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Eugenol, Isolated from the Essential Oil from *Lonicera japonica* Flower Buds, Could Increase the Oxidative Stability of Sunflower Oil in the Deep-Frying Procedure of *Youtiao*

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Abstract: In order to assess the sunflower oil (SFO) oxidative stability that was added by the essential oils extracted from *Lonicera japonica* flower buds (LJEO) during deep-frying at 180 °C for 30 h, we clarified the compound/compounds of LJEO that improved the oxidative stability of SFO. The results displayed that the addition of LJEO (0.06 g/kg) could significantly restrict the elevation or the reduction in the levels of total polar compounds (TPC), thiobarbituric acid (TBA), conjugated dienes and conjugated trienes, and the values for polymer, viscosity and the color of SFO during the whole period. Meanwhile, the reduction in the sensory attributes, including flavor, taste, crispness and overall acceptability of the fried product, *youtiao*, was obviously restricted as well. After the bioassay-guided fractionation of LJEO and repeated deep-frying at 180 °C for 30 h, one of its chemical constituents, eugenol, was demonstrated to be the very compound that did significantly inhibit the oxidative rancidity of the SFO. Therefore, eugenol may be employed as potential effective natural antioxidants to inhibit the oxidative rancidity of SFO during its deep-frying procedures.

Keywords: *Lonicera japonica*; eugenol; sunflower oil; oxidative stability; sensory attributes

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1. Introduction

In recent years, as one type of the secondary metabolite from green spice and medicinal plants with special tempting fragrance, essential oils have been attracting much attention all over the world [1]. As is known, essential oils are constituted by complex mixtures of low molecular weight natural compounds, including phenols, terpenes, ketones, alcohols, aldehydes, hydrocarbons, etc., [2]. Due to the existence of these natural compounds, essential oils have exhibited a variety of biological effects in animal and cellular models, such as anti-insect, anticancer, antioxidant, antimicrobial, and anti-inflammatory effects [3]. For example, the essential oil extracted from *Rosmarinus officinalis* could inhibit the inflammation symptoms of several animal models, including paw edema induced by carrageenin, ear edema induced by croton seed oil, and granuloma tissue induced by cotton pellet [4]. At the same time, the potential relationship between the administration of essential oils extracted from *Lavendula angustifolia* and *Melaleuca alternifolia* and the improvement of pediatric endocrine disorder of animal models has been repeatedly disclosed and reviewed by Hawkins et al. [5]. With regard to modern applications, essential oils are employed as aromatic agents in the physical therapy of white-collar workers, as nutrient supplements in the daily feeding of livestock animals, and as antioxidant agents in the transportation of poultry meats and vegetable oils [6]. For instance, by the direct addition method, the essential oils obtained from spice plants showed promising antioxidant effects in minimizing

the oxidative rancidity in diverse meat matrices, such as beef, pork, and chicken [7,8]. The addition of essential oils from medicinal plants into sunflower oil (SFO) not only improved their oxidative stability during the accelerated storage periods and deep-frying procedures, but also increased the sensorial attributes of themselves and their fried products, including *Maye*, *Caijiao,* and *Youmotou* [9–13].

Lonicera japonica Thunb. (Caprifoliaceae family), also known as *Ren Dong Hua* or *Jin Yin Hua*, is a famous spice and medicinal plant native to East Asian countries [14]. In China, *L. japonica* has been largely cultivated in the Xinmi County and the Fengqiu County of Henan province for more than 1000 years, and the flower buds of the plants have been employed in the traditional Chinese medicine (TCM) for the treatment of cough, fever, arthritis, sore throat, and flu infection [15]. *L. japonica* and more than 500 prescriptions of TCM incorporating *L. japonica* have been documented in the *Chinese Pharmacopoeia* (1977, 1985, 1990, 1995, 2000, 2005, 2015, and 2020 versions) with the name "金 银 花" since 1963. Recently, with the tendency of "Homology of Medicine and Food", the flower buds of *L. japonica* have been applied in everyday beverages, including herbal tea, scented tea, and carbonated drinks [16]. In European countries, *L. japonica* is cultivated and employed as a renowned ornamental groundcover for its evergreen leaves, beautiful flowers, and, above all, luscious fragrance [17]. In the modern investigations, a large number of secondary metabolites have been isolated from *L. japonica*, such as iridoids, flavonoids, triterpenoids, polysaccharides, organic acids, and essential oils [18]. Meanwhile, the natural compounds/crude extracts from *L. japonica* were demonstrated to display a variety of biological effects, including antiviral, antitumor, antioxidant, antiallergic, antidiabetic, antiplatelet, antibacterial, and anti-inflammatory effects [19]. For example, one polysaccharide isolated from *L. japonica* flower buds harvested from the Fengqiu County of Henan province (China) was demonstrated to reveal hypoglycemic effect and hypolipidemic effect in streptozotocin-induced animal models [20]. Moreover, the essential oils and other extracts of *L. japonica* could restrain the growth of food-borne and food spoilage bacteria and improve the cold-stored porcine patty quality by inhibiting the oxidation of lipid and myofibrillar protein [21,22].

SFO, as the fourth most-consumed vegetable oils behind soy bean oil (first), rape-seed oil (second) and cotton-seed oil (third) in China, is quite popular with urban white-collar workers because of its primrose yellow color, slight fragrance, and affluent nutrition [23]. As reported, the purchasing amount of SFO was increased to 1.64 million tons in 2019 from 0.98 million tons in 2015, with an increase rate of >10% [24]. SFO is always involved in the preparation of all kinds of Chinese soups and dishes and all sorts of fried products by frying procedures [13]. Nevertheless, SFO also suffers from the intractable problem of all vegetable oils, i.e., oxidative rancidity, due to the high content of unsaturated fatty acids (UFAs), above all, polyunsaturated fatty acids (PUFAs). In order to overcome the troublesome problem, plenty of synthetic antioxidants, including *tert*-butyl hydroquinone (TBHQ), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) have been added to SFO. Generally, the production companies always add TBHQ to SFO before its appearance in supermarkets [25]. Nevertheless, owing to the potential carcinogenic and teratogenetic effects of these synthetic antioxidants, the natural antioxidants from spice and medicinal plants including essential oils have been frequently investigated in the past few years. After all, the natural antioxidants are considered to be safe [26].

Until now, the applications for *L. japonica* essential oil (LJEO) as natural antioxidants in the deep-frying procedure of SFO have never been explored and reported. Consequently, in the present study, the antioxidant effect of LJEO was investigated in the deep-frying procedure using SFO, together with the sensorial evaluation of the fried products, *youtiao*, and the antioxidant effect of one of its natural compounds, eugenol, obtained by bioassay-guided fractionation.

2. Materials and Methods

2.1. Materials and Chemicals

The *L. japonica* flower buds (10.0 × 5 kg) harvested in March 2021 in Fengqiu County, China were bought from Yuzhou Chinese Medicine Market (Xuchang, China). The *L. japonica* flower buds were identified by Prof. Dongying Wang working at College of Food Science and Engineering, Henan University of Technology (Zhengzhou, China), and their voucher specimens were deposited in Laboratory for Production Technology of Special Oilseeds (Henan University of Science and Technology, Zhengzhou, China). SFO (50.0 × 6 kg), manufactured using sunflower seeds imported from Bulgaria by China-Europe Railway Express in 2020, was purchased from Dongsheng Limited Company (Shangqiu, China). Wheat flour (50.0 × 2 kg) manufactured using wheat grain cultivated in Xihua County (China) was purchased from Longxing Limited Company (Zhoukou, China). In the production of *youtiao*, 100.0 g of fresh wheat flour and 50.0 g of drinking water were manually combined to obtain one original fluid dough, and then the original fluid dough was produced with one full-automatic dough making machine (Model 350, Hangfan, Xingtai, China) to obtain one fresh *youtiao* (15.0 cm in length; 2.5 cm in width and 2.5 cm in height). Subsequently, to acquire the fried *youtiao* (22.5 cm in length; 3.5 cm in width and 3.5 cm in height), the deep-frying procedure at 180 °C using SFO of *youtiao* was commenced and continued for about 5 min. Furthermore, TBHQ, BHA, and BHT and the other solvents of analytical or HPLC grade were supplied by Senbo Limited Company (Zhengzhou, China).

2.2. Chemical Analysis of LJEO

The extraction procedure of LJEO was carried out according to our previous method [27]. For the chemical analysis of LJEO, a gas chromatography-mass spectrometry (GC-MS) system of Shimadzu GC-17A (Kyoto, Japan) attached by a fused silica capillary column ZB-1 MS (30.0 m × 0.25 mm i.d. × 0.25 mm f.t.; Phenomenex, Torrance, CA, USA). The oven temperature was programmed: increased to 150 °C from 50 °C at 3 °C/min and remained at 150 °C for 10 min, and then increased to 250 °C from 150 °C at 10 °C/min while the temperature of injector and detector were set at 220 °C and 280 °C, respectively. The flow rate of carrier gas, Helium (He), was set at 1.0 mL/min, and the split ratio was 1:50. The authentication of the individual compounds of LJEO was performed by one-by-one comparison of retention indices (RI) with that of the NIST Chemistry WebBook 2018 and the literature data [22]. The relative amount (RA) of each compound of LJEO was expressed as percentage for the area of the individual peak, relative to that of the total peaks.

2.3. Selection of Concentration for Antioxidants

In accordance with Table 1, LJEO, TBHQ, BHA, and BHT were, one after another, directly and artificially added into SFO at different concentrations (0.00 g/kg, 0.03 g/kg, 0.06 g/kg, 0.09 g/kg, 0.12 g/kg, and 0.15 g/kg), to prepare a sequence of (5.0 kg each) SFO samples, and all the SFO samples were maintained in the experimental refrigerator (4 °C) [11,12]. Soon afterwards, so as to acquire the optimal concentrations for LJEO and these synthetic antioxidants in the subsequent deep-frying procedures for *youtiao*, all the SFO samples were applied in one deep-frying procedure at 180 °C of shorter time (5.0 h) with a deep-frying kettle (Model ZG-BK-ZL-81, Chigo, Foshan, China). When the SFO sample temperature had just reached 180 °C, the shorter deep-frying procedure could be manually started. During the whole deep-frying period, the level of SFO sample acid value (AV) was determined after every hour, following the procedure of the Chinese National Standard 5009.229 in 2016. Meanwhile, the levels of iodine value (IV), *p*-anisidine value (AnV), and peroxide value (PV) of SFO samples were determined, following the procedure of the National Standards of China 5532 in 2008, 5009.227 in 2016, and 24,304 in 2009, respectively.

Table 1. The amounts of the antioxidants added to sunflower oil samples.

Antioxidant [a]	Addition Amount (Antioxidant/Sunflower Oil, g/kg)					
	0	1	2	3	4	5
LJEO	0	0.03	0.06	0.09	0.12	0.15
TBHQ	0	0.03	0.06	0.09	0.12	0.15
BHA	0	0.03	0.06	0.09	0.12	0.15
BHT	0	0.03	0.06	0.09	0.12	0.15

[a] LJEO: The essential oil from the flower buds of *L. japonica*; TBHQ: *tert*-butyl hydroquinone; BHA: butylated hydroxyanisole; BHT: butylated hydroxytoluene.

The results of these experiments showed that the levels for AV, PV, and AnV were comparative lower and the level for IV was comparative higher, when the LJEO, TBHQ, BHA, and BHT were added directly and artificially into SFO at 0.06 g/kg, 0.09 g/kg, 0.12 g/kg, and 0.12 g/kg, respectively. Therefore, in the subsequent deep-frying processes of *youtiao*, the concentrations for LJEO, TBHQ, BHA, and BHT were respectively chosen as 0.06 g/kg, 0.09 g/kg, 0.12 g/kg, and 0.12 g/kg.

2.4. Deep-Frying Procedure of Youtiao

In accordance with the laboratory findings mentioned above, LJEO, TBHQ, BHA, and BHT were directly and artificially added into SFO (each 10.0 kg) at 0.06 g/kg, 0.09 g/kg, 0.12 g/kg, and 0.12 g/kg to prepare SFO samples (named LJEO-0.06, TBHQ-0.09, BHA-0.12, and BHT-0.12, respectively), together with SFO samples (non-added sample, control). All these investigated SFO samples were also maintained in the experimental refrigerator (4 °C). Subsequently, all of them were severally applied to a deep-frying procedure (30 h) at 180 °C of the fried products, *youtiao*, which was started when the SFO sample temperature had just reached 180 °C. For its deep-frying, each fresh *youtiao* was fried in SFO for about 5 min so that about 360 *youtiao* were obtained for all these investigated SFO samples. In the whole deep-frying period, the levels for total polar compounds (TPC) and thiobarbituric acid (TBA) of the investigated SFO samples were assessed every 6 h on the base of the National Standards of China GB/T5009.202 in 2016 and GB5009.181 in 2016, respectively, while the levels for conjugated dienes and conjugated trienes of them were assessed on the base of the National Standard of China GB/T 22500 in 2008. Moreover, the values for polymer and viscosity of the investigated SFO samples were assessed using our previous methods, together with the values of L^* and b^* [13,14]. In the meantime, the sensorial evaluation of *youtiao* was carried out every 6 h.

2.5. Sensorial Evaluation of Fried Youtiao

For the sensorial evaluation, the fried *youtiao* was cut into 30 pieces, and they were numbered at random in three-digit numbers from 001–030 [28]. Before the sensorial evaluation, 1 coordinator, 3 assistants, 30 participants (15 male and 15 female) were engaged from the graduate students from College for Health Care of Chinese Medicine, Guangzhou Food and Drug Training School (Guangzhou, China). In order to accelerate the sensorial assessment, all of them were instructed by Prof. Dongying Wang, who is a leading expert in Sensory Evaluation Centre of National Engineering Research Center for Wheat & Corn Further Processing (Zhengzhou, China), and an evaluation sheet was designed, to make sure they were quite familiar with the specific details of the whole sensorial evaluation and the accurate meaning of the sensorial attributes, including flavor, taste, crispness, and overall acceptability of fried *youtiao*. When sensorial evaluation started, the 3 assistants send fried *youtiao* piece by piece to the 30 participants. After being smelled for 30 s and masticated for 30 s, the participants could give one score to each piece of fried *youtiao* about its flavor, taste, crispness, and overall acceptability using a 10-point hedonic scale, where 10 represented extremely acceptable and 1 represented extremely unacceptable.

The results exhibited that, together with the oxidative stability decrease in SFO, the sensorial properties reduction in fried *youtiao* was also significantly increased when SFO

was added LJEO at 0.06 g/kg so that the very compound/compounds in LJEO that did exhibit antioxidant effect in the whole deep-frying procedure should be illustrated in detail by bioassay-guided fractionation.

2.6. Bioassay-Guided Fractionation of LJEO

As exhibited in our previous studies [13,14,29], the very compound/compounds in LJEO that did reveal effective antioxidant effects in the deep-frying procedure of *youtiao* using SFO was/were isolated using preparative silica gel TLC plates via the process below. In the first step, using the *n*-hexane/ethyl acetate solution system (80:20, *v*/*v*), one piece of silica gel TLC plate (analytical) on the aluminum foil (8.0 μm in gel particle size; 2.0 mm in layer thickness; GF_{254}, Guangzhou, China) was employed to analyze all the natural compounds in LJEO. In the second step, the silica gel TLC plate (analytical) was completely dried by means of an electric oven and absolutely sprayed by means of DPPH radical methanol solution (200 μmol/L), so that its background was turned purple and the natural compounds of LJEO with antioxidant effect were turned into bright yellow spots, and these spots were supposed to be natural compounds with antioxidant activity, such as scavenging DPPH radical activity. In the third step, using the *n*-hexane/ethyl acetate solution system (80:20, *v*/*v*) as well, another piece of silica gel TLC plate (preparative) on the aluminum foil was employed to analyze the natural compounds with antioxidant effect in LJEO. Herein, the bright yellow spots with the corresponding and analogical retention factor (Rf) values were isolated with ether (100%) solvent. In the fourth step, the natural compounds with potential and effective antioxidant effects in LJEO isolated were technically purified by the silica gel TLC plates (preparative) on the aluminum foil with *n*-hexane/ethyl oxide solution system (83:17, *v*/*v*), and the natural compounds with potential and effective antioxidant effects in LJEO were finally acquired. The natural compounds with antioxidant effects in LJEO were identified by ^{1}H-NMR (Bruker Ascend™, Bruker Karlsruhe, Germany) and HRESIMS (Agilent 6540, Santa Clara, CA, USA), and they were identified to be three known compounds (1,8-cineole with the Rf value 0.78, eugenol with the Rf value 0.74 and spathulenol with the Rf value 0.65).

2.7. Antioxidant Employment of in Deep-Frying Procedure of Youtiao

In order to confirm the antioxidant effects in the deep-frying procedure of these compounds of LJEO of *youtiao* using SFO, together with SFO samples mentioned above (TBHQ, 0.09 g/kg, TBHQ and non-added sample, Control), 1,8-cineole (0.06 g/kg × 4.11% = 2.47 mg/kg, CI-2.47), eugenol (0.06 g/kg × 7.22% = 4.33 mg/kg, EU-4.33), and spathulenol (0.06 g/kg × 7.45% = 4.47 mg/kg, SP-4.47) were separately and directly added into SFO to prepare SFO samples (each 10.0 kg; named CI-2.47, EU-4.33 and SP-4.77, respectively). Soon afterwards, the deep-frying procedure was repeated and all the physico-chemical properties mentioned above were assessed using the same method as well.

2.8. Statistical Analysis

The experimental data exhibited in all the tables and figures were expressed in means ± standard deviation (SD), and the experimental data presented in tests were only expressed in means, unless otherwise stated. In the statistical analysis, the analysis of variance (ANOVA) was carried out with GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA), and the significant levels were on the base of the confidence levels of 99% ($p < 0.01$, highly significant) and 95% ($p < 0.05$, statistically significant).

3. Results and Discussion

3.1. Chemical Composition of LJEO

With the rapid progress of physical therapy, the essential oils of spice and medicinal plants, including *L. japonica*, were considered as one of important research hotspots of researchers [30]. The extraction and chemical analysis of LJEO have been carried out for plenty of times, and the extraction yields and chemical compositions of LJEO were different

because of the different sources of plant material and methods of chemical extraction [31]. In the present investigation, 124.2 g of LJEO was extracted from 10.0 kg of flower buds of *L. japonica* by steam distillation method, with an extraction yield of 1.24%. As shown in Table 2, 36 chemical constituents were identified in LJEO, with the main chemical constituents caryophyllene oxide (19.17%, *w*/*w*), *cis*, *trans*-farnesol (7.71%), spathulenol (7.45%), eugenol (7.22%), *trans*-linalool oxide (6.48%), epiglobulol (5.14%), *β*-ionone (4.14%), and 1,8-cineole (4.11%). All these identified chemical constituents were consistent with that of the cited literature, while the relative amounts of them were quite different between the two investigations [22]. Naturally, the origins of plant material and ways of chemical extraction always play important roles in the chemical compositions of essential oils, after all, the environmental factors, including sunshine, moisture, and mineral elements in soil for the harvest places of plant material, could influence the biological characteristics of *L. japonica* [32]. In addition, although there are plenty of extraction methods for essential oils, the different experimental temperature and different evaporation efficiency of extraction equipment can bring about the missing of essential oils so that the extraction yields and chemical compositions are influenced [33].

Table 2. Chemical composition of the essential oil from the flower buds of *L. japonica*.

No.	RI [a]	RI [b]	Compound Names	RA [c] (%)
1	884	884	*cis*-4-Heptenal	0.39
2	961	961	1-Octen-3-ol	0.85
3	1003	1003	*p*-Cymene	0.32
4	1005	1005	1, 8-Cineole	4.11
5	1017	1017	Acetophenone	2.17
6	1036	1036	Benzyl alcohol	0.56
7	1061	1061	*cis*-Linalool oxide	0.92
8	1076	1076	Linalool	1.47
9	1085	1085	*trans*-Linalool oxide	6.48
10	1136	1136	Phenylethyl alcohol	2.42
11	1159	1159	*α*-Terpineol	1.23
12	1163	1163	Citronellyl acetate	1.01
13	1182	1182	Decanal	0.56
14	1200	1200	Dodecane	0.38
15	1238	1238	Geraniol	1.15
16	1287	1287	Undecanal	4.17
17	1368	1368	Geranyl acetate	3.25
18	1372	1372	*cis*-Jasmone	0.77
19	1380	1380	Eugenol	7.22
20	1397	1397	Dodecanal	3.28
21	1414	1414	*β*-Caryophyllene	1.54
22	1431	1431	Geranylacetone	1.70
23	1438	1438	Aromadendrene	0.26
24	1448	1448	*β*-Ionone	4.14
25	1529	1529	Elemol	0.94
26	1550	1550	Spathulenol	7.45
27	1567	1567	Caryophyllene oxide	19.17
28	1574	1574	Epiglobulol	5.14
29	1626	1626	*α*-Cadinol	0.65
30	1660	1660	Junipher camphor	0.85
31	1697	1697	*cis*, *trans*-Farnesol	7.71
32	1769	1769	Tetradecanoic acid	0.64
33	1816	1816	Hexahydrofarnesylacetone	0.74
34	1869	1868	Pentadecanoic acid	1.53
35	1968	1968	Hexadecanoic acid	0.87
36	2175	2175	Octadecanoic acid	1.41
		Total		97.45

[a] Retention index on ZB-1 capillary column in this exploration. [b] Retention index on ZB-1 capillary column in the reported literature. [c] Relative area (peak area/total peak area).

3.2. Optimization of Concentration for Antioxidants

For the deep-frying procedures using all kinds of vegetable oils, the application for the antioxidants were considered as a practical and economic method for the inhibition of the oxidative deterioration of vegetable oils and, subsequently, the sensorial reduction of fried products. The amount of antioxidants added to the oils not only affects their availability to some extent but also affects their toxicological aspects. Therefore, the appropriate concentrations of all sorts of antioxidants employed in the deep-frying procedures should be optimized [34]. As is known, it is necessary to conduct one shorter deep-frying procedure of more than 5.0 h to make sure of the effectiveness of antioxidants and choose the optimized concentrations of them [35]. In the present investigation, as exhibited in Figure 1, when LJEO was added at 0.06 g/kg, the levels for AV value (a), PV value (b), and AnV value (c) were 0.88 mg KOH/kg, 8.42 meq O_2/kg and 9.1 g I_2/100 g, respectively, while the level for IV value (d) was 138.7. Compared with the levels for AV value, PV value, AnV value, and IV value when LJEO added at other concentrations, the optimized concentration of LJEO was considered to be 0.06 g/kg. Meanwhile, the optimized concentrations of the synthetic antioxidants TBHQ, BHA, and BHT were considered to be 0.09 g/kg, 0.12 g/kg, and 0.12 g/kg, respectively. The results displayed that, as one crude extract of flower buds of *L. japonica* containing 36 natural compounds, LJEO might reveal antioxidant effect by means of the antioxidant effect of at least one natural compound or the synergistic effect of two or more natural compounds in it [36]. The optimized concentration of LJEO was lower than that of TBHQ, BHA, and BHT, and the possible reason was these common synthetic antioxidants are easily destroyed and evaporated during the deep-frying procedure and the chemical constituents of the antioxidant plant essential oils always have higher thermally stability [37].

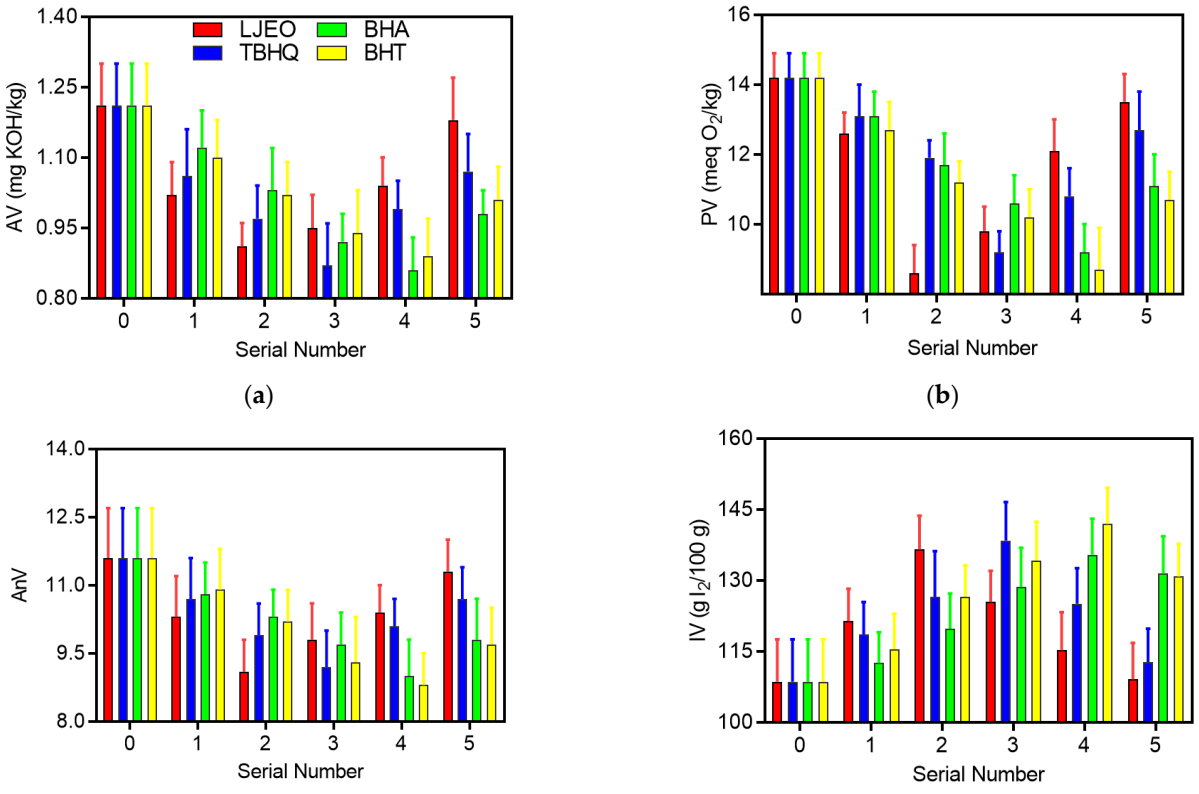

Figure 1. The acid value (AV, (**a**)), peroxide value (PV, (**b**)), *p*-anisidine value (AnV, (**c**)) and iodine value (IV, (**d**)) of sunflower oil added by LJEO, TBHQ, BHA, and BHT at 0.03, 0.06, 0.09, 0.12, and 0.15 g/kg during the frying, respectively. Values are expressed as means ± SD (n = 10).

3.3. Changes for Physico-Chemical Properties of SFO during Deep-Frying Procedure

In the deep-frying procedure oxidative deterioration of vegetable oils, the polar compounds are generated by the thermal, hydrolytic, and oxidative alteration so that the level for TPC is regarded as an indicator of oil deterioration [38]. The level for TBA is regarded as the indicator of malondialdehyde (MDA), which is the main secondary product of the oxidative deterioration of vegetable oils [39]. In the meantime, the conjugated dienes produced at the early stage of lipid oxidation are found to have absorption at 232 nm, while the conjugated trienes produced at the secondary stage of lipid oxidation have absorption at 268 nm. Therefore, to some extent, the absorbance of the UV spectrum at 232 nm and 268 nm are regarded as the levels for conjugated dienes and conjugated trienes, respectively [40]. In the present investigation, as displayed in Figure 2, the levels of TPC (a) and TBA (b), and the absorption of UV spectrum at 232 nm (c) and 268 nm (d) of the control SFO sample were prominently increased from 65.51 g/L, 0.35 mg/kg, 2.41, and 2.32 to 232.63 g/L, 1.51 mg/kg, 12.61, and 9.84, respectively, at the end of the deep-frying procedure. For the LJEO-0.06 SFO sample, the levels for TPC and TBA and the absorptions at 232 nm and 268 nm were also gradually increased, but they were prominently decreased from 232.6 g/L, 1.51 mg/kg, 12.6, and 9.8 to 106.42 g/L, 0.74 mg/kg, 7.43, and 4.66, respectively, compared with the control sample ($p < 0.05$ or $p < 0.01$). Meanwhile, all these physical-chemical properties of the SFO samples TBHQ-0.09, BHA-0.12, and BHT-0.12 were obviously increased as well, and they were much higher than that of LJEO-0.06 SFO sample in the whole deep-frying period. The antioxidant effects of essential oils extracted from spice and herb plants in the accelerated storage and deep-frying procedures were displayed once again, and the similar experimental results had been reported by others, where the addition of plant essential oils not only improved the oxidative rancidity of all kinds of vegetable oils but also added some nutritional attributes to them [41,42].

In the deep-frying procedure, the oxidative deterioration of vegetable oils and the free radicals produced from them by means of the cleavage of hydro-peroxides always react to generate all sorts of polymers, and the relevant viscosity is always increased due to the polymerization and generation of high molecular weight compounds [43]. In the meantime, the external color of the different kinds of vegetable oils is turned darker and darker with the passage of time. Many practitioners of fried product production have considered the variation for the color of vegetable oils as an important indicator for physico-chemical properties of vegetable oils [44]. Therefore, the variation in the color of vegetable oils has been widely applied as an objective index to measure the quality of used vegetable oils. In the present investigation, as displayed in Figure 3, the values for polymer (a), viscosity (b) and b^* (c) of the control SFO sample were dramatically increased from 1.71 g/L, 4.85×10^{-2} Pa·S, and 2.66 to 17.15 g/L, 10.33×10^{-2} Pa·S, and 17.26, respectively, at the end of the deep-frying procedure, while the value of L^* (d) was dramatically decreased from 74.22 to 31.57. For the LJEO-0.06 SFO sample, the values for polymer, viscosity, and b^* were also gradually increased, but they were dramatically decreased from 17.15 g/L, 10.33×10^{-2} Pa·S, and 17.26 to 6.33 g/L, 5.46×10^{-2} Pa·S, and 6.36, respectively, compared with the control sample ($p < 0.05$ or $p < 0.01$). Additionally, the value of L^* was dramatically increased from 31.57 to 59.75 ($p < 0.05$). Meanwhile, all these physico-chemical properties of the SFO samples—TBHQ-0.09, BHA-0.12, and BHT-0.12—were obviously increased as well, and they were much higher or lower than that of the LJEO-0.06 SFO sample in the whole deep-frying period. Herein, the antioxidant effect of LJEO was demonstrated for the first time, and the antioxidant effect of the plant *L. japonica* was first endowed to its essential oils [45]. In spite of the essential oils, there is still one crude extract; it can be employed as promising effective antioxidant agents for the deep-frying procedures of SFO and the transportation of some high-in-fat foods [21].

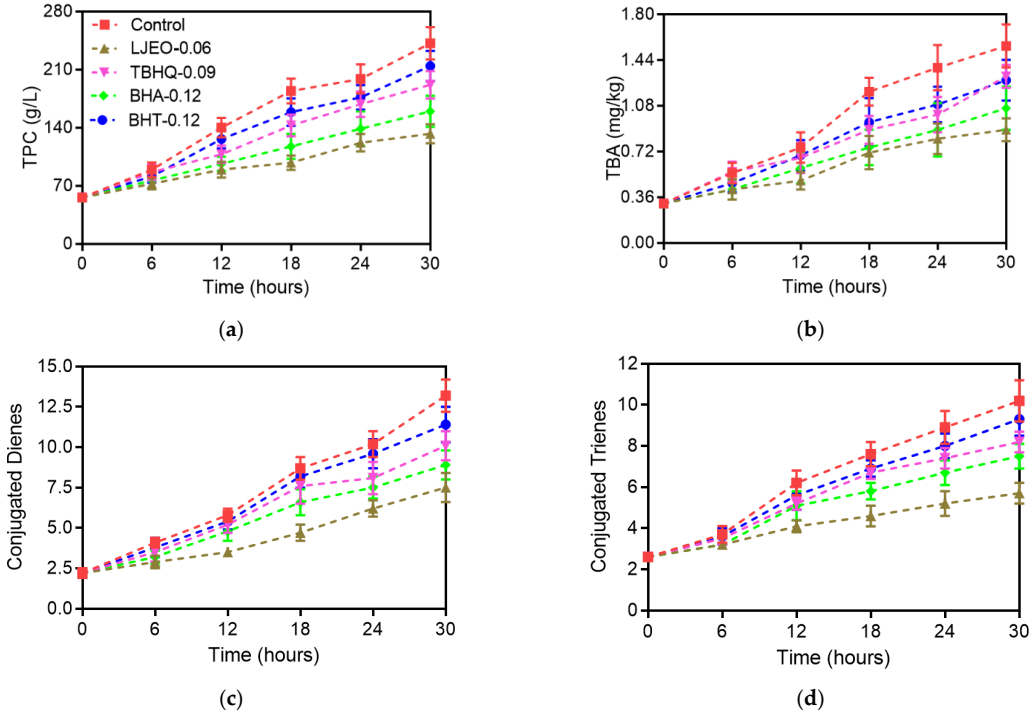

Figure 2. The values for the total polar compounds (TPC, (**a**)), thiobarbituric acid (TBA, (**b**)), conjugated dienes (**c**) and conjugated trienes (**d**) of sunflower oil added by nothing, LJEO (0.06 g/kg), TBHQ (0.09 g/kg), BHA (0.12 g/kg), and BHT (0.12 g/kg) during the deep-frying of *youtiao* for 30 h. Values are expressed as means ± SD (n = 10).

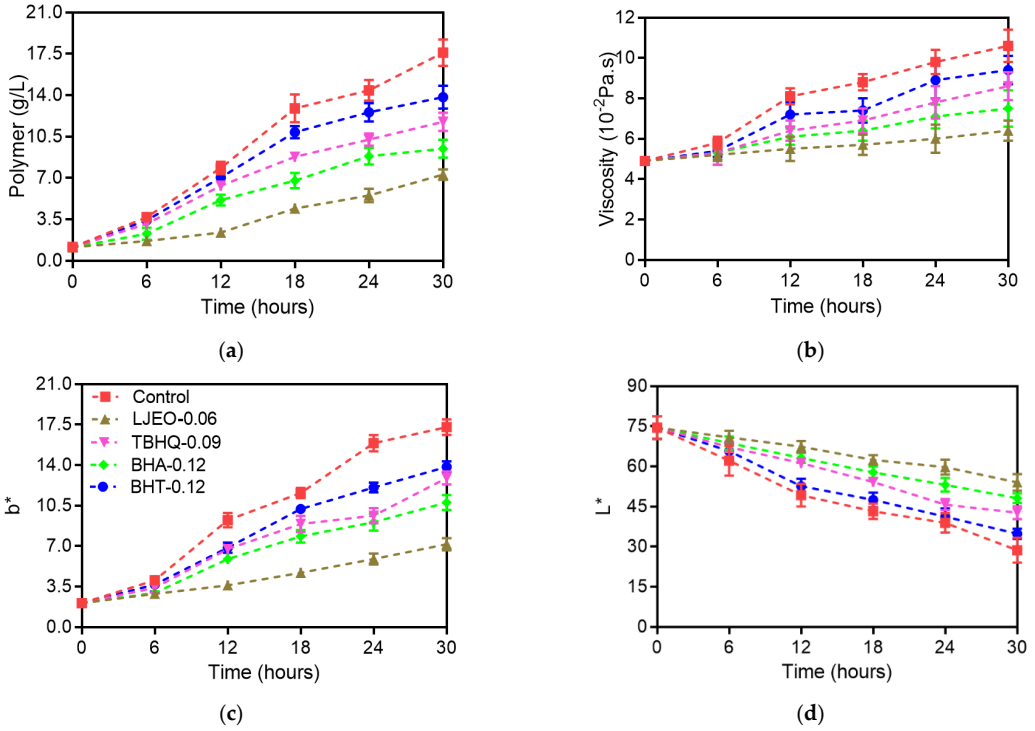

Figure 3. The values for the polymer (**a**), viscosity (**b**), *b** (**c**), and *L** (**d**) of sunflower oil added by nothing, LJEO (0.06 g/kg), TBHQ (0.09 g/kg), BHA (0.12 g/kg), and BHT (0.12 g/kg) during the deep-frying of *youtiao* for 30 h. Values are expressed as means ± SD (n = 10).

3.4. Changes for Sensorial Attributes of Youtiao during Deep-Frying Procedure

As is known, the addition of spice and herb plants can change their special sensorial attributes of high-in-fat foods; after all, the aroma compounds in the plants with some special fragrance always transfer from the plants to the foods [46]. Besides, before the choosing of new foods by consumers, the sensorial attributes of them should be assessed by the producers so that they can estimate the market potentials [47]. Therefore, the performance for the sensorial evaluation of the fried products, *youtiao*, is quite indispensable. In the present investigation, most of natural compounds of LJEO were evaporated and disappeared when the temperature of SFO was high. However, there may be one or more compounds that still remains in the fried products. As exhibited in Table 3, for the control SFO sample, the scores for the sensorial attributes of *youtiao*, including overall acceptability, crispness, taste, and flavor, were all reduced with the passage of time in the deep-frying process from 8.9, 8.8, 8.3, and 8.7 at hour 0 to 4.3, 4.3, 4.2, and 4.0 at hour 30, respectively. For the LJEO-0.06 SFO sample, compared with the SFO control sample, the scores for the sensorial attributes of *youtiao*, including overall acceptability, crispness, taste, and flavor, were only reduced from 8.9, 8.8, 8.3, and 8.7 at hour 0 to 6.5, 6.7, 6.5, and 6.6 at hour 30 ($p < 0.05$ or $p < 0.01$), respectively. For the other SFO samples, the scores of these sensorial attributes were quite similar to that of the SFO control sample. The results indicated that the remained aroma compounds from *L. japonica* apparently enhanced the sensorial properties of fried products, and the direct addition of essential oils into vegetable oils was considered as one effective way to improve the sensorial attributes of fried products, which was in agreement with that of our previous investigations [11–13].

Table 3. The influence of the essential oil from the flower buds of *L. japonica* on the sensory properties of *Youmotou*, such as flavor, taste, crispness, and overall acceptability.

Attributes	Hour	Control	LJEO-0.06	TBHQ-0.09	BHA-0.12	BHT-0.12
Flavor	0	8.9 ± 0.8	8.9 ± 0.8	8.9 ± 0.8	8.9 ± 0.8	8.9 ± 0.8
	6	8.3 ± 0.7	8.4 ± 0.7	8.0 ± 0.7	8.1 ± 0.7	8.2 ± 0.9
	12	6.9 ± 0.7	7.8 ± 0.9	6.8 ± 0.7	6.6 ± 0.7	6.8 ± 1.5
	18	6.1 ± 0.8	7.3 ± 0.8 [c]	6.5 ± 0.6	6.2 ± 0.6	6.5 ± 0.6
	24	5.2 ± 0.7	7.1 ± 0.8 [c]	5.4 ± 0.5	5.6 ± 0.6	6.3 ± 0.5
	30	4.3 ± 0.6	6.5 ± 0.7 [d]	4.4 ± 0.7	4.3 ± 0.7	4.4 ± 0.5
Taste	0	8.8 ± 1.2	8.8 ± 1.2	8.8 ± 1.2	8.8 ± 1.2	8.8 ± 1.2
	6	8.3 ± 0.7	8.4 ± 0.7	7.8 ± 0.8	7.9 ± 0.6	7.5 ± 0.6
	12	7.4 ± 0.9	7.7 ± 0.7	7.1 ± 0.6	7.3 ± 0.8	7.1 ± 0.6
	18	6.3 ± 0.8	7.2 ± 0.9	6.5 ± 0.6	6.2 ± 0.7	5.9 ± 0.4
	24	5.2 ± 0.7	6.9 ± 0.6 [c]	5.4 ± 0.5	5.3 ± 0.7	5.2 ± 0.6
	30	4.3 ± 0.7	6.7 ± 0.6 [d]	4.7 ± 0.7	5.2 ± 0.4	4.2 ± 0.6
Crispness	0	8.3 ± 0.8	8.3 ± 0.8	8.3 ± 0.8	8.3 ± 0.8	8.3 ± 0.8
	6	7.6 ± 0.8	7.9 ± 0.5	7.8 ± 0.5	7.7 ± 0.7	7.5 ± 0.8
	12	6.5 ± 0.9	7.5 ± 0.5	6.4 ± 0.6	6.4 ± 0.4	6.3 ± 0.9
	18	5.7 ± 0.7	7.1 ± 0.8 [c]	6.0 ± 0.9	6.0 ± 0.5	6.1 ± 0.7
	24	5.1 ± 0.6	6.7 ± 0.9 [c]	5.6 ± 0.5	5.3 ± 0.7	5.1 ± 0.7
	30	4.2 ± 0.3	6.5 ± 0.6 [d]	3.7 ± 0.8	4.0 ± 0.5	4.3 ± 0.3
Overall acceptability	0	8.7 ± 1.4	8.7 ± 1.4	8.7 ± 1.4	8.7 ± 1.4	8.7 ± 1.4
	6	8.0 ± 0.7	8.3 ± 0.8	7.8 ± 0.7	7.6 ± 0.7	8.3 ± 0.9
	12	6.8 ± 0.6	8.0 ± 0.6 [c]	6.5 ± 0.8	6.5 ± 0.9	7.0 ± 0.5
	18	5.5 ± 0.8	7.6 ± 0.6 [d]	5.7 ± 0.7	5.4 ± 0.9	5.8 ± 0.7
	24	4.7 ± 0.6	7.0 ± 0.9 [d]	4.5 ± 0.6	4.6 ± 0.7	4.4 ± 0.4
	30	4.0 ± 0.6	6.6 ± 0.6 [d]	4.1 ± 0.6	4.0 ± 0.5	4.0 ± 0.8

[c] As compared to control group at the same time: $p < 0.05$. [d] As compared to control group at the same time: $p < 0.01$.

3.5. Antioxidant Effect of Eugenol in SFO during Deep-Frying Procedure

As one secondary metabolite composed by plenty of natural compounds, essential oils are considered to be crude extracts, and thus their biological effects cannot be repeated [48].

After all, the origins of plant materials and ways of chemical extraction always play important roles in the chemical compositions of them [32]. Therefore, in the deep-frying procedure of SFO, the very compound/compounds that does/do exert antioxidative effect should be verified by bioassay-guided fractionation. In the present exploration, as revealed in Figure 4, compared with hour 0, the levels for TPC (a) and TBA (b) and the absorption at 232 nm (c) and 268 nm (d) of the SFO control sample at hour 30 were observably elevated during the whole deep-frying procedure ($p < 0.01$), while the levels for TPC and TBA and the absorption at 232 nm and 268 nm of the SFO sample EU-4.33 at hour 30 were observably reduced to 83.45 g/L, 73.15 mg/kg, 7.62, and 4.22, respectively, compared with the SFO control sample. In Figure 5, compared with hour 0, the value for polymer (a), viscosity (b), and b^* (c) of the SFO control sample at hour 30 were memorably elevated during the whole deep-frying procedure ($p < 0.01$), while the value for L^* (d) was memorably reduced ($p < 0.01$). For the SFO sample EU-4.33 at hour 30, the values for polymer, viscosity, and b^* were memorably reduced to 6.77 g/L, 6.07×10^{-2} Pa·S, and 4.83 ($p < 0.05$), respectively, while the value for L^* was memorably elevated to 58.54, compared with the SFO control sample ($p < 0.05$). The results revealed that eugenol was regarded as the very compound that did exhibit antioxidant effect in the oxidative deterioration of the deep-frying procedure of SFO for *youtiao*, which is consistent with our previous study where the antioxidative effect of the *Coriandrum sativum* essential oil was also attributed to eugenol [13].

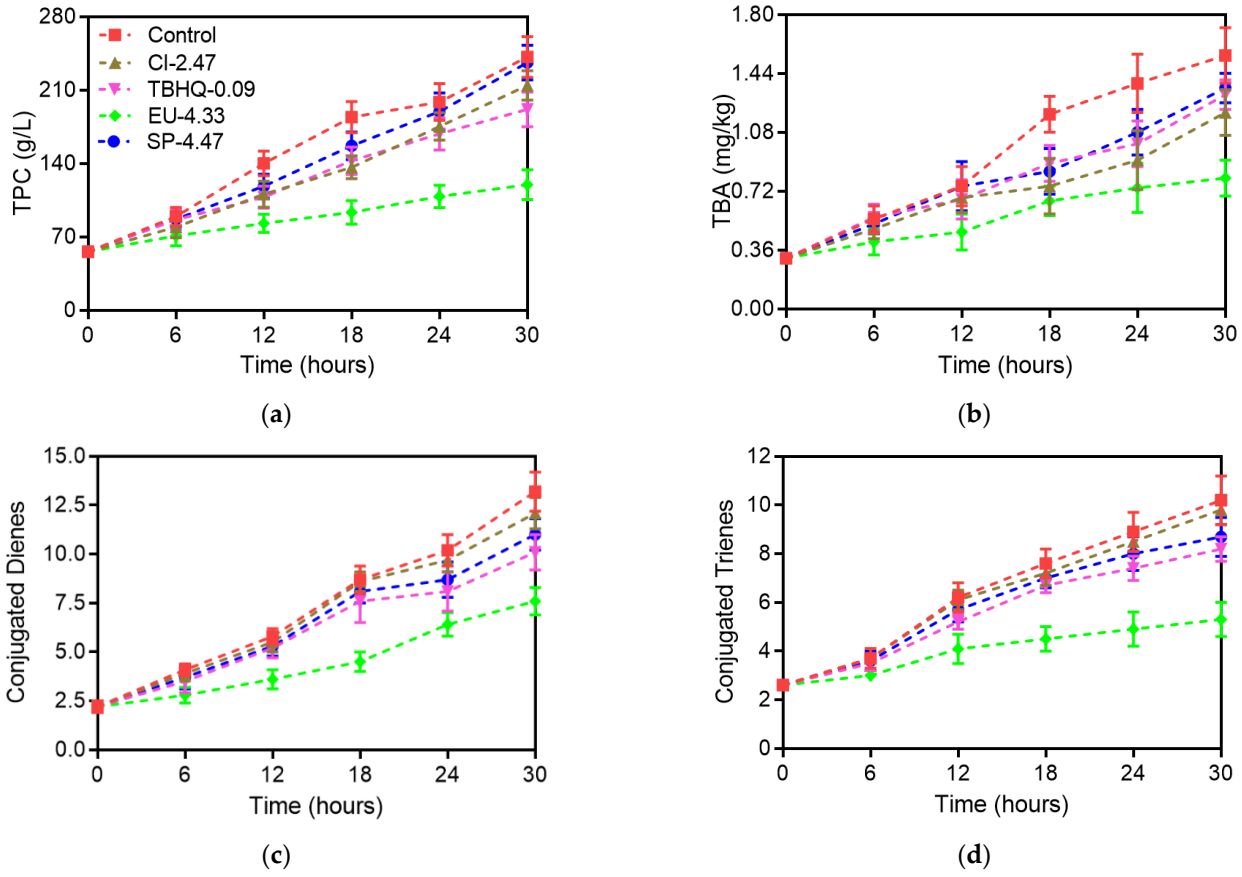

Figure 4. The values for the total polar compounds (TPC, (**a**)), thiobarbituric acid (TBA, (**b**)), conjugated dienes (**c**) and conjugated trienes (**d**) of sunflower oil added by nothing, 1,8-cineole (2.47 mg/kg, CL-2.47), TBHQ (0.12 g/kg, TBHQ-0.09), eugenol (4.33 mg/kg, EU-4.33), and spathulenol (4.47 mg/kg, SP-4.47) during the deep-frying of *youtiao* for 30 h. Values are expressed as mean ± SD (n = 10).

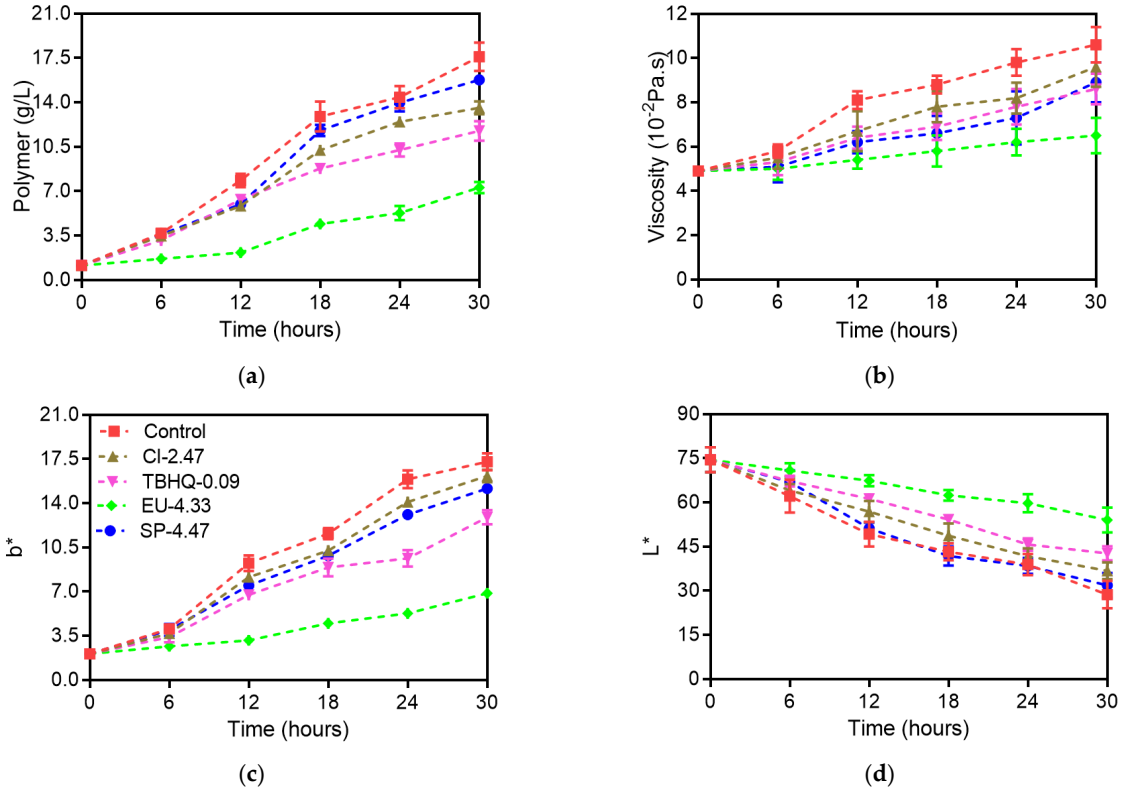

Figure 5. The values for the polymer (**a**), viscosity (**b**), *b** (**c**), and *L** (**d**) of sunflower oil added by nothing, 1,8-cineole (2.47 mg/kg, CL-2.47), TBHQ (0.12 g/kg, TBHQ-0.09), eugenol (4.33 mg/kg, EU-4.33), and spathulenol (4.47 mg/kg, SP-4.47) during the deep-frying of *youtiao* for 30 h. Values are expressed as mean ± SD (n = 10).

4. Conclusions

In summary, compared with non-added SFO, the direct and artificial addition of LJEO at 0.06 g/kg to SFO could obviously improve the deep-frying procedure oxidative stability at 180 °C for 30 h. Additionally, the addition of LJEO at the concentration of 0.06 g/kg to SFO also could prominently restrain the decrease in the sensorial attributes of *youtiao*, including overall acceptability, crispness, taste, and flavor of *youtiao*. The subsequent bioassay-guided chromatograph of LJEO and the repeated deep-frying procedure of SFO showed that one of its chemical constituents, eugenol, was considered to be the very compound that did exhibit an antioxidant effect. Currently, both the reaction mechanism and food toxicology of the compound eugenol during the deep-frying procedure are being investigated in our laboratory. When all these experiments are accomplished, eugenol will be employed as a potential natural antioxidant to take the place of the traditional synthetic antioxidants during the deep-frying procedure.

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