM method, it is difficult to transform the flavoring materials from spice and herb plants into vegetable oils because of the non-homogenous distribution of their leaves and stems in the co-pressing/malaxation processes [15]. The EM method often raises some problems such as the potential dangers of organic solvents, the disposal of flavoring materials in high-temperature extraction and organic solvent evaporation [16]. Therefore, the maceration method is the most frequently used, although it is a lengthy process. Above all, there is novel equipment that can be employed to shorten the time required, such as microwave and ultrasound [12].

Spearmint (*Mentha spicata* L.), belonging to the Lamiaceae family, is mainly distributed in Africa and some temperate European and Asian countries. As a famous medicinal, spice and herb plant, spearmint has been applied in clinics for the treatment of headache, influenza, stomachache, hemorrhoids and respiratory diseases for quite a long time [17]. In the Henan province of China, spearmint is employed in the preparation of some delicious soups, noodles and cuisines [18]. For example, the natural ethanol extract from spearmint was demonstrated to play an important role in the biogenic amine formation of sardine fillets [19]. The essential oil extracted from spearmint was also demonstrated to improve the chemical, microbial and sensory attributes of minced camel meat during its refrigerated storage [20]. Interestingly, the plant of spearmint was applied in the preparation of flavored SFO in our previous investigation by the EM method [7]. Until now, there have been few articles published about the preparation of flavored SFO using the conventional MM.

Consequently, in the present study, the SFO samples flavored by spearmint were prepared by the conventional MM and innovative MM using ultrasound. Furthermore, in order to ensure the high edible safety and high popularity of these SFO samples, their oxidative stability, microbial survival and sensory attributes were scientifically investigated in detail.

2. Materials and Methods

2.1. Materials and Chemicals

Sunflower seeds (100.0 kg) imported by China-Europe Railway Express from Bulgaria in 2021 were bought from Dongsheng Co. Ltd., Shangqiu, China, and the SFO samples em-

ployed in the experiments were manufactured by the cold-pressing method. Whole plants of *M. spicata* L. (spearmint, 100.0 kg) cultivated in Guangwu Vegetable Base of Xingyang, China, were harvested by Mr. Hongxin Li and purchased from Kexuedadao Shop of Dennis Supermarket, Zhengzhou, China. The specimens of the plant were identified by Prof. Dongying Wang and deposited in the Laboratory for Special Oilseed Manufacture Technology, Henan University of Science and Technology, Zhengzhou, China. All the chemical reagents of HPLC or analytical grade were provided by Senbo Co. Ltd., Zhengzhou, China.

2.2. Preparation of Flavored SFO Samples

According to the method of Assami et al., the SFO samples flavored by spearmint with the ratio 5% (*w*/*w*) were prepared by MM, including conventional MM and innovative MM [21]. For the conventional MM method, 1.0 kg of the whole plants of spearmint (roots removed) was washed with 1.0 L of NaOCl solution (0.1%) for 10 min and 1.0 L of Milli-Q water solution for 30 min, sheared into spearmint slices about 1.0 cm in length by a table knife (FT-RL11, Fangtai, Ningbo, China) and subjected to sodium chloride solution (10.0 mL, 1.0%) treatment for 30 min. Afterwards, the spearmint slices were cleaned with filter papers and placed in a draught cupboard for 24 h. Finally, the spearmint slices were completely immersed in 19.0 kg of refined SFO, and the obtained mixture was adequately shaken until homogenized by a table concentrator (StabS2, RadoBio, Shanghai, China) at approximately 25 °C for 7 successive days to acquire the flavored SFO sample MM-07. After maceration, the shaken mixture was completely filtered to eliminate the solid residues. By the same method, the flavored SFO was homogenized for successive 14 days, acquiring the MM-14 sample. For the innovative MM, a similar sono-extraction reactor (JH2000W20, Jinghao, Hangzhou, China) as that reported by Veillet et al. was produced and employed [16]. The operational conditions of flavored SFO using the sono-extraction reactor were as follows: 1.0 kg of spearmint slices was blended with 19.0 kg of refined SFO to obtain the flavored SFO sample. During the maceration procedure at approximately 25 °C for 10 min, the ultrasound intensity of the reactor was about 1.0 W/cm^2, with a frequency of 25 kHz (UM; the optimization procedure of the operational conditions for the ultrasound-assisted maceration will be published elsewhere). Meanwhile, 20.0 kg of the SFO used in the preparation of flavored SFO samples was applied at 25 °C as a control SFO sample (Control). The prepared SFO samples were prepared in triplicate and deposited in 25.0 L brown bottles. Immediately, all the obtained flavored SFO samples were preserved in a laboratory refrigerator (4 °C) for the subsequent experiments.

2.3. Quality Parameter Determination

The acid value (AV), peroxide value (PV) and *p*-anisidine value (AnV) of the investigated SFO samples were determined according to the Chinese National Standard GB/T5009.229 in 2016, GB/T5009.227 in 2016 and GB/T24304-2009/ISO6885 in 2006, respectively. Moreover, in accordance with the Chinese National Standard GB/T22500 in 2008, the absorbance values of the SFO samples at 232 nm (K_{232}) and 268 nm (K_{268}) were measured.

The levels for the chlorophyll and carotenoid content of the investigated SFO samples were determined according to the method of Ammar et al. [22]. Briefly, 7.5 mL of SFO sample and 17.5 mL of cyclo-hexane were poured into a Falcon tube. After being vortexed for 5 min by a vortex mixer, the absorbance values of the blended mixture were measured at 670 and 470 nm using a UV spectrophotometer (UT-6, Yipu, Shanghai, China) for the determination of the chlorophyll level and carotenoid level, respectively. The chlorophyll level was calculated by the following equation: Chlorophyll (mg/kg) = ($Abs_{670} \times 10^6$)/(613 × 100 × density). The carotenoid level was calculated by the following equation: Carotenoid (mg/kg) = ($Abs_{470} \times 10^6$)/(2000 × 100 × density).

The contents of the total polyphenols were determined using the Folin–Ciocalteu reagent according to the method of Jabri-Karoui and Marzouk [23]. Briefly, 10.0 g of SFO samples and 10.0 g of aqueous methanol solution ($MeOH/H_2O$, 80/20, *v*/*v*) were poured

into a centrifuge tube and vortexed for 5 min by a vortex mixer. After centrifuging at 6000 rpm for 10 min, the methanol phase of the solvent was recovered. Soon afterwards, Folin–Ciocalteu reagent was added, and the content for the total polyphenols of the SFO samples was determined by measuring the absorbance at 765 nm using the UV spectrophotometer and expressed as gallic acid equivalents (mg of GAE/kg of SFO).

The contents of the tocopherols of the investigated SFO samples were determined according to the method of Redondo-Cuevas et al. [24]. Firstly, tocopherols were extracted as follows: 20.0 mg of SFO, 280.0 μL of water and 400.0 μL of ethanol were poured into a tube (10 mL) and intensively mixed by a shaking process for 30 s. After the addition of 100.0 μL of echinone and 700.0 μL of hexane (containing BHT), the tube containing the mixture was shaken for 10 min and centrifuged for 5 min. Soon afterwards, 600.0 μL of the supernatant hexane phase of the mixture was removed, dried down in the speed vacuum for 10 min, dissolved in 200.0 μL of DEA (60% of acetonitrile, 20% of 1,4 dioxane and 20% of ethanol, v/v), and shaken for 10 min to ensure it blended well. Secondly, HPLC analysis of tocopherols was carried out by a Waters 717 plus Autosampler Module (Waters, Milford, MA, USA) attached with a C18 ODS silica gel analytical column (250 m × 4.6 mm, i.d., 5.0 μm, p.s., Beckman, Ultrasphere). In the HPLC analysis, echinone was employed as the internal standard, while the injection volume was 150 μL and the flow rate was 1.0 mL/min. Together with the total tocopherol content, the α-, γ- and δ-tocopherol contents were determined with the blended tocopherol standard containing these tocopherols at appropriate concentrations, and the results were all expressed in μg/g.

2.4. Antioxidant Effect

The antioxidant effect of the investigated SFO samples was explored by three methods: DPPH radical scavenging assay, β-carotene bleaching assay and reducing power assay [25].

2.5. Microbial Survival

The enumeration of viable osmophilic yeasts and xerophilic molds in the investigated SFO samples was carried out using Ciafardini et al.'s method, and the total number of yeasts and molds was expressed as CFU/mL [26,27]. The measurement of *Listeria monocytogenes* of the investigated SFO samples was performed [28]. Briefly, 25.0 mL of SFO sample was transferred to 225.0 mL of sterile Half Fraser broth to acquire the 10-fold dilution of SFO sample, and then, 0.1 mL of Half Fraser broth cultured at 30 °C for 24 h was aseptically inoculated into 10 mL of Fraser broth. Soon afterwards, the culture was incubated at 37 °C for 48 h. A loopful cultured Fraser broth was streaked onto Oxford and ALOA agar and incubated at 37 °C for 24 h. Moreover, the measurement of the viable total number of bacteria by inoculating the SFO sample on a culture media incubated at 30 °C was carried out, and the total number of isolated bacteria was expressed as CFU/mL.

By means of inoculating the SFO samples on Violet Red Bile Glucose agar, the measurement of the viable bacteria of the Enterobacteriaceae family was performed [28]. Briefly, after incubating at 37 °C for 24 h, typical Enterobacteriaceae bacteria-grown colonies in red/pink were counted. In the plates with enterobacterial growth, 5 typical colonies were isolated on CASO agar (37 °C for 24 h). Furthermore, the biochemical confirmation was performed with the API 20 E kit (BioMérieux, Lyons, France) and oxidase assay kit (MERCK, Germany) according to their instructions. The total number of viable bacteria was expressed as CFU/mL.

2.6. Sensorial Evaluation

The evaluations of the sensorial properties of the investigated SFO samples were carried out in accordance with Meng et al.'s method, including taste, flavor, appearance and overall acceptability [29,30].

2.7. Accelerated Storage

As described in previous explorations [31], the accelerated storage of these SFO samples was carried out as follows. Firstly, the SFO samples (each 0.5 L), control, MM-07, MM-14 and UM were severally deposited in brown bottles and refrigerated at −4 °C. Secondly, for the accelerated storage, the SFO samples were put into a thermo-tank (F202, Shuli, Shanghai, China) at 65 °C, and the storage period was 30 days. As is known, the accelerated storage for 24 h of vegetable oils at 65 °C is equal to routine storage for 1 month at normal temperature [32]. In order to monitor the oxidative stability of SFO samples during the whole period, their physicochemical attributes for them were measured every 6 days. Among them, AV and PV of the investigated SFO samples were determined in accordance with the Chinese National Standard GB/T5009.229 in 2016 and GB/T5009.227 in 2016, respectively. The specific extinction values K_{232} and K_{268} were evaluated according to the Chinese National Standard GB/T22500 in 2008.

2.8. Statistical Analysis

Unless otherwise stated, the experimental data exhibited in tests are expressed in means, while the experimental data exhibited in tables and figures are expressed in means ± standard deviation (SD, n = 10). Subsequently, the analysis of variance (ANOVA) was used in statistical analysis by means of GraphPad Prism 8.0, San Diego, CA, USA.

3. Results and Discussion

3.1. Physicochemical Properties of Flavored SFO

Because of the variation in technical routes and operation procedures of the production methods, the SFO samples obtained by the different methods mentioned above should possess different physicochemical properties [33]. As is known, in vegetable oil degradation, the triglyceride oxidation can produce primary oxidation products and secondary oxidation products, while the triglyceride hydrolysis can produce free fatty acids (FFAs) that can be determined by AV value [34]. As the main primary oxidation products, the generation of lipid hydroperoxides can induce the elevation of PV value, and they can be decomposed into non-volatile and volatile secondary oxidation products, including carbonyl, aldehydes and ketones, which can be determined by AnV value [35]. Furthermore, the absorbance at 232 nm and 268 nm provides the determination for the levels of K_{232} and K_{268}, which displays the manufacture degree of the primary and secondary oxidation products [36]. In the present investigation, although the physicochemical properties including AV, PV, AnV, K_{232} and K_{268} of the flavored SFO samples MM-07, MM-14 and UM were slightly higher than those of the control, there were no statistical significances among them (Table 1). All three flavored SFO samples were in accordance with the Chinese National Standard GB 2761 in 2018. Although the differences in the production methods were objective and substantial, the flavored SFO samples obtained all had high physicochemical properties. In the comparison of production methods, the MM method of 7 days was long enough to macerate the spearmint plants when we consider the economic cost, and the UM method was supposed to be a powerful and efficient method for the fabrication of flavored SFO of spearmint. The results were in agreement with the previous explorations, where the UM method was employed in the manufacture of SFO flavored with basil, rosemary and pink pepper [37,38].

Table 1. The physicochemical properties of SFO samples [A].

Sample [B]	AV (mg/g)	PV (g/100 g)	AnV	K_{232}	K_{268}
Control	0.31 ± 0.02 [a]	0.12 ± 0.02 [a]	1.17 ± 0.16 [a]	2.46 ± 0.09 [a]	1.07 ± 0.05 [a]
MM-07	0.33 ± 0.04 [a]	0.14 ± 0.02 [a]	1.20 ± 0.11 [a]	2.58 ± 0.13 [a]	1.19 ± 0.06 [b]
MM-14	0.42 ± 0.04 [a]	0.14 ± 0.02 [a]	1.24 ± 0.19 [a]	2.84 ± 0.09 [b]	1.25 ± 0.07 [b,c]
UM	0.36 ± 0.03 [a]	0.15 ± 0.03 [a]	1.33 ± 0.13 [a]	2.97 ± 0.16 [b]	1.32 ± 0.09 [c]

[A] The values are expressed in means ± SD (n = 10). [B] Control: SFO sample produced by the cold-pressing method; MM-07: SFO sample produced by a maceration procedure for 7 days; MM-14: SFO sample produced by a maceration procedure for 14 days; UM: SFO sample produced by an ultrasound-assisted maceration for 10 min. a–c, Means within a column with different letters are significantly different ($p < 0.05$).

3.2. Chlorophylls, Carotenoids and Polyphenols of Flavored SFO

In recent years, various studies about flavored vegetable oils have explored the levels of chlorophylls and carotenoids because they play important roles in maintaining the oxidative stability of flavored vegetable oils and are considered to be mainly responsible for their external appearance [39]. Moreover, employed as radical scavenging and metal chelating agents, polyphenols are also important because they are effective in inhibiting human diseases and elevating oil shelf-life [40]. Therefore, chlorophylls, carotenoids and polyphenols are considered to be useful indicators of the nutrition, freshness and authenticity of flavored vegetable oils [41]. In the present investigation, the contents of chlorophylls, carotenoids and polyphenols of SFO samples MM-07, MM-14 and UM were all much higher than that of the control. As shown in Table 2, the chlorophylls, carotenoids and polyphenols of SFO sample UM were 9.81 mg/kg, 45.33 mg/kg and 56.16 mg/kg, respectively, which were close to those of SFO samples MM-07 and MM-14. The differences in pigment concentrations of these SFO samples can be explained by a series of biological factors of spearmint plants, such as genotypic potential, maturity period, harvest condition and agricultural practices (sunlight, irrigation and temperature), as well as a number of technical factors of the production method such as preparation time, apparatus model and the addition proportion of SFO and spearmint plants [25]. This increase in polyphenols can be attributed to the addition of spearmint plants which had higher polyphenol content. Certainly, the elevation of the chlorophylls, carotenoids and polyphenols observed in SFO samples could have positive impacts on their shelf-life and human health, as well [42].

Table 2. The contents of chlorophylls, carotenoids and polyphenols in SFO samples [A].

Samples [B]	Chlorophylls (mg/kg)	Carotenoids (mg/kg)	Polyphenols (mg/kg)
Control	0.08 ± 0.01 [a]	01.31 ± 0.05 [a]	10.14 ± 0.07 [a]
MM-07	8.77 ± 0.58 [b]	35.31 ± 0.66 [b]	47.73 ± 0.41 [b]
MM-14	9.49 ± 0.70 [b,c]	43.26 ± 0.81 [b,c]	52.65 ± 0.55 [b,c]
UM	9.81 ± 0.67 [c]	45.33 ± 0.73 [c]	56.16 ± 0.39 [c]

[A] The values are expressed in means ± SD (n = 10). [B] Control: SFO sample produced by the cold-pressing method; MM-07: SFO sample produced by a maceration procedure for 7 days; MM-14: SFO sample produced by a maceration procedure for 14 days; UM: SFO sample produced by an ultrasound-assisted maceration for 10 min. a–c, Means within a column with different letters are significantly different ($p < 0.05$).

3.3. Tocopherols of Flavored SFO

As is known, tocopherols in vegetable oils serve as potential antioxidants that can postpone their own oxidative alteration. The higher the levels of tocopherols, the greater stability of vegetable oils can be expected [43]. In the present investigation, both the maceration procedures using the traditional method and the innovative method could bring about a significant increase in tocopherol contents of SFO samples compared with the control. As shown in Table 3, the contents of α-tocopherol, γ-tocopherol, δ-tocopherol and total tocopherols for the SFO sample UM were 666.58 μg/mL, 38.33 μg/mL, 8.63 μg/mL and 712.94 μg/mL, respectively, indicating that the maceration using ultrasound could not only shorten the needed time, but also boost the levels of the tocopherols to a great extent.

The results displayed that tocopherols were present at higher concentrations in the flavored SFO samples than in the control sample, and the preservation of tocopherols was related to the addition of the natural antioxidants from the spearmint plants during the maceration procedures where they can be dissolved in SFO samples and employed as a stabilizer for them [33].

Table 3. The contents of tocopherols in SFO samples [A].

Samples [B]	α-Tocopherol (μg/mL)	γ-Tocopherol (μg/mL)	δ-Tocopherol (μg/mL)	Total (μg/mL)
Control	552.17 ± 48.73 [a]	22.36 ± 2.14 [a]	4.74 ± 0.08 [a]	578.62 ± 52.46 [a]
MM-07	589.36 ± 51.28 [b]	24.41 ± 2.03 [b]	5.09 ± 0.14 [b]	618.17 ± 57.13 [b]
MM-14	612.34 ± 56.19 [c]	25.65 ± 1.94 [c]	5.21 ± 0.16 [c]	642.87 ± 48.78 [c]
UM	666.58 ± 31.87 [d]	38.33 ± 2.32 [d]	8.63 ± 0.09 [d]	712.94 ± 36.95 [d]

[A] The values are expressed in means ± SD (n = 10). [B] Control: SFO sample produced by the cold-pressing method; MM-07: SFO sample produced by a maceration procedure for 7 days; MM-14: SFO sample produced by a maceration procedure for 14 days; UM: SFO sample produced by an ultrasound-assisted maceration for 10 min. a–d, Means within a column with different letters are significantly different ($p < 0.05$).

3.4. Antioxidant Effect of Flavored SFO

The antioxidant effect of the flavored SFO samples was evaluated by the commonly-used DPPH radical scavenging assay, β-carotene bleaching assay and reducing power assay. As exhibited in Table 4, compared with the control (51.78% for DPPH radical scavenging, 18.46% for β-carotene linoleate bleaching and 0.139 for OD value at 700 nm), the SFO samples MM-07 (73.12% for DPPH radical scavenging, 32.34% for β-carotene linoleate bleaching and 0.152 for OD value at 700 nm), MM-14 (91.45% for DPPH radical scavenging, 64.75% for β-carotene linoleate bleaching and 0.167 for OD value at 700 nm) and UM (93.36% for DPPH radical scavenging, 68.32 for β-carotene linoleate bleaching and 0.175 for OD value at 700 nm) revealed apparent antioxidant effects. These results should be attributed to the increased concentrations of natural antioxidants, including chlorophylls, carotenoids and polyphenols, because of the employment of spearmint plants, so that the increase in the scavenging effect against free radicals occurred. Notably, the positive impacts of flavored vegetable oils on human health and the correlations between flavored vegetable oils and plenty of diseases had previously been investigated [25].

Table 4. The antioxidant effect of SFO samples [A].

Samples [B]	DPPH Radical Scavenging (%)	β-Carotene Linoleate Bleaching (%)	Reducing Power (OD Value at 700 nm)
Control	51.78 ± 1.42 [a]	18.46 ± 0.39 [a]	0.139 ± 0.012 [a]
MM-07	73.12 ± 1.56 [b]	32.34 ± 0.44 [b]	0.152 ± 0.026 [b]
MM-14	91.45 ± 1.32 [c]	64.75 ± 0.57 [c]	0.167 ± 0.027 [c]
UM	93.36 ± 1.47 [c]	68.32 ± 0.41 [c]	0.175 ± 0.020 [d]

[A] The values are expressed in means ± SD (n = 10). [B] Control: SFO sample produced by the cold-pressing method; MM-07: SFO sample produced by a maceration procedure for 7 days; MM-14: SFO sample produced by a maceration procedure for 14 days; UM: SFO sample produced by an ultrasound-assisted maceration for 10 min. a–d, Means within a column with different letters are significantly different ($p < 0.05$).

3.5. Microbial Survival in Flavored SFO

Although the preparation of flavored vegetable oils using spice and herb plants can give health benefits to humans, they can also introduce some potential dangers by means of the presentation of micro-organisms [41]. As reported, the micro-organisms of spice and herb plants can be transferred to flavored vegetable oils during maceration. Such micro-organisms, mainly formed from yeasts and molds, are able to influence the chemical characteristics of the vegetable oils owing to hydrolytic enzymes [44]. In the present investigation, to ensure the edible safety of these investigated SFO samples, the microbiological examination of yeasts, molds, *L. monocytogenes* and the bacteria of the

Enterobacteriaceae family were carried out, and the results are exhibited in Table 5. As seen, the micro-organism colonies of yeasts, molds and the bacteria of the Enterobacteriaceae family for the SFO samples MM-07, MM-14 and UM were obviously reduced with the passage of time, while the micro-organism colonies of them for the control were negligible. For *L. monocytogenes*, there were almost no micro-organism colonies in all these SFO samples. The reason for the reduction in the micro-organism colonies of yeasts, molds and the bacteria of the Enterobacteriaceae family may be the potential antimicrobial ingredients in the vegetable oils. The cleanout of spearmint plants using NaOCl and Milli-Q water in the pretreatment could throw *L. monocytogenes* micro-organism colonies away. The results are consistent with Odeh et al., who explored the existence of micro-organisms in flaxseed oils obtained by the infusion with the spice/herb plants of chili, basil, fennel, oregano and rosemary. The investigation herein also confirmed the hypothesis that the spice and herb plants in vegetable oils can influence the micro-organism survival by means of changing their living environments [28].

Table 5. Microorganism colonies in SFO samples during 90-day storage [A].

Samples [B]	Yeasts (CFU/mL)				Molds (CFU/mL)				*L. monocytogenes* (CFU/mL)				Enterobacteriaceae (CFU/mL)			
	0	30	60	90	0	30	60	90	0	30	60	90	0	30	60	90
Control	<1	<1	<1	<1	<1	<1	<1	<1	n.d.	n.d.	n.d.	n.d.	<1	<1	<1	<1
MM-07	12	<1	<1	<1	6	<1	<1	<1	n.d.	n.d.	n.d.	n.d.	6	<1	<1	<1
MM-14	14	<1	<1	n.d.	7	<1	<1	n.d.	n.d.	n.d.	n.d.	n.d.	9	<1	<1	<1
UM	15	n.d.	n.d.	n.d.	9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	9	<1	<1	n.d.

[A] The values are expressed in means. [B] Control: SFO sample produced by the cold-pressing method; MM-07: SFO sample produced by a maceration procedure for 7 days; MM-14: SFO sample produced by a maceration procedure for 14 days; UM: SFO sample produced by an ultrasound-assisted maceration for 10 min.

3.6. Sensory Evaluation of Flavored SFO

A traditional evaluation is the sensory evaluation of flavored vegetable oils; after all, the reason for expediting their applications is their special sensory properties [45]. In addition, if the sensory evaluation is not performed before its presentation in supermarkets, we cannot predict the consumption amount and decide the production amount [46]. In the present investigation, the scores for sensory attributes of all the flavored SFO samples, including taste, flavor and overall acceptability, were higher than the control, while the score for sensory attribute appearance was much lower than that of the control, as exhibited in Table 6. The cause of this phenomenon was the addition of spearmint plants which contained chlorophylls and carotenoids in the preparation of these flavored SFO samples. The scores for taste, flavor and overall acceptability of flavored SFO samples were much higher than the control, so we could expect the prospective future of them after the desorption of the green color, and the application in the form of vegetable oils may provide an interesting opportunity for spice and herb plants. In a word, the employment of spice and herb plants in powders always affects the mentality and even the appetite of the consumers [47].

Table 6. The sensory evaluation of SFO samples [A].

Samples [B]	Taste	Flavor	Appearance	Overall Acceptability
Control	6.56 ± 0.42 [a]	6.12 ± 0.36 [a]	8.33 ± 0.47 [a]	5.87 ± 0.31 [a]
MM-07	8.31 ± 0.36 [b]	8.57 ± 0.28 [b]	6.65 ± 0.52 [b]	7.69 ± 0.25 [b]
MM-14	8.69 ± 0.27 [b]	8.41 ± 0.41 [b]	6.45 ± 0.65 [b]	8.23 ± 0.51 [b]
UM	8.07 ± 0.32 [b]	8.30 ± 0.37 [b]	6.01 ± 0.34 [b]	7.92 ± 0.44 [b]

[A] The values are expressed in means ± SD (n = 60). [B] Control: SFO sample produced by the cold-pressing method; MM-07: SFO sample produced by a maceration procedure for 7 days; MM-14: SFO sample produced by a maceration procedure for 14 days; UM: SFO sample produced by an ultrasound-assisted maceration for 10 min. a and b, Means within a column with different letters are significantly different ($p < 0.05$).

3.7. Accelerated Storage of Flavored SFO

The application of accelerated storage for the quality control and shelf-life estimation of vegetable oils is frequently performed. Naturally, the exploration of the accelerated storage for vegetable oil products is necessary and essential to predict their expiration dates before they are consumed by people, although such experiments usually take a long time and much energy [48]. In the present investigation, as revealed in Figure 1, the levels for AV, PV, K_{232} and K_{268} of the SFO samples were increased during the whole period, while the levels of the SFO samples MM-07, MM-14 and UM were still lower than that of the control. In the comparison of the three flavored SFO samples, the oxidative stability of SFO sample UM was considered to be better than SFO samples MM-07 and MM-14; after all, the levels for AV, PV, K_{232} and K_{268} of SFO sample UM were all lower than those of SFO samples MM-07 and MM-14. The results of the accelerated storage were in accordance with the measured results mentioned above. The preparation of flavored SFO using ultrasound-assisted maceration could result in higher levels of chlorophylls, carotenoids, tocopherols and polyphenols employed as internal antioxidants. According to the Chinese National Standard GB 2761 in 2018, where the legal levels for AV and PV are set as 4.0 mg/g and 0.25 g/100 g, respectively, the predicted shelf-life of the three flavored SFO samples is longer than 30 months, indicating that the employment of spearmint plants in flavored SFO preparation has significant potential and prospects [49].

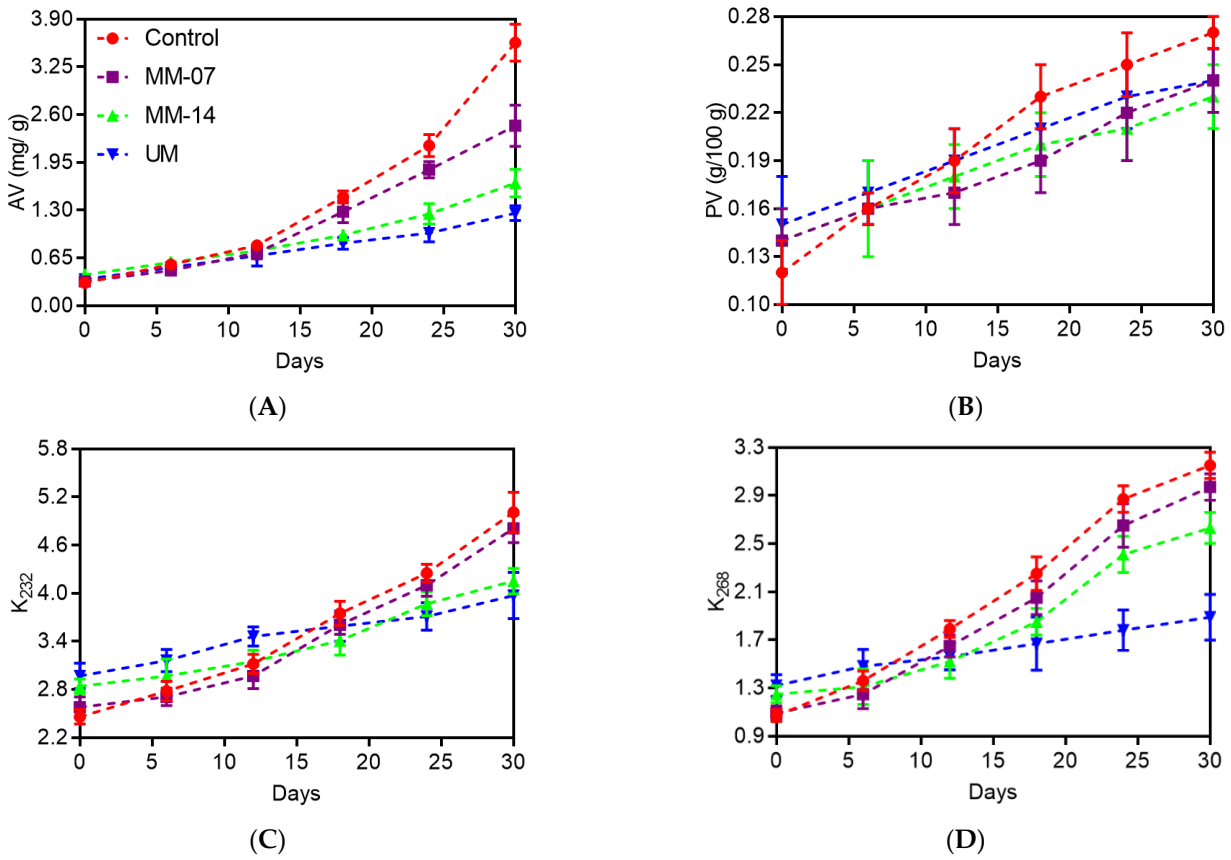

Figure 1. The variation in the levels of AV (**A**), PV (**B**), K_{232} (**C**) and K_{268} (**D**) in the accelerated storage at 65 °C for 30 days. Control: SFO sample produced by the cold-pressing method; MM-07: SFO sample produced by a maceration procedure for 7 days; MM-14: SFO sample produced by a maceration procedure for 14 days; UM: SFO sample produced by an ultrasound-assisted maceration for 10 min.

4. Conclusions

Flavored SFO preparation using spearmint plants was demonstrated to be achievable and effective. Between the traditional maceration and innovative maceration, ultrasound-assisted maceration is an important and effective method in preparing flavored SFO. The physicochemical properties, microbial survivals, sensorial attributes and shelf-life of the flavored SFO prepared using spearmint plants with ultrasound-assisted maceration were all improved over the control. Therefore, the preparation of flavored SFO products is feasible, and the ultrasound-assisted maceration is promising for the future.

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