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Physicochemical characteristics, oxidative stability, and micro-biological properties of blended French sauce with olive and soybean oils and augmented with olive leaf polyphenolic extract obtained via ultrasound-assisted extraction

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Abstract: This study was carried out in three phases. During the first stage, the physicochemical properties of French sauce prepared from different blends of soybean and olive oils were evaluated. In the second stage, the oxidative stability of the optimum sauce sample from the first stage enriched with various amounts of olive leaf polyphenolic extract (OLE) (obtained via ultrasound-assisted extraction) was investigated over 90 days of storage. During the third stage, the microbiological and sensory properties of the samples containing the optimum amounts of OLE, as a substitution for the synthetic preservatives, were studied. According to the results, addition of olive oil at higher levels (75 and 100%) could affect the physicochemical properties of the sauce as compared to the control sample. It was also found that the addition of olive oil (up to 50%) would not significantly impact the sauce properties. Regarding the OLE enrichment in the samples, it was found that high levels of OLE could improve the oxidative stability of the samples. Based on the results of the experiments in the third phase, it was found that OLE could be used as a preservative instead of the commercial ones. Overall, this study suggests the potential use of olive oil and olive leaf extract in the preparation of French sauce to boost its nutritional value and its stability.

Keywords: Functional Food; Sauce; Olive Oil; Olive Leaf; Polyphenolic Compounds

1. Introduction

Sauces and salad dressings are oil in water (O/W) emulsions that have drawn the attention of the consumers for their daily diets. Sauces are being used to improve the flavor, taste, and appearance of many food products and among them French sauce is one of the most known ones [1]. Generally, these types of products are called cold-sauces as any amount of heating treatments would result in an emulsion-breakdown [2]. Increasing demand for the health-promoting products has led salads to get special attention as healthy food products. This, in turn, has promoted the use of sauces as one of the main commodities in our daily diets. French sauce has been a matter of interest among scientists. For instance, Rasmussen et al. [3] showed the potential use of honey as a good substitute for EDTA and commercial sweeteners. In an another study, de Melo et al. [4] evaluated the stability and nutritional index of French sauce samples with the use of mannoprotein from spent brewer's yeast. A typical French sauce usually consists of egg yolk, vinegar, oil, and tomato paste. In addition, several ingredients such as paprika, mustard, and garlic powder are needed as the main participants for sauces' specific taste and flavor. Moreover, texturizing agents are normally used to form the consistency of the sauce [5]. In sauces' formulation, the type and nature of the used oil are very important. In sauces and dressings oil phase plays a vital role in the stability and general appearance of them. Additionally, the nutritional value of sauces can be considered as a direct function of their oil phase. Using oils with high amounts of saturated fatty acids can easily lead to several health problems such as obesity, cardiovascular disease, etc. [6].

Olive oil is a source of beneficial components such as polyphenolics, hydrocarbons, sterols, pigments, volatile compounds, vitamins, and minerals [7]. Olive oil is relatively resistant to oxidation because of its noticeable amount of

mono-unsaturated fatty acids (MUFAs) as well as presence of natural antioxidants such as α-tocopherol and phenolic compounds, like tyrosol, hydroxytyrosol, caffeic acid and others [8]. Among the MUFAs, oleic acid contributes to 58-83% in the fatty acids of olive oil, which has been proven to provide multiple beneficial impacts such as anti-inflammatory and anti-cancer effects [9]. Because of a wide variety of health benefits of olive oil, it can be used as a new oil phase in preparation of O/W model emulsions such as French sauce, mayonnaise, etc. In general, finding a new oil phase with functional and health properties which does not alter the physical and sensorial properties of the final product, has been a matter of interest among researchers for many years [10].

One of the most important factors in sauces and salad dressings is their stability. Because of their relatively high content of oil and perishable ingredients such as egg yolk, they are likely to be spoiled quickly [6]. Thus, in order to preserve them to maintain for a long time, the use of preservatives are usual in the sauces' industry. The presence of commercial preservatives like sorbate and benzoate salts is a major issue in these types of products; as they can cause several health problems such as allergic reactions or different cancers [11]. Using natural preservatives instead of synthetic ones has growing rapidly. For example, Rafiee et al. [12] used nanoliposomes which contained polyphenolic compounds of pistachio green hull as natural preservative agents in mayonnaise formulation. In an another study, Bruzewicz et al. [13] used baicalin (from baical skullcap root) to prepare mayonnaise samples to improve their shelf-life.

Nowadays, food waste products are considered as an excellent source of bioactive compounds such as polyphenolics, protein residues, and fibers. Therefore, the possibility of using them in a wide-variety of food formulations and also to reduce the environmental burden is of high interest for both food technologists and environmental activists. Using food sectors' by-products provides major benefits to the large world population by supplying additional food sources at lower costs [14,15]. Olive leaves, which are the by-products of olive processing industries, are considered to be rich sources of polyphenolic compounds, which have been used for food, medicine, and cosmetic applications [16]. Oleuropein, which is classified as a phenolic bitter compound, is found abundantly in olive leaves and offers several health benefits such as cardio-protection, neuro-protection, anti-aging, anti-inflammatory, anti-cancer, antioxidant, and anti-microbial activities [17,18]. Tyrosol is another polyphenolic compound that is found in olive leaves at high quantities. Şahin and Bilgin [19] reported that the antioxidant activity of hydroxyl tyrosol is ten times as high as that of the green tea polyphenolics. Because of the anti-microbial properties of olive leaves, they could be a promising natural ingredient help to enhance the shelf-life of emulsion-based products. Additionally, Due to the high polyphenolic content of olive leaves, they can also improve the overall nutritional value of products. Thus, the nutritional value of these products are crucial to be assessed [20].

Physicochemical as well as oxidative and microbiological properties are the most practical methods for analyzing emulsion-based products (mainly sauces) in terms of their nutritional value and stabilities. Considering the above points, the objectives of the current study are to find the optimum amount of olive oil in combination with soybean oil (which is widely used in sauce industries) in the formulation of French sauce and to further apply olive leaf extract (OLE) to improve the shelf-life of French sauce. The possibility of using OLE instead of commercial synthetic preservatives (benzoate and sorbate salts) are also evaluated.

2. Materials and Methods

2.1. *Materials*

Egg yolk, sugar, salt, vinegar, citric acid, tomato paste, paprika, garlic, and mustard powders were purchased from a local supermarket in Tehran, Iran. Soybean oil, xanthan and guar gums were provided by Behrouz Nik Industrial Company (Karaj, Iran). Olive oil without any additive was purchased from Massoud Oil Company (city of Roudbar, Guilan province of Iran). Olive leaves were harvested from an olive tree in Karaj, Iran at mid-June. All chemicals were at analytical grade and obtained from Merck (Darmstadt, Germany). Commercial French sauce was purchased from a local supermarket in Tehran, Iran and was from Bijan Industrial Company.

2.2. *Basic analysis of oils*

Basic parameters of olive oil and soybean oil (Acidity, Peroxide value, Iodine value, and Saponification value) were measured according the AOCS official methods [21]. Total phenolic content of oils were measured according to [22] and pigments content (chlorophylls and carotenoids) were determined using the method of [23]. Table 1 shows the basic parameters of oils used in this study.

Table 1. Chemical analysis of soybean and olive oils (shown as SBO and OO, respectively) used in the experiments

	AV	PV	SV	IV	TPC	ChC	CaC
SBO	0.1 ± 0.0	2.8 ± 0.6	195 ± 2	134 ± 1	4 ± 0.3	0.001 ± 0.000	0.025 ± 0.000
OO	1.5 ± 0.3	18.7 ± 0.2	191 ± 3	79 ± 2	178 ± 8	7.41 ± 0.00	2.16 ± 0.00

AV: acid value in mg NaOH/kg oil, PV: Peroxide value in meq O_2/kg oil, Saponification value in mg KOH/ g oil, IV: Iodine value in g I_2/ 100 g oil, TPC: Total phenolic content in mg GAE/ kg oil, ChC: Chlorophyll content in mg pheophytin, CaC: Carotenoid content in mg lutin.

2.3. *Preparation and characterization of olive leaf extract*

To obtain olive leaf extract (OLE), ultrasound-assisted extraction technique (UAE) was applied according to the procedure outlined by Difonzo et al. [16]. At first, 25 g of olive leaves were placed in an oven at 120 °C until the moisture content of leaves reached below 1%. Then, the leaves were powdered in a mixer (IKA-WEKE M20, Germany). After that, distilled water was added to the powdered leaves in the ratio of 1:20 (w/v, respectively). Then, the leaves and water blend was placed in an ultrasonic bath (Universal Ultrasonic Cleaner, DSA 100-SK2, China) and exposed to ultrasound waves for 90 min at 35±5 °C. Then, the mixture was centrifuged (Hettich-Universal 320, Germany) (25 °C, 30 min, and 4,000 ×g). At the end, the supernatant was carefully separated and lyophilized. The obtained and lyophilized OLE was then stored at -20°C for the rest of experiments.

The method reported by Şahin and Şamlı [24] was used with minor modifications to measure total phenolic content (TPC) of OLE. The samples' absorbance were measured at 765 nm. The results were expressed as mg Gallic acid equivalent (GAE) per gram of the dried olive leaves (mg GAE/g dried olive leaf). Gallic acid at different concentrations (0.170-0.150 mg/ml) was used to plot the calibration curve. A regression coefficient of 0.9983 was obtained for the calibration curve. The TPC of the obtaining extract was determined as 27 ± 0.6 (mg GAE/ g dry leaf).
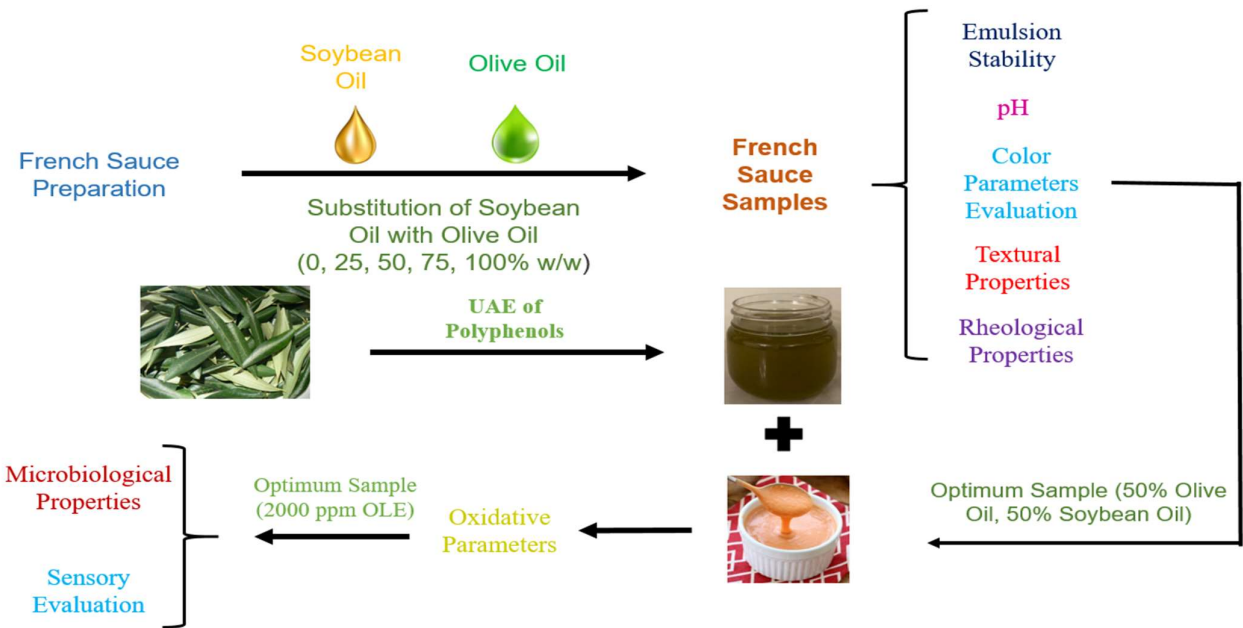

Figure 1. An illustration of schematic overview of this research

2.4. *Overview of the experiments*

This research was carried out in three phases as illustrated in Figure 1. In the first stage, French sauce samples were produced using different blends of olive and soybean oils; then in the second stage, the optimum sample from stage one was selected and oxidative effects of OLE in sauce samples were evaluated. In the third phase, the optimum amount of OLE selected and its effects on microbiological properties as well as sensory properties of French sauce were evaluated. The tests of each phase are explained below:

2.5. *First phase of the research*

2.5.1. Preparation of French sauce samples

The following proportion of the compounds (w/w) was used for the preparation of French Sauce according to the method of Mizani et al. [1] with some modifications: egg yolk (20%), vinegar (10%), mustard powder (2.0%), garlic powder (0.16%), paprika powder (0.4%), tomato paste (4.0%), citric acid (0.1%), sugar (6.0%), salt (2.0%), xanthan gum (0.64%), guar gum (0.26%), sodium benzoate (0.063%), potassium sorbate (0.012%), water (16.4%), oil (38%). Olive and soybean oils were used at five different ratios (0, 25, 50, 75, and 100%, w/w). The sample containing 100% of soybean oil and 0% of olive oil was used as the control sample. For preparation of samples, first, egg yolk was stirred to produce a considerable amount of foam. Then, vinegar, mustard powder, and citric acid were added and completely dissolved. Then, tomato paste and other ingredients except for gums were inserted and completely mixed. After that, xanthan and guar gums were added to make a proper texture. Finally, oil and water were added while mixing the components to generate a suitable emulsion structure in the samples.

2.5.2. Emulsion stability

Emulsion stabilities of French sauce samples were measured using the method reported by Mun et al. [25] with slight modifications. Briefly, falcon tubes containing 15 g of sauce samples were placed in a water bath (80 °C, 30 min). Then, the samples were cooled down for ~10 min, followed by centrifugation at 3,000 ×g for 10 min. After centrifugation, the oil layer was carefully removed. Finally, the samples were weighed without the oil layer. Emulsion stability of the samples were determined using the equation 1:

$$\text{Emulsion Stability (\%)} = \frac{F_0}{F_1} \times 100 \qquad (1)$$

where F0 and F1 are the sample weights after and before centrifugation, respectively.

2.5.3. Color measurements

Color parameters (a*, b*, L*) were recorded at 25 °C using a Chroma Meter (CR-400, Japan). All parameters were measured in triplicate.

2.5.4. Texture profile analysis

Using a texture analyzer TA-XT2 (Stable Micro Systems, UK), firmness and consistency levels of the sauce samples were measured following the procedure described by Patil and Benjakul [26] at 25 °C. Briefly, samples were transferred to the containers up to the 125-ml levels and a disc with a 35-mm diameter was used for the compression. The speed was fixed at 1 mm/sec, until reaching the depth of 40 mm. To measure firmness and consistency, the force-time curve was used, in which the maximum point of force curve and the area of the curve were used as the firmness and consistency values for the samples successively.

2.5.5. Rheological properties

A rheometer (Physica MCR 301, Anton Paar, Austria) was used to determine the rheological properties of French sauce samples with parallel geometry (diameter: 25 mm, gap: 1 mm) using the probe PP 40/S (Austria). First, the strain sweep from 0.01 to 100% at the constant frequency of 1.0 Hz was accomplished to determine the linear viscoelastic range. Then, the frequency sweep test in the 0.1-100 Hz frequency range was obtained under a constant strain of 0.5% and 25 °C.

2.5.6. pH determination

A 5% (w/v) sample solution was prepared and the pH values of the samples were measured using a pH-meter (Crison-GLP 22, Germany) at 25 °C. All tests were carried out in triplicate.

2.6. *Second phase of the research*

2.6.1. Characterization of enriched French sauce containing 50:50 of olive/soybean oils and different amounts of Olive leaf extract

For further analysis, sauce samples were made using 50% of olive oil and 50% of soybean oil in the formulation (According to the obtained results, this blend of oils was selected as the optimum sample, so it used for the rest of our experiments). Then, OLE was added to sauce samples in different levels (500, 1000, 1500, and 2000 ppm). A sample without the addition of OLE was used as the control. Sauces' oxidative stability tests were then carried out every 15 days for a 3-month storage at 25 °C.

2.6.2. Oxidative Stability Evaluation

Acid value (AV), peroxide value (PV) and p-anisidine value (PAV) were measured according to the official recommended methods of AOCS [21]. Then, following equations were applied to determine the values of each oxidative parameter.

$$AV\ (\%) = \frac{N \times V \times 28.2}{W} \qquad (2)$$

where V, N, and W stand for the volume of NaOH, normality of NaOH, and sample weight, respectively.

$$PV\ (meq\ O_2\ /\ Kg\ Oil) = \frac{(S-B) \times N \times 1000}{W} \qquad (3)$$

where S and B are the volumes of titrant used for sample and blank (sample without the oil), respectively. N is the normality of sodium thiosulfate and W is the sample weight.

$$PAV = \frac{1.2 \times (A_2 - A_1) \times 25}{W} \qquad (4)$$

where A_1 is the initial absorbance of sample and A_2 is absorbance of the sample after 10 min of exposure to *p*-anisidine solution, and W is sample weight.
Total oxidation value (Totox) of each sample was calculated using the equation 5:

$$Totox\ Value = (2 \times PV) + PAV \qquad (5)$$

2.7. *Third phase of study*
In the last part of the study, the optimum amount of OLE was selected and microbiological properties of a sauce sample with optimum amount of OLE/without commercial preservatives and a sample with commercial preservatives/without OLE were analyzed. Sensory evaluations were also carried out on those samples with a commercial product.

2.7.1. Microbiological Properties

Total plate counts for the mesophilic aerobic bacteria, yeast and molds, and presence of E. coli and lactic acid bacteria of two French sauce samples with the substitution of commercial preservatives/OLE were determined in seven stages (at the beginning and every 15 days up to the three months of storage). For each analysis, first, 10 g of French sauce sample was weighed in a test tube and homogenized with peptone water (90 ml). Then, suitable dilutions of each suspension (1 ml) were poured and spread onto total count plate and selective agars' plates (Merck, Darmstadt, Germany). Total plate count of aerobic mesophilic bacteria were carried out by means of a standard plate count agar and incubated at 30 °C for 72 hr. Yeast and molds were measured via YGC (Yeast extract glucose chloramphenicol agar), which were

incubated at 25 °C for 6 days. In order to determine the presence of E. coli, 1 ml of sample's suspension was added to 9 ml of lauryl sulfate broth (LSB) and incubated at 37 °C for 48h. Acid-tolerant microorganisms were identified using Orange serum agar (OSA) at 45 °C, in which OSA was added to 1 ml of sample and after mixing it was left to solidify at room temperature, after which, it was incubated at 30 °C for 5 days.

2.7.2. Sensory Analysis

Sensory analysis of three samples (with and without OLE and a commercial product) were performed according to the method explained by Liu, Xu, & Guo [27]. Briefly, a 9- point hedonic scale was used (1=the poorest and 9=the best). The characteristics of samples (appearance, odor, texture, color, taste, and overall acceptance) were evaluated by 25 panelists (at 21-30 years of age). Bread and water were provided between testing the samples to reset the remaining taste from the previous samples.

2.8. Statistical Analysis

Statistical analysis among different experiments were carried out using the SPSS program for windows (version 25.0, SPSS Inc., Chicago, IL, USA). The evaluation of the differences among the treatments were performed applying a one-way analysis of variance (ANOVA) with Duncan's test at P<0.05.

3. Results and Discussion

3.1. *Emulsion Stability*

Emulsion stability is an important factor in emulsion-based systems. This factor is related to some phenomena such as coalescence, flocculation, and Ostwald ripening [25]. In sauces and salad dressings, proteins can make a layer around oil droplets. The generated layer by proteins can play a protective role against the accumulation of oil droplets in one area [28]. The results of emulsion stability of samples are shown in Table 2. According to the results, all samples showed a very high emulsion stability (99.4-99.9%) at day 0 of production. As sauces and salad dressings contain high amounts of oil in their formulations, particles cannot move easily through their matrix, thus the stability of these products are usually high [29]. The stability levels of the samples were slightly decreased as the ratio of olive oil increased. The sample containing 100% olive oil showed the lowest stability (99.4%). But, the control sample showed the highest stability level (99.9 %.). From the statistical point of view, addition of olive oil up to 50% could not cause a meaningful difference with control sample both on day 0 and on day 90 of storage time. Olive oil generally contains less PUFAs than soybean oil. The partial reduction in the stability of emulsion with increasing amount of olive oil is due to the oils' nature fatty acid profile. Based on the explanation of Magnusson et al. [30], in their study a positive correlation was obtained between the amount of unsaturated fatty acids and the emulsion stability. There was a decreasing trend in the emulsion stabilities of the samples during the storage time, but the trend was much obvious in the samples containing 75% and 100% olive oil. This decreasing trend could be due to the sensitivity of saturated fatty acids to the shear stress, so the crystalline structure of them will be damaged more easily [31].

3.2. *pH Analysis*

pH is one of the most important parameters in the evaluation of sauces and salad dressings. A high pH value of a product usually causes serious damages such as growing of pathogen microorganisms (e.g., *Staphylococcus aureus*). Constant pH mentoring is one of the most critical factors in preparing a new formulation of emulsion-based products [32]. The results of pH test are shown in Table 3. Regarding the obtained results, the initial pH values (day 0) of the samples did not show significant differences. However, they decreased during the storage period of 90 days. Sample containing 100% olive oil showed the lowest pH value (3.89), while the sample with 50% olive oil showed the highest pH of 4.05. Similar to the findings of the current study, Worrasinchai et al. [32] reported a declining trend over the 64 days of storage for the pH value in mayonnaise samples. The reduction in pH during the storage time could be related to the breakdown of ester groups and generation of acidic groups [33]. Also, the growth and activity of some microorganisms mainly lactic acid bacteria can cause a drop in the pH [32].

Table 2. Emulsion Stability of French sauce samples prepared at different ratios of olive and soybean oils (1: sample with 100% of soybean oil, 2: sample with 75% of soybean oil and 25% of olive oil, 3: sample with 50% of soybean oil and 50% of olive oil, 4: sample with 24% of soybean oil and 75% of olive oil, and 5: sample with 100% of olive oil)

Storage Period (Days)	Sample				
	1	2	3	4	5
0	99.9 ± 0.1^{Aa}	99.9 ± 0.1^{Aa}	99.9 ± 0.1^{Aa}	99.6 ± 0.1^{Ab}	99.4 ± 0.0^{Ac}
15	99.9 ± 0.0^{Aa}	99.7 ± 0.2^{ABa}	99.8 ± 0.1^{Aa}	99.1 ± 0.1^{Ab}	98.9 ± 0.1^{Ab}
30	99.8 ± 0.0^{Aa}	99.5 ± 0.1^{Bab}	99.5 ± 0.1^{ABab}	98.1 ± 0.8^{Bb}	98.8 ± 0.1^{Ac}
45	99.7 ± 0.1^{ABa}	99.5 ± 0.0^{Ba}	99.2 ± 0.2^{Ba}	96.9 ± 0.1^{Cb}	97.81 ± 0.7^{Bc}
60	99.6 ± 0.2^{Ba}	98.8 ± 0.0^{Cb}	98.4 ± 0.1^{Cb}	96.7 ± 0.7^{Cc}	95.8 ± 0.1^{Cd}
75	98.4 ± 0.1^{Ca}	98.5 ± 0.1^{CDb}	98.1 ± 0.1^{Cc}	96.7 ± 0.1^{Cd}	95.5 ± 0.5^{Ce}
90	98.4 ± 0.3^{Ca}	98.3 ± 0.3^{Da}	98.1 ± 0.8^{Ca}	96.6 ± 0.7^{Cb}	95.4 ± 0.5^{Cc}

- The results are given as means ± SD, n = 3.
- In each row, values with the same letter (lower case) are not significantly different.
- In each column, values with the same letter (upper case) are not significantly different.

Table 3. pH values of French sauce samples prepared at different ratios of olive and soybean oils (1: sample with 100% of soybean oil, 2: sample with 75% of soybean oil and 25% of olive oil, 3: sample with 50% of soybean oil and 50% of olive oil, 4: sample with 24% of soybean oil and 75% of olive oil, and 5: sample with 100% of olive oil)

Storage Period (Day)	Sample				
	1	2	3	4	5
0	4.10 ± 0.10^{Aa}	4.08 ± 0.08^{Aa}	4.10 ± 0.05^{Aa}	4.10 ± 0.1^{Aa}	4.13 ± 0.02^{Aa}
15	4.08 ± 0.08^{Aa}	4.03 ± 0.05^{Aa}	4.09 ± 0.01^{Aa}	4.06 ± 0.05^{ABa}	4.12 ± 0.00^{Aa}
30	4.05 ± 0.05^{ABa}	4.04 ± 0.05^{Aa}	4.09 ± 0.00^{Aa}	4.06 ± 0.05^{ABa}	4.11 ± 0.00^{Aa}
45	3.89 ± 0.09^{Bb}	3.70 ± 0.00^{Ba}	3.90 ± 0.10^{ABb}	4.03 ± 0.05^{ABc}	4.11 ± 0.01^{Ac}
60	3.60 ± 0.10^{Cab}	3.50 ± 0.1^{Ca}	3.83 ± 0.28^{BCbc}	4.01 ± 0.00^{Ac}	4.00 ± 0.00^{Bc}
75	3.50 ± 0.1^{CDa}	3.46 ± 0.05^{Ca}	3.63 ± 0.15^{Ca}	3.97 ± 0.06^{Bb}	3.90 ± 0.10^{Cb}
90	3.36 ± 0.06^{Da}	3.45 ± 0.05^{Ca}	3.86 ± 0.05^{ABCb}	3.97 ± 0.06^{Bb}	3.90 ± 0.10^{Cb}

- The results are given as means ± SD, n = 3.
- In each row, values with the same letter (lower case) are not significantly different.
- In each column, values with the same letter (upper case) are not significantly different.

3.3. *Color Analysis*

The collected data of color parameters including L* (lightness), b* (yellowness), and a* (redness) during the storage period are presented in Figure 2. On day 0, all samples showed different values for color parameters. Lightness is the most important color parameter in sauce products; as it directly affects consumers' choices [25]. Results showed that L* factor had the highest value in control sample indicating that it was the most transparent sample among others. This value decreased as the relative ratio of olive oil increased, such that samples with 75% and 100% olive oil had major differences with other samples. The differences in the L* value are due to the different light scattering of the components

from different samples. The scattering and absorption of samples are largely dependent on the reflective index, size and dispersion of droplets, concentration, and presence of chromophobic materials [34]. As the soybean oil used in the current study was much paler than the olive oil (as soybean oil had much lower of pigments than olive oil (Table 1)), the control sample showed the maximum transparency (L*=51). But, the sample containing 100% olive oil showed the highest turbidity (L*=40). Regarding the yellowness and redness factors, it can be clearly seen that the values increased with the increasing the ratio of olive oil. Control sample showed the lowest values for b* and a* (25 and 6, respectively). In terms of b* value, there were no apparent differences among all samples on day 0 of production. The differences in the a* values, especially in the samples containing 75% and 100% olive oil, are due to the intrinsic pigments of olive oil, as olive oil contains much higher amount of pigments than soybean oil [35]. On day 90 of storage, the lightness and yellowness values for all samples were lowest, while the redness was somewhat higher. The rates of decrease in the samples containing higher levels of olive oil were higher if compared to that the of control sample. The polyphenolic content of olive oil is much higher than soybean oil (178 compared to 4 mg Gallic acid equivalent / kg oil, (Table1)). As a result of oxidation of polyphenolic compounds, the L* value can decrease faster in the samples containing higher levels of olive oil. Additionally, the changes in the droplet sizes for the samples with different ratios of olive/soybean oils can be another reason for such changes. Generally, droplets get larger as the time proceeds towards the end of storage period and therefore influences the light scattering of the samples [26]. This explanation also was confirmed by our emulsion stability results (Table 2), where a slight reduction was observed in the emulsion stability during the storage time. The b* values of all samples were decreased on day 90 as a result of samples' pigment oxidation [36]. Carotenoids (especially xanthophylls, which can be found in egg yolk and oils) are more sensitive to oxidation [37]. As a consequence, in samples containing higher levels of olive oil the degradation was much sharper than the control. Badr [38] reported that the carotenoid content tend to decrease in liquid egg yolk during the storage, but the rate of decrease at low temperatures is noticeably faster than room temperature. The increase in the a* value during the storage time could be related to the oxidation of lipid components [26].
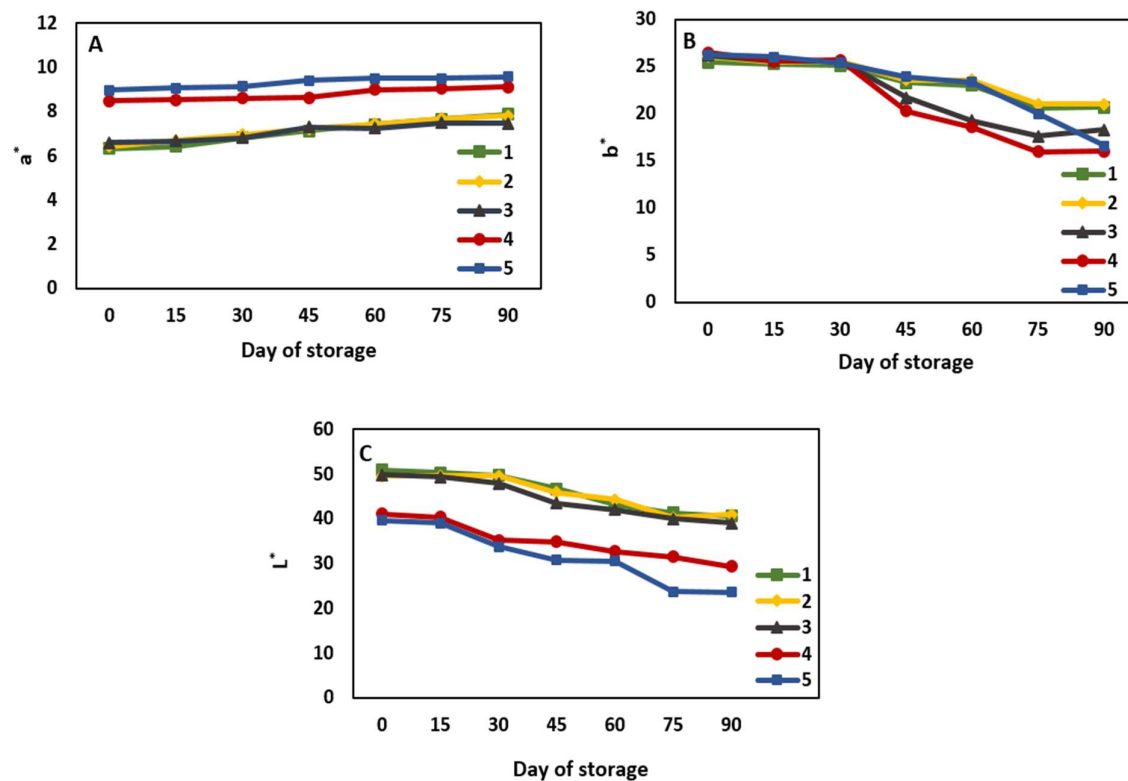

Figure 2. Color parameter (A: a*, B: b*, and C: L*) changes in French sauce samples prepared with different blends of olive/soybean oil (1: sample with 100% of soybean oil, 2: sample with 75% of soybean oil and 25% of olive oil, 3: sample with 50% of soybean oil and 50% of olive oil, 4: sample with 24% of soybean oil and 75% of olive oil, and 5: sample with 100% of olive oil)

3.4. *Textural Properties*

The data obtained for the changes in the consistency and firmness of French sauce samples over the 90 days of storage are given in Table 4. On day 0, a decreasing trend was observed with an increase in the ratio of olive oil, which might be due to the lower amounts of unsaturated fatty acids in the samples containing higher levels of olive oil. Patil et al. [26] and Kupongsak et al. [39] reported similar results for the textural properties of mayonnaise at different ratios of fish/coconut and rice bran/coconut oil, respectively.

During the study period (90 days), both the firmness and consistency dropped for all French sauce samples (Table 4). This fact could be related to some phenomenon such as coalescence, where oil droplets tend to join together and build larger particles [40]. These findings agree well with the results of the emulsion stability for the French sauce (Table 2).

Table 4. Textural properties of French sauce samples prepared with different blends of olive/soybean oil (1: sample with 100% of soybean oil, 2: sample with 75% of soybean oil and 25% of olive oil, 3: sample with 50% of soybean oil and 50% of olive oil, 4: sample with 24% of soybean oil and 75% of olive oil, and 5: sample with 100% of olive oil)

Sample	Firmness (g)			Consistency (g.sec)		
	Day 0	Day 45	Day 90	Day 0	Day 45	Day 90
1	163 ± 9^{Aa}	154 ± 3^{Aa}	128 ± 3^{ABb}	2507 ± 22^{Aa}	2303 ± 16^{Ab}	1938 ± 16^{Ac}
2	154 ± 5^{ABa}	146 ± 7^{Aa}	127 ± 3^{ABb}	2474 ± 15^{Aa}	2284 ± 18^{Ab}	1921 ± 22^{Ac}
3	161 ± 5^{Aa}	152 ± 2^{Aa}	131 ± 8^{Ab}	2407 ± 12^{Ba}	2180 ± 21^{Bb}	1952 ± 28^{Ac}
4	145 ± 4^{BCa}	130 ± 2^{Bb}	121 ± 9^{ABb}	2034 ± 27^{Ca}	1952 ± 17^{Cb}	1509 ± 11^{Bc}
5	134 ± 7^{Ca}	121 ± 4^{Cb}	116 ± 3^{Bb}	1829 ± 14^{Da}	1522 ± 11^{Db}	1488 ± 17^{Bc}

• The results are given as means ± SD, n = 3.
• In each row, values with the same letter (lower case) are not significantly different.
• In each column, values with the same letter (upper case) are not significantly different.

3.5. *Rheological Properties*

The changes in the viscoelastic properties of French sauce samples are shown in Figure 3 (Storage modulus) and Figure 4 (Loss modulus). For all samples, loss modulus (G'') was lower than the storage modulus (G'). This indicates that all samples have an elastic behavior rather than a viscous behavior. Also, samples showed linear viscoelastic responses with increasing the angular frequency indicating that all prepared French sauces can be considered as a gel-like network [41].

On day 0, the storage moduli for all samples increased slightly with an increase in the angular frequency (Figure 3). The sample containing 50% olive oil and the control indicated the highest and lowest storage modulus, respectively. This shows that the sample with 50% olive oil had the best power of structural uniformity [37], meaning that it required higher shear stress to flow. This subject is a matter of importance in the pouring ability of sauces in the factories as well as packages.

In terms of loss modulus (Figure 4), there were no clear differences among the samples. But, the sample containing 50% olive oil showed the highest value. Higher values of loss modulus indicate that the sample containing 50% olive oil had a better behavior of viscoelasticity compared to the others. On days 45 and day 90, all samples showed a slight decrease in the storage modulus compared to day 0. This means that the elastic behavior and the structural uniformity have dropped in all samples and samples have more liquid-like behaviors compared to the freshly-prepared samples on day 0. These findings are in agreement with the emulsion stability data, where the stability was reduced as the bonds between the oil droplets started to weaken. No apparent changes were found in terms of loss modulus during the storage time (Figure 4 a, b, and c).

The flow curves of prepared French sauce samples with different ratios of olive oil and soybean oil are presented in Figure 5. The shear stress of all samples soared as shear rate increased. However, there was no linearity in the relationship between the shear rate and shear stress for any of the sauce samples. Thus, all sauce samples can be considered as non-Newtonian liquids [42].

On day 0, the sample containing 50% olive oil showed the highest shear stress. This shows that it had the highest viscosity compared to the other samples; as, viscosity can be defined as the ratio of shear stress and shear rate. On days 45 and 90, all samples showed lower shear stress values compared to the freshly prepared samples on day 0. Generally, higher values of shear stress as a function of shear rate are preferred based on the consumers' data [43]. Such products could cause better mouth feel, but they could have some limitations considering the pumps and the fillers that are needed industrially [26].
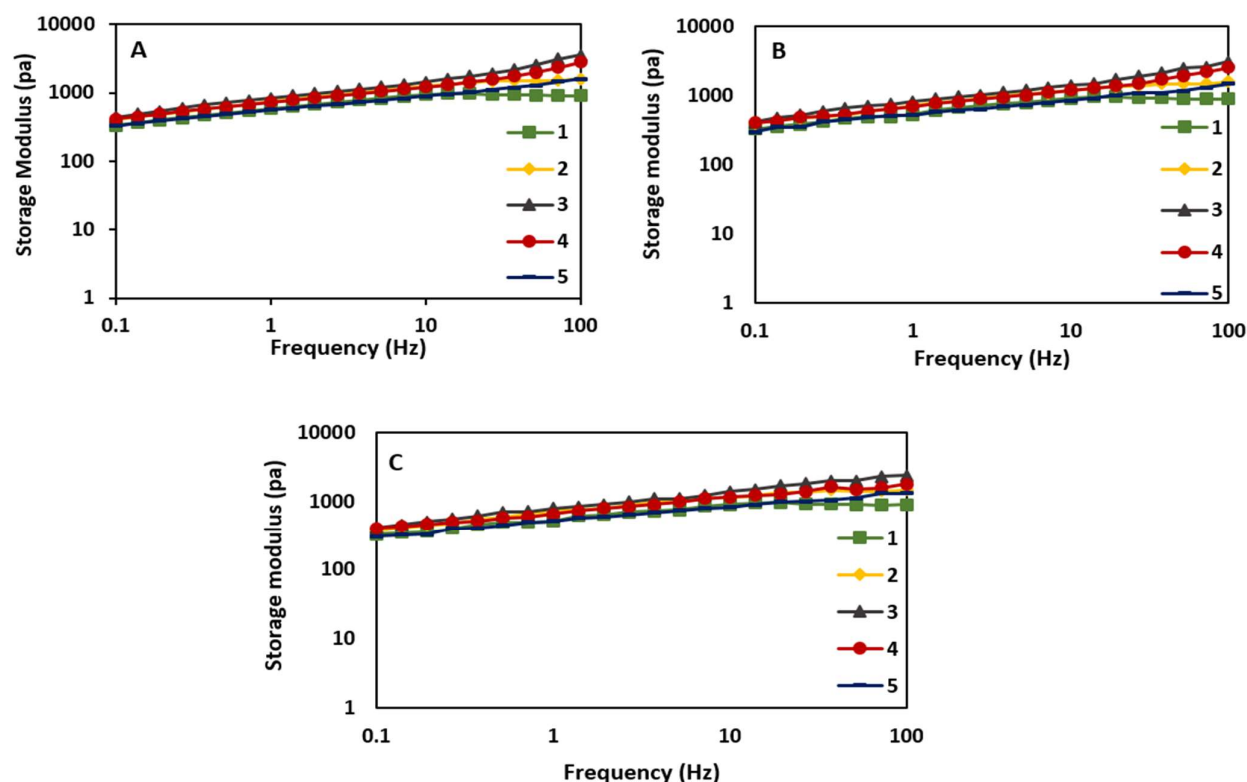

Figure 3. Storage modulus (G') of French sauce samples prepared with different blends of olive/soybean oil (A: Day 0, B: Day 45, and C: Day 90 of storage) (1: sample with 100% of soybean oil, 2: sample with 75% of soybean oil and 25% of olive oil, 3: sample with 50% of soybean oil and 50% of olive oil, 4: sample with 24% of soybean oil and 75% of olive oil, and 5: sample with 100% of olive oil)
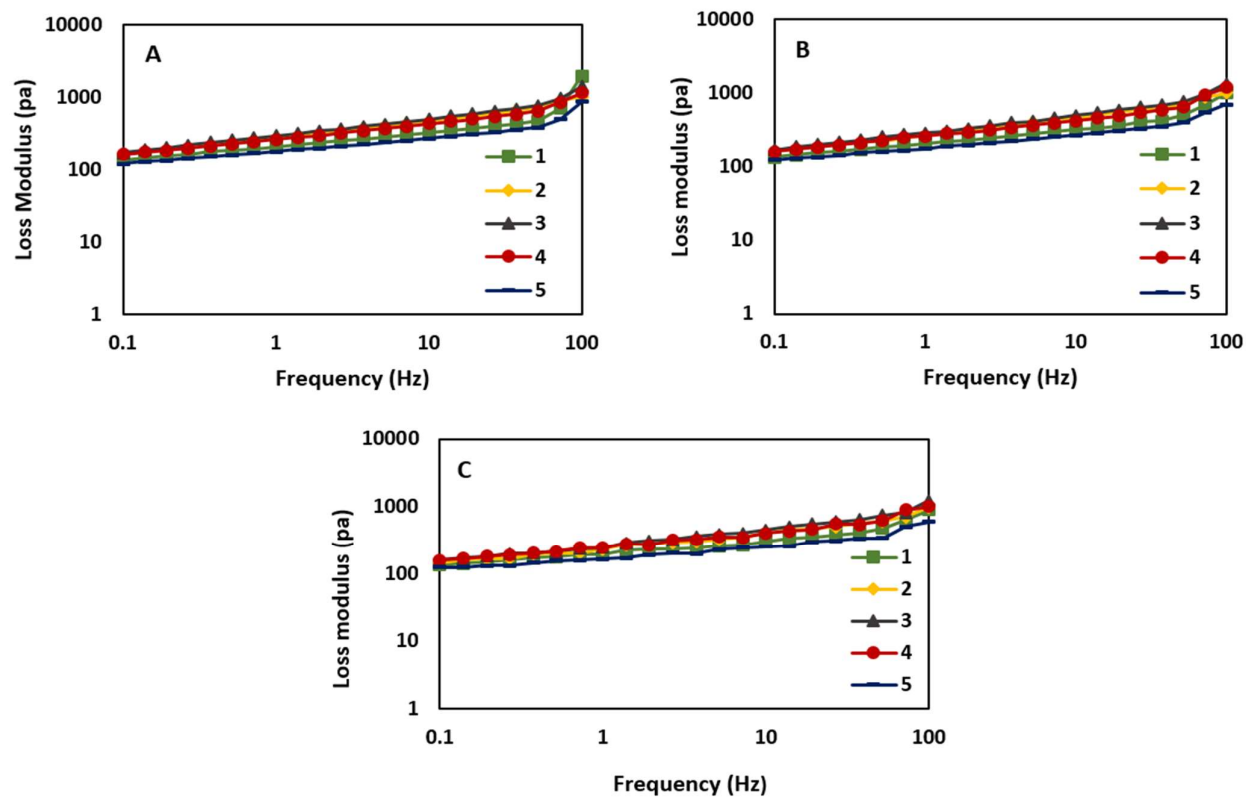
Figure 4. Loss modulus (G") of French sauce samples prepared with different blends of olive/soybean oil (A: Day 0, B: Day 45, and C: day 90 of storage) (1: sample with 100% of soybean oil, 2: sample with 75% of soybean oil and 25% of olive oil, 3: sample with 50% of soybean oil and 50% of olive oil, 4: sample with 24% of soybean oil and 75% of olive oil, and 5: sample with 100% of olive oil)

Figure 5. Flow curves of French sauce samples prepared with different blends of olive/soybean oil (A: Day 0, B: Day 45, and C: Day 90 of storage) (1: sample with 100% of soybean oil, 2: sample with 75% of soybean oil and 25% of olive oil, 3: sample with 50% of soybean oil and 50% of olive oil, 4: sample with 24% of soybean oil and 75% of olive oil, and 5: sample with 100% of olive oil)
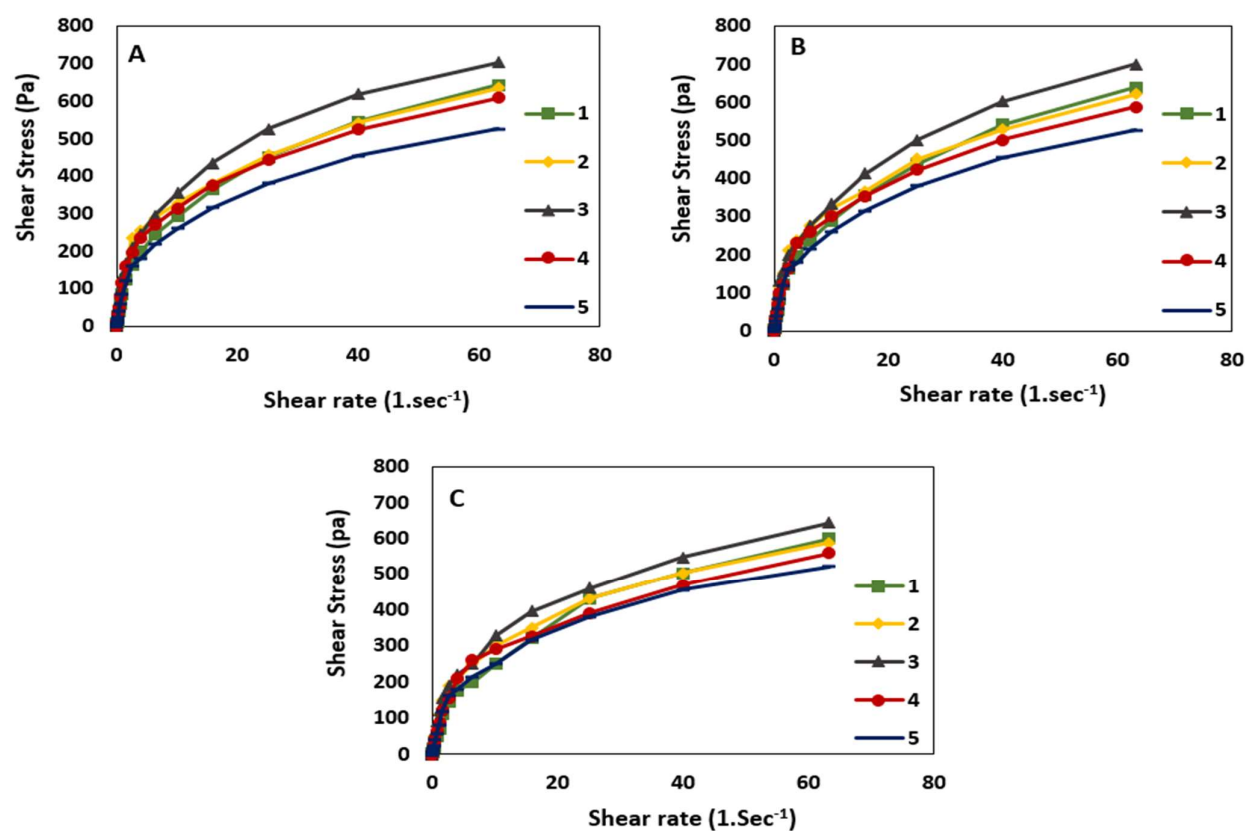
3.6. Stability Evaluation

3.6.1. Oxidative Parameters

AVs of extracted lipid phases from the samples are shown in Table 5. Generally, AV measures the free fatty acids, which are made through the hydrolysis of lipids in the presence of water [44]. According to Table 5, AV was increased in all samples during the storage for 90 days, but the rates of increase were different among the different samples. The control sample (with no added OLE) showed the highest changes during the storage, for which the AV was 1.1 at the beginning, but reached to its maximum (5.0) at the end of the studied period. The increase in the AV during the storage could be related to the activity of the hydrolytic enzymes in the eggs due to the acidic conditions of the sauce samples [45]. Additionally, the growth and activity of acid tolerant bacteria especially lactic acid bacteria could also impact on the free fatty acid formation in the aqueous phase of the samples [33]. The AV for the samples containing OLE, especially those with higher levels (1500 and 2000 ppm), increased at much lower rates than that of the control. Such effects can probably be mainly attributed to the polyphenolic compounds in the OLE, which was measured at 27 mg GAE/g dry leaf.

The results for the changes in the PV during the storage are shown in Table 6. The PV for all of the samples showed an initial increase followed by a slight decrease and then another increase afterward. The slight decrease especially around day 30 and 45 of storage is due to the breakdown of hydroperoxides that can form other compounds and the high increase rate in samples around day 75 and 90 could be the result of boosting oxidative reactions [46]. Samples with higher amounts of OLE showed lower levels of PV than the control sample. While the sample containing 2,000 ppm of OLE indicated the lowest PV value of 5.0 on day 90, the control sample showed the highest PV (9.0). These findings confirms the positive effect of OLE, especially its high and unique polyphenolic profile [47], on the sample's shelf-life. The obtained results are in agreement with those of other related studies in this area [48,49].

Generally, oxidation process has a detrimental impact on the products with high fat levels such as sauces. The reactions taking place in the interface of oil-water phases can speed up the process of oxidation [50].

PAV is another factor that measures the secondary oxidative compounds, which are responsible for the undesirable flavors and odor in the products. These oxidation reactions can lead to the formation of numerous compounds especially 2-alkenals and 2,4-dienals [49]. Generally, high levels of PAV indicates the deterioration of lipids in the food matrix [51].

The results of PAV of samples during the storage period are presented in Table 7. There was an increasing trend in the PAV during the storage time of 90 days. Such trend could be related to the acceleration of oxidative processes with time. Samples containing higher levels of OLE (1500 and 2000 ppm) showed lower increases in the PAV. Timm-Heinrich et al. [52] reported that some polyphenolic compounds can minimize the production of oxidative products such as hexanal and heptadienal. On day 90 of storage in the current study, the control sample had the highest PAV, while the sample containing 2,000 ppm of OLE showed the lowest PAV (12.6 and 5.7, respectively).

Total oxidation value (totox) is an index that is directly linked to both PV and PAV. Therefore, hydroperoxides and their primary and secondary oxidation products are reported by this parameter. The results of changes in the totox values of samples during the storage period are depicted in Figure 6. The totox values for all samples increased by time. However, the samples with higher amounts of OLE (1,500 and 2,000 ppm) increased at lower rates (Figure 6). However, the control sample and sample with 500 ppm OLE experienced higher changes in terms of totox value. On day 90 of storage, the control sample indicated the highest totox value of 30.6 while the sample containing 2,000 ppm OLE showed the lowest totox value of 15.8. Overall trend for the changes in the totox value (Figure 6) is similar to those of PV and PAV (Tables 6 & 7, respectively). The decreasing effect of OLE on totox values in the samples treated with high amounts of OLE can be related to to the presence of polyphenolic groups in the extract that have inhibitory effects against the generation of primary and secondary oxidation products [49].
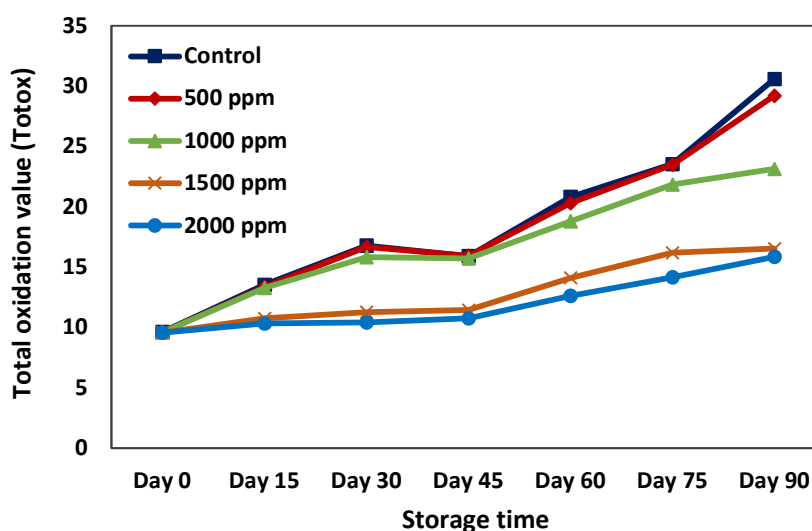

Figure 6. Changes in the totox value of French sauce samples enriched by different levels of olive leaf extract (OLE) (up to 2,000 ppm) during 90 days of storage.

Table 5. Acid values obtained for the samples of French sauce enriched by different amounts of olive leaf extract (OLE).

Storage Period (Day)	OLE Concentration (ppm)				
	0	**500**	**1000**	**1500**	**2000**
0	1.1 ± 0.0^{Aa}	1.1 ± 0.0^{Aa}	1.1 ± 0.0^{Aa}	1.1 ± 0.0^{Aa}	1.1 ± 0.0^{Aa}
15	1.2 ± 0.0^{Bab}	1.2 ± 0.0^{Ab}	1.2 ± 0.01^{Aab}	1.1 ± 0.1^{Aa}	1.1 ± 0.0^{Aa}
30	1.4 ± 0.0^{Ca}	1.4 ± 0.0^{Ba}	1.3 ± 0.0^{Aa}	1.3 ± 0.0^{Ba}	1.3 ± 0.0^{Ba}
45	2.3 ± 0.0^{Db}	2.3 ± 0.1^{Cab}	2.3 ± 0.0^{Bab}	2.3 ± 0.0^{Cb}	2.2 ± 0.0^{Ca}
60	3.1 ± 0.0^{Ec}	3.1 ± 0.1^{Dc}	3.1 ± 0.0^{Cc}	2.9 ± 0.0^{Db}	2.6 ± 0.1^{Da}
75	3.7 ± 0.1^{Fb}	3.6 ± 0.1^{Eb}	3.6 ± 0.4^{Db}	3.0 ± 0.0^{Da}	2.9 ± 0.0^{Ea}
90	4.9 ± 0.0^{Gd}	5.0 ± 0.0^{Fd}	4.7 ± 0.1^{Ec}	3.7 ± 0.2^{Eb}	3.1 ± 0.1^{Fa}

- The results are given as means $\pm$ SD, n = 3.
- In each row, values with the same letter (lower case) are not significantly different.
- In each column, values with the same letter (upper case) are not significantly different.

Table 6. Peroxide values of French sauce samples enriched by olive leaf extract (OLE).

Storage Period (Day)	OLE Concentration (ppm)				
	0	**500**	**1000**	**1500**	**2000**
0	3.1 ± 0.0^{Aa}	3.1 ± 0.0^{Aa}	3.1 ± 0.0^{Aa}	3.1 ± 0.0^{Aa}	3.1 ± 0.0^{Aa}
15	3.8 ± 0.0^{Ba}	3.8 ± 0.0^{Ba}	3.7 ± 0.0^{BCb}	3.4 ± 0.0^{Bc}	3.2 ± 0.1^{Bd}
30	4.4 ± 0.0^{Ca}	4.3 ± 0.1^{Ca}	3.9 ± 0.04^{Cb}	3.6 ± 0.1^{Dc}	3.3 ± 0.0^{Bd}
45	3.9 ± 0.1^{Bab}	3.9 ± 0.1^{Ba}	3.8 ± 0.0^{Bb}	3.5 ± 0.1^{Cc}	3.3 ± 0.0^{Bd}
60	4.9 ± 0.1^{Da}	4.7 ± 0.1^{Db}	4.5 ± 0.1^{Dc}	4.2 ± 0.1^{Ed}	3.8 ± 0.0^{Ce}
75	6.1 ± 0.1^{Ea}	6.1 ± 0.0^{Ea}	5.9 ± 0.1^{Eb}	5.1 ± 0.1^{Fc}	4.5 ± 0.1^{Dd}
90	8.9 ± 0.0^{Fa}	8.3 ± 0.2^{Fb}	6.1 ± 0.1^{Fc}	4.9 ± 0.0^{Gd}	5.0 ± 0.1^{Ed}

- The results are given as means $\pm$ SD, n = 3.
- In each row, values with the same letter (lower case) are not significantly different.
- In each column, values with the same letter (upper case) are not significantly different.

Table 7. p-Anisidine values of French sauce samples enriched by different amounts of olive oil (OLE).

Storage Period (Day)	OLE Concentration (ppm)				
	0	500	1000	1500	2000
0	3.3 ± 0.0[Aa]	3.3 ± 0.01[Aa]	3.3 ± 0.0[Aa]	3.3 ± 0.0[Aa]	3.3 ± 0.0[Aa]
15	5.9 ± 0.1[Bc]	5.8 ± 0.0[Bc]	5.84 ± 0.1[Bc]	4.0 ± 0.1[ABb]	3.8 ± 0.1[Ba]
30	8.0 ± 0.0[Cb]	7.9 ± 0.1[Cb]	7.9 ± 0.8[Cb]	3.9 ± 0.9[ABa]	3.84 ± 0.1[Ba]
45	8.1 ± 0.0[Cb]	8.0 ± 0.0[Cb]	8.0 ± 0.0[Cb]	4.4 ± 0.1[Ba]	4.2 ± 0.3[Ca]
60	10.9 ± 0.0[Dd]	10.8 ± 0.0[Dd]	9.8 ± 0.6[Db]	5.6 ± 0.6[Ca]	5.0 ± 0.0[Da]
75	11.3 ± 0.0[Ed]	11.2 ± 0.0[Ed]	10.0 ± 0.0[Dc]	6.0 ± 0.0[CDb]	5.1 ± 0.1[Da]
90	12.6 ± 0.0[Fd]	12.6 ± 0.0[Fd]	10.9 ± 0.5[Ec]	6.6 ± 0.7[Db]	5.7 ± 0.2[Ea]

- The results are given as means ± SD, n = 3.
- In each row, values with the same letter (lower case) are not significantly different.
- In each column, values with the same letter (upper case) are not significantly different.

Table 8. Total plate counts (cfu/g) of French sauce samples during the 90 days of storage period.

Storage Period (Day)	Sample 1			Sample 2		
	Yeast and molds	*E. coli*	Lactic acid bacteria	Yeast and molds	*E. coli*	Lactic acid bacteria
0	<100	nd	Nd	<100	nd	nd
15	<100	nd	Nd	<100	nd	nd
30	<100	nd	Nd	<100	nd	nd
45	<100	nd	Nd	<100	nd	nd
60	<100	nd	Nd	<100	nd	4.0×10^2
75	<100	nd	Nd	<100	nd	4.2×10^2
90	<100	nd	Nd	<100	nd	4.2×10^2

Sample 1 contains 2,000 ppm of OLE and no commercial preservatives.
Sample 2 contains the commercial preservatives, sodium benzoate, 0.063%, w/w, and potassium sorbate, 0.012%, w/w, without the addition of olive leaf extract (OLE).

3.6.2. *Microbiological properties*

The results of total plate counts for mesophilic aerobic bacteria of French sauce samples with and without using OLE are depicted in Figure 7. Both samples showed increases in the total plate count during the storage time. But, the sample containing OLE at 2000 ppm level indicated lower value than the control. On day 0, total plate count of sample 1 (i.e., that with no OLE added) was higher (300 cfu/g) than that in sample 2 (containing OLE at 2000 ppm) (250 cfu/g). But, around day 15 of the storage, total plate count for sample 1 surpassed that of sample 2. Slight reductions were observed in the total counts of both samples around day 30 and 60 of storage, which could be due to the dissociation of acetic acid in the oil phase resulting in the depletion of microorganisms for a while [33]. The inhibitory effects of OLE on the growth of aerobic microorganisms can be due to its high polyphenolic compounds that have shown to have antibacterial and preservative effects in other studies [18,53]. Pourkomailian [54] reported that in samples with high amount of oil

phase, microorganisms usually stay in the oil phase and therefore they are not influenced by the pH effect of the aqueous phase.
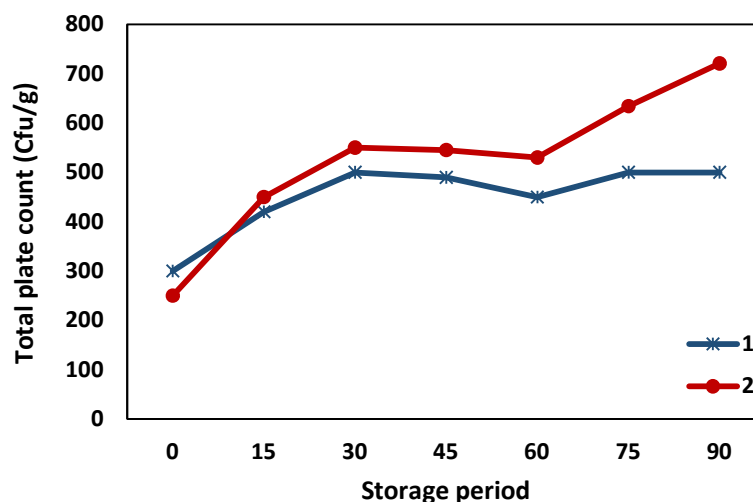

Figure 7. Total plate counts of mesophilic aerobic bacteria of French sauce samples (1: sample with 2000 ppm of OLE and no commercial preservatives; 2: Sample with commercial preservatives , sodium benzoate, 0.063% w/w, and potassium sorbate, 0.012% w/w, without the addition of OLE)

A set of comparative microbiological data are shown in Table 8 for a sample containing 2,000 ppm of OLE and no commercial preservatives and a sample containing sodium benzoate at 0.063%, w/w, and potassium sorbate at 0.012%, w/w, as commercial synthetic preservatives, without the addition of OLE. According to these data, *E. coli* was not detected in either sample during the entire storage period. Also, the number of yeast and molds were similar in both samples (Table 8). Regarding acid tolerant bacteria, specifically lactic acid bacteria, they grew after day 60 of storage in sample 2, while in sample 1, they were not observed during the whole study period. These findings agree with the results of AV of samples in section 3.6 and confirms the protective role of olive leaves polyphenolic content against acid tolerant bacteria, which could have undesirable effects on the samples.

3.7. *Sensory evaluation*

The results of sensory evaluation of French sauce samples with and without the enrichment by OLE at 2,000 ppm and a commercial French sauce are presented in Figure 8. No significant differences were found among the samples in terms of their appearances, colors, and texture. Apparently, this level of OLE does not significantly influence these sensorial attributes. Additionally, the use of olive oil at 50% did not change these parameters either. The sample without OLE showed the lowest score in the odor, but there was no major difference between the samples. But, the commercial product gained the best score for the odor. With regards to the taste scores, the commercial product obtained the best score, while the sample containing OLE had the lowest score, which was due to the intrinsic taste of OLE which could even be perceived at low concentrations. Regarding the overall acceptance scores, the commercial product received the highest average score of 7.2 but the sample without OLE resulted in a score of 6.4, which was significantly different with the sample containing OLE receiving a score of 5.7.
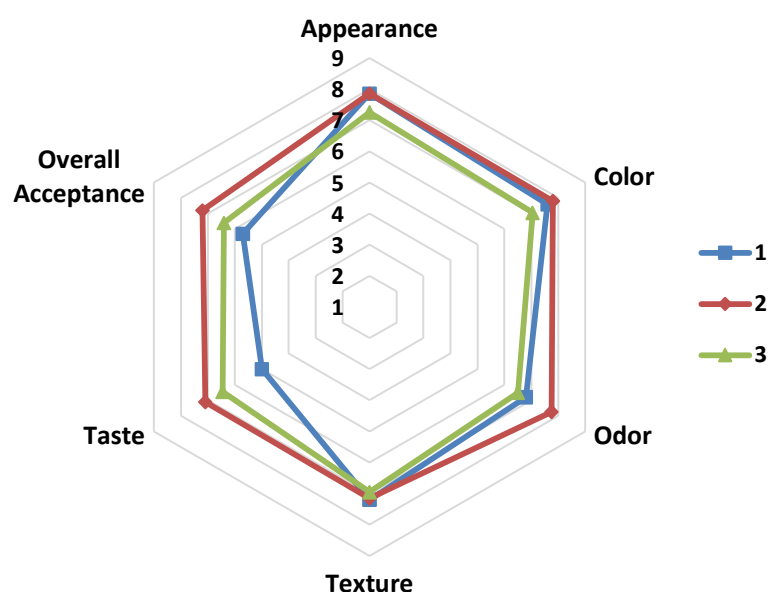
Figure 8. Sensory evaluation of French sauce samples (1: sample with OLE, 2: Commercial French sauce, and 3: Sample without OLE).

4. Conclusion

Major objective of this study was to produce a functional product (French sauce) replacing part of soybean oil, which is currently used in the formulation of such products, with olive oil. Additionally, to improve the health aspects of the sauce, the enrichment of OLE in sauce samples was investigated. Based on the results, there was a slight reduction in the emulsion stability of samples with increasing the amount of olive oil especially at higher levels (75 and 100%). Moreover, the type and amount of oils also affected the color parameters due to the different amount of pigments available in two studied oils. Textural and viscoelastic properties of samples were affected by the addition of olive oil. It was found that the sample prepared with 50% olive oil and 50% soybean oil was the best sample. Regarding the microbiological tests, it was understood that OLE was able to retard the growth of microorganisms during the storage time as well as prevention of the growth of lactic acid bacteria at the end of the studied shelf-life time. So, OLE was found to be a promising alternative to commercial synthetic additives that are usually used in the formulation of sauces. Sensory analysis showed that OLE can influence the taste and odor even at small little amounts, so further studies are needed to mask the intrinsic taste and odor of OLE in commercial food products.

Author Contributions: Karamatollah Rezaei was the principal investigator and in-charge of the entire project. Mohammad Amin Aliyari was the graduate student and carried out the experiments and prepared the draft of the manuscript under the supervision of the principal investigator.

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

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