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Edible Protein Films Containing Bay Leaves (*Laurus Nobilis* L.) and Sage (*Salvia Officinalis*) Extracts: Preventing Oxidation And Application On Refrigerated Cooked Meatballs

Defne Yaprağı ve Adaçayı İçeren Yenilebilir Filmler: Oksidasyonun Engellenmesi ve Soğukta Muhafa Pişmiş Köftelere Uygulanması.

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Abstract

The aim of this study was to determine the effect of whey protein isolate-based edible films incorporated with extracts from bay leaves (D, Laurus nobilis L.) or sage (S, Salvia officinalis.) on oxidative changes of cooked beef patties during refrigerated storage at 2ºC for 7 days. Results showed that TBA values of the patties with S-EF were lower than the TBA values of the patties with EF. Higher antioxidant activity by SA-F than D-EF, S-EF and EF was determined with a 2,2'-diphenyl-2-picrylhydrazyl radical-scavenging assay (DPPH). Total phenolic compound content significantly increased in beef patties with incorporated extracts compared to the EF-counterparts. Incorporation of phytochemicals from D or S into the edible films significantly reduced ($p<0.05$) the extent of lipid oxidation as measured by assorted methods in cooked meatballs. This study suggests that the addition of natural antioxidants from D or S into EFs is an effective strategy for retarding oxidative changes in ready-to-eat meat products.

Keywords: *Edible films, antioxidant activity, bay leaves and sage, meatballs, refrigerated storage*

Öz

Bu çalışmanın amacı, defne yaprağı (D, Laurus nobilis L.) veya adaçayı (S, Salvia officinalis) ekstraktları ile birleştirilen peynir altı suyu proteini izolatı bazlı yenilebilir filmlerin 7 gün boyunca 2°C'de soğutularak saklanan pişmiş dana köftelerinin oksidatif değişiklikleri üzerindeki etkisini belirlemektir. S-EF'li köftelerin TBA değerleri, EF. D-EF'li köftelerin TBA değerlerinden daha düşük olduğu bulunmuştur, SA-F, 2,2'-diphenyl-2-picrylhydrazyl radikal yakalama metoduna (DPPH) göre D-EF, S-EF ve EF gruplarından daha fazla antioksidan aktivite göstermiştir. Toplam fenolik bileşik içeriği, ekstrakt eklenmiş yenilebilir film gruplarında, ekstrakt eklenmemiş yenilebilir film gruplarına göre daha önemli ölçüde artmıştır.

Yenilebilir filmlere (EFs) D veya S'dan elde edilen doğal antioksidan ekstraktlarının dahil edilmesi, pişmiş köftelerde çeşitli yöntemlerle ölçülen lipid oksidasyonunun derecesini önemli ölçüde azaltmıştır ($p<0.05$). Bu çalışma, bir antioksidan aktif paketleme olarak F'lere D veya S EF'lerin eklenmesinin et ve et ürünlerindeki oksidatif değişiklikleri geciktirmede etkili olduğunu göstermektedir.

Anahtar Kelimeler: *Yenilebilir Filmler, Antioksidan aktivite, Defne yaprağı ve Adaçayı, Köfte, Soğuk muhafaza*

1. Introduction

Meat and meat products are one of the most vulnerable food items. Especially, oxidation process is common cause of food deterioration which result in color changes, texture modifications, development of off-flavor, and loss of nutritional value and quality of meat products[1]. The shelf-life has to be extended to fullfill marketing requirements. Therefore, edible films and/or coatings offer a novel method of preserving and packaging these foods.

Edible films and coatings have attracted much interest in recent years because of their advantages, that include ability to be used as edible packaging materials instead of synthetic films[2]. Edible packaging systems could enhance the oxidative stability and storage quality of meat and meat products by reducing the rate of moisture loss, oxidation reactions and colour deterioration[3, 4]. In order to prevent lipid oxidation during meat storage, antioxidant substances such as plant extracts or essential oils have been integrated into edible films and coatings. [4, 5]. Protein based edible films incorporated with oregano essential oils (EOs), pimiento or a mixture improved oxidative stability of beef muscle [6]. Maltodextrin based calcium alginate edible films were developed by Kalem, Bhat [5] by using an extract from *Terminalia arjuna*.

Sage (*Salvia officinalis*) is a variety of aromatic herb which has been planted widely throughout much of the world. It is not only used as a raw material in the pharmaceutical and cosmetic industries but also used to improve flavors of foods[7]. Sage's antioxidant effect was attributed to phenolics such carnosic acid and rosmarinic acid [1, 8]. Bay leaves (*Laurus nobilis* L.) is native to the Mediterranean region, currently, the plant is cultivated in many Mediterranean countries. Bay leaves are strongly aromatic and in many countries used in soups, stews, sauce, pickles, sausages, and also an essential ingredient of the herb mixes. Bay leaves have attracted renewed interest because of the antimicrobial [9-12] and antioxidant properties [13, 14]. Sage has been shown to antioxidant properties by many researchers. [1, 15] and bay leaves in meat products [12, 16, 17]. However, only limited data exist on the application of antimicrobial edible films incorporated with extracts in real food systems.

The focus of this research was to detect the effects of whey protein isolate (WPI) films combined with bay leaves (D, *Laurus nobilis* L.) and sage (S, *Salvia officinalis*.) extracts on oxidative stability of cooked meatballs during refrigerated storage.

2. Material and methods

2.1. Materials

WPI (98% protein content) was provided by Davisco Foods International Inc. (BiPRO®, Le Sueur, MN, USA). Chemicals were acquired from Merck Co. (Darmstadt,Germany). Bay leaves (*Laurus nobilis L.*) and sage (*Salvia officinalis.*) were supplied from Defne Dış Ticaret ve Tarım Ürünleri A.Ş.Izmir, Turkey. All materials were food grade quality. Minced beef rib meat was obtained from a commercial slaughterhouse.

2.2. Extract preparation

Dried bay leaves and sage extracts were prepared according to the method described by Akcan, Estévez [3] and Total phenolics' of concentrated extracts were previously shown as gallic acid equivalent for bay leaves (81.68 mg/100 g GAE) and sage (63.49 mg/100 g GAE)[3].

2.3. Whey protein isolate-based film preparation

Edible films (F) were prepared according to the method described by Akcan, Estévez [3]. Following that films were peeled and stored in a vacuum desicator for onward use.

2.4. Patty Preparation and Film Application

Patties prepared and cooked as explained by Akcan, Estévez [3] then cooked patties were divided into six treatments. Treatment groups consisted of 1) control samples (K), 2) samples coated with films without addition of extract (EF), 3) samples coated with 2% D extract added films (D2-EF), 4) samples coated with 4% D added films (D4-EF), 5) samples coated with 2% S added films (S2-EF) and 6) samples coated with 4%S added films (S4-EF).

Patties were placed into petri dishes and film material prepared as described above were applied upper and bottom surfaces of the petri dishes except control (K). Samples stored in closed petri dishes at 2 ± 1 ºC for 7 days and sampled on days of 1, 4 and 7 for further analyses. 30 samples were used for each treatments during the storage time.

2.5. 2, 2-Diphenyl-2-Picrylhydrazyl Radical-Scavenging

2,2-Diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay was employed was used in accordance with the procedure given by Akcan, Estévez [3].

2.6. Total phenolics

The phenolic content of both the extracts and the meat products was determined using a modified Folin–Ciocalteu method of that described by [3], Escarpa and González [18].

Results were given as milligrams of Gallic acid equivalent (GAE) per milliliter of extract (mg GAE mL^{-1}).

2.7. Peroxide value (PV)

Peroxide value was determined according to AOAC method 965.33 AOAC (2007) and expressed as meq O_2/kg lipid.

2.8. Conjugated dienes

Conjugated dienes were determined according to the method described in details by [3]. Results were reported as absorbance $E^{1\%}_{1cm}$, which was given by the formula: $E^{1\%}_{1cm}(234)$: Aλ × (C*d)-1, where "Aλ" is the absorbance at 234 nm, "C" the concentration of the lipid solution in iso-octane (g 100 ml^{-1}) and "d" is the length of the cell (cm).

2.9. p-Anisidine value (P-Av)

p-Anisidine value was determined by the IUPAC (1989). Extracted lipid (0.5 g) was dissolved in 25 mL n-hexane and absorbance of mixture was measured at 350 nm using a UV–vis spectrophotometer (A1). p-Anisidine reagent was added to 5 mL of the mixture and held in dark for 10 min before absorbance reading (A2) at the same wavelength. The value of p-Av was calculated using the following:

$$p - Av = \frac{25(1.2(A2-A1))}{Sample\ weight} \quad (1)$$

2.10. Thiobarbituric Acid Value

TBARS were determined according to extraction method described by [3]. TBARS numbers were calculated as mg of malonaldehyde per kg of meat (mgMA kg-1 meat).

2.11. Color measurement

Total color differences (ΔE∗) values of samples were measured using CIE L ∗, a ∗, b ∗ values on each storage day. Color Flex A60-1010-615 Manual Version 23 Hunter Lab spectrocolorimeter equipped with the light source illuminant D65 (10° standard observer) was used for measurement.

2.12. Statistical analysis

The study was repeated two times, and the measurements of each measured parameter were carried out in duplicate. Mean values for measured parameters were calculated and compared by analysis of variance using the SPSS software for windows (SPSS 21.0 for Windows; SPSS Inc. Chicago, IL, USA). Storage data values were analyzed using two-way ANOVA with treatment and storage time as main effects. Significant differences between the average means were tested using the Duncan test. Differences among mean values were considered significant when $P < 0.05$. The average values were reported along with standard error (± standard error).

3. Results

3.1. Total phenolics and antioxidant activity results

Overall changes in total phenolics during cold storage of patties are seen in Table 1. On the first day of the storage phenolics content was significantly ($p<0.05$) higher in S4-EF, S2-EF, D4-

EF treatments and D2-EF than in EF and K samples. These results indicate that treatments with S and D extracts had significantly ($p<0.05$) higher phenolics content during storage period. The overall changes in total phenolics indicated a significant ($p<0.05$) decrease during the storage. At day 7 the highest total phenolics content was found in D4-EF, S2-EF and S4-EF groups.

The DPPH assay is used to predict antioxidant activity based on the process by which antioxidants prevent lipid oxidation, resulting in DPPH radical scavenging and further determining free radical scavenging capacity. The antioxidant activity of samples against the DPPH (%D) ranged from 45.55 to 92.02%. Meatball samples were significantly ($p<0.05$) affected by the WPI. Especially LA2-WPF and LA4-WPF groups showed higher antiradical activity than the other groups.

Table 1. Effects of edible films on total phenolic compound (mg GAE $100g^{-1}$) of patties during refrigerated storage*

	Storage Period (Day)		
	1	4	7
Treatment		DPPH (%D)	
K	64.35±2.09^{eA}	51.99±1.75^{cB}	49.29±0.7^{eC}
EF	70.71±2.96^{dA}	55.02±3.53^{cB}	45.55±1.82^{dC}
D2-EF	75.81±3.34^{cA}	65.59±4.05^{bB}	57.3±2.09^{cC}
D4-EF	82.23±2.26^{bA}	65.43±2.97^{bB}	65.22±3.94^{bB}
S2-EF	89.35±2.1^{aA}	75.76±2.99^{aB}	68.04±1.74^{bC}
S4-EF	92.02±1.45^{aA}	80.4±4.69^{aB}	73.75±1.54^{aC}
Treatment		Total Phenols	
K	120.53±2.89 ^{eA}	44.90±1.95^{eB}	22.13±3.69^{dC}
EF	122.99±9.64 ^{eA}	48.68±3.87^{eB}	29.37±3.09^{cC}
D2-EF	155.99±4.35 ^{dA}	124.60±7.44^{dB}	60.05±2.01^{bC}
D4-EF	173.41±5.94 ^{cA}	156.34±4.31^{cB}	84.60±5.02^{aC}
S2-EF	229.21±8.79^{bA}	196.96±5.77^{bB}	82.26±2.19^{aC}
S4-EF	264.70±12.97^{aA}	210.02±4.41^{aB}	80.36±2.02^{aC}

*The mean ± standard error.

$^{a-e}$Treatments within the same storage period with the same superscripts are not different. $^{A-C}$Storage periods within the same treatment with the same superscripts are not different. K: Control (no film coated) EF: Samples coated with films without addition of extract. D2-EF: Samples coated with 2% D extract added films. D4-EF: Samples coated with 4% D added films. S2-EF: Samples coated with 2% S added films. S4-EF: Samples coated with 4% S added films.

3.2. Peroxide Value

PVs and CDs indicate the primary oxidation products. Mean PVs (Table 2) were significantly ($p<0.05$) higher in C, followed by F group. Treatments with extract added films had significantly lower PVs. The lowest PV was found in S4-EF samples. During all storage periods PV

was significantly higher in control and F samples. The PV of the control increased until 4th day and decreased thereafter. In other samples PVs decreased with increasing storage days.

3.3. Conjugated diene (CD) value

The evaluation on the results from CD analyses (Table 2) showed that all of the samples presented significant increases ($P < 0.05$) during 4 days of refrigeration and then decreased until the end of storage.

3.4. para-Anisidine value (p-Av)

One of them is p-anisidine which is used for aldehydes (secondary products) generated by the breakdown of peroxides (Table 3). As it could be seen on the Table p-anisidine value was not significantly different ($p>0.05$) between F, S2-EF and S4-EF groups at day 1. On the contrary D2-EFand D4-EFgroups had significantly ($p < 0.05$) lower p-anisidine value at day 1 among the all groups. It is showed that bay leave extract was effective on p-anisidine value. Also coating with edible films effected ($p<0.05$) p-anisidine values of cooked beef patties during the storage. Regarding the cooked samples, heat treatment caused a decrease in peroxide concentration, which were then transformed into secondary oxidation products. This resulted in an increase of the p-anisidine value

Table 2. Effect of whey based films prepared with bay leave or sage extracts on peroxide (meq O_2 kg^{-1}) and conjugated diene (absorbance $E_{1cm}^{1\%}$) values of cooked meatballs during refrigerated storage.*

	Storage Period (Day)		
	1	4	7
Treatment		Peroxide value	
K	12.55± 0.93^{aC}	33.10± 1.83^{aA}	24.83± 2.95^{aB}
EF	10.39± 0.73^{bC}	20.75± 0.80^{bA}	17.24± 1.76^{bB}
D2-EF	3.65±0.30^{cdB}	8.22±0.40^{dA}	8.32±0.22^{cA}
D4-EF	2.90±0.26^{dC}	4.60±0.32^{eB}	8.33±0.18^{cA}
S2-EF	3.72±0.21^{cC}	12.12± 1.12^{cA}	7.63±0.73^{cB}
S4-EF	1.70±0.17^{eC}	8.93±0.64^{dA}	6.56±0.87^{cB}
Treatment		CD value	
K	12.59±0.98^{bC}	28.19±1.28^{aA}	15.41±0.56^{aB}
EF	11.63±0.46^{bC}	22.41±1.47^{bA}	14.42±1.11^{aB}
D2-EF	11.84± 0.20^{bB}	20.78±1.00^{bcA}	7.37±0.45^{bcC}
D4-EF	11.29±0.93^{bB}	14.89±1.68^{dA}	6.13±0.78^{dC}
S2-EF	14.14± 1.78^{aB}	19.75± 1.82^{cA}	8.37±0.68^{bC}
S4-EF	11.79± 0.77^{bA}	13.82± 2.12^{dA}	7.03±0.77^{cdB}

*The mean ± standard error.

$^{a–e}$Treatments within the same storage period with the same superscripts are not different. $^{A–C}$Storage periods within the same treatment with the same superscripts are not different. K: Control (no film coated) EF: Samples coated with films without addition of extract. D2-EF: Samples coated with 2% D extract added films. D4-EF: Samples coated with 4% D added films. S2-EF: Samples coated with 2% S added films. S4-EF: Samples coated with 4% S added film

3.5. TBA

TBARS value is an important indicator for warmed over flavor (WOF) in meat products which is unwelcomed flavor for the consumers. The threshold for WOF in cooked meats ranges 1.0-2.0 (mg MDA /kg sample), but of course it depends on a number of factors. TBA values over 7 days of refrigerated storage are shown in Table 3. TBARS values for all treatments were significantly ($p<0.05$) lower than those for the control during the storage period. S2-EF and S4-EF were the most effective ($p<0.05$) at reducing the values of TBA, followed by D2-EFand D4-EF. Also compared to control, F group had significantly lower TBARS values during storage ($p<0.05$). This result indicates that lipid oxidation was effectively retarded by films and extract added films during and immediately after cooking.

No significant difference ($P > 0.05$) in L* value was observed between the treatments at day 1 (Table 4) except for D2-EFtreatment. There was an increase ($P < 0.05$) in lightness at the end of the storage period in all treatments. BL2-F, D4-EFand S4-EF had the lowest L* values ($P < 0.05$) at the end of storage (day 7).

A general decrease was observed in a* value for cooked patties treated with extract added films ($P < 0.05$). At end of the storage, samples coated with extract added films had lower a* values compared with C and F groups (Table 4). Especially D4-EFand S4-EF groups had the lowest a* values which means that the concentration of NAEs had a decreasing effect on a* value.

Storage period had no effect on b* values of patty samples ($p<0.05$) (Table 4). S4-EF group had higher b* values at end of storage ($p<0.05$).

3.6. Instrumental Color

Table 3. Effect of whey based films prepared with bay leave or sage extracts on para-anisidine (p-av) and TBA (mg MDA kg^{-1}) value of cooked meatballs during refrigerated storage.*

	Storage Period (Day)		
	1	4	7
Treatment		p-AV	
K	4.04±0.54^{aC}	9.51±0.26^{aB}	19.76± 1.26^{aA}
EF	2.86±0.37^{bC}	6.59±0.61^{bB}	17.37± 0.45^{bA}
D2-EF	1.78±0.14^{cC}	2.75±0.09^{eB}	14.70± 0.44^{cA}
D4-EF	1.61±0.21^{cC}	3.29±0.05^{dB}	16.65± 0.57^{bA}
S2-EF	2.46±0.03^{bC}	4.17±0.10^{cB}	14.64± 0.56^{cA}
S4-EF	2.00±0.14^{bC}	2.63±0.44^{eB}	11.10± 0.48^{dA}
Treatment		TBA	
K	0.32±0.13^{aB}	0.79±0.05^{aA}	0.96±0.13^{aA}
EF	0.17±0.02^{bC}	0.63±0.06^{bB}	0.88±0.08^{abA}
D2-EF	0.13±0.02^{bC}	0.54±0.06^{cB}	0.76b±0.06^{cA}
D4-EF	0.16±0.02^{bC}	0.56±0.05^{cB}	0.79±0.02^{bA}
S2-EF	0.15±0.01^{bC}	0.38±0.05^{dB}	0.65±0.10^{cA}
S4-EF	0.14±0.04^{bC}	0.40±0.03^{dB}	0.65±0.04^{cA}

*The mean ± standard error.

[a–e]Treatments within the same storage period with the same superscripts are not different. [A–C]Storage periods within the same treatment with the same superscripts are not different. K: Control (no film coated) EF: Samples coated with films without addition of extract. D2-EF: Samples coated with 2% D extract added films. D4-EF: Samples coated with 4% D added films. S2-EF: Samples coated with 2% S added films. S4-EF: Samples coated with 4% S added film

4. Discussion and Conclusion

4.1. Total phenolics and antioxidant activity results

Matkowski, Zielińska [19] reported a total phenolic material content in sage extracts consistent with that from the present study (62.2 mg GAE 100 g-1). The concentration of phenolic compounds in bay leave extracts reported by Hinneburg, Damien Dorman [20] is also comparable to our results (92 mg GAE 100 g-1). Small differences may result from differences in the plant variety, extraction time, temperature, solvent, phenolic acid equivalent and extraction method [20, 21]. Similar to our research, Leheska et al. (2006) observed an increase in total phenolics of precooked pork breakfast sausages prepared with fruit purees. The presence of phenolic compounds in meat samples without added plant extracts may be attributed to the phenolic structures of aromatic amino acids. Devatkal, Narsaiah [10] observed a decrease in phenolic contents of raw chicken patties prepared with kinnow rind powder and pomegranate rind powder and pomegranate seed powder. Decrement in total phenolic content in chicken patties at 4th day of storage was also reported by Devatkal, Narsaiah [10]. Park [22] indicated the ability of films to maintain the functionality of some compounds (plasticizers, antimicrobials, antioxidants) within the matrix. The loss observed may be explained by the degradation of these compounds in assorted reactions including redox reactions in which these compounds may neutralize reactive oxygen species generated during meat storage.

Contrary to our results, Koşar, Dorman [23] reported that methanol extracts with acid treatment from LA and SA had DPPH radical-scavenging activity, with SA possessing higher antioxidant activity. Although few studies are available in the literature regarding the measurement of DPPH value in meat products, Akcan, Estévez [3] reported that cooked meatballs coated with laurel and sage leaves extracts incorporated edible films had higher %D values compared to the control group ($p<0.05$). Fasseas, Mountzouris [15] noted that addition of SA EOs (3%) to porcine ground meat samples in the cooked state and to bovine ground meat samples in the raw state significantly reduced the antioxidant activity at the end of storage at 4˚C which agreed our results. The studies by Mielnik, Olsen [24] also indicate that addition of the grape seed extract which has a high cafeic acid concentration to cooked turkey meats increased the anti radical scavenging activity as compared to control groups. Hinneburg, Damien Dorman [20] studied antioxidant activity of hydrodistilled extracts of herbs and spices. They reported that extracts obtained from LA displayed the highest antioxidant activity in the ABTS and DPPH radical scavenging assays than in the iron-chelating assay. These results may indicate that phytochemicals from LA may exert antioxidant activity through a radical scavenging activity rather than through a metal-chelating activity.

4.2. Peroxide value

The permissible upper limit for lipid peroxides in oil or fat is 5–10 mmol/kg[25]. Other than oil and lipids, peroxide limits are rarely set[26].El-Alim, Lugasi [27] found that the EOs of L. nobilis obtained from hydrodistillation had a notable inhibitory effect on the linoleic acid peroxidation. In analyzed patties, after 7 days of storage a varied and disproportional increase in the levels of both PV and AV was observed. This may indicate that the rate of hydroperoxide decomposition was higher than the rate of formation at that point of storage [28]. Similar to our results, Karpińska, Borowski [29] reported that several spices and sage added to fried turkey meatballs showed decreased PVs after 4 days of storage at 4˚C. Previous reports showed that whey-coated sausages had a lower PV than the controls [30] which agreed with our results. Similar to our study Coskun, Calikoglu [31] investigated that soy based edible films with oregano or thyme EOs incorporated effects on ground beef patties and they found that incorporated films had lower PVs as compared to control.

4.3. Conjugated diene (CD) value

Almost immediately after peroxides are formed, the non-conjugated double bonds (C=C–C–C=C) that are present in natural unsaturated lipids are converted to conjugated double bonds (C=C–C=C). The formation of conjugated diene, which parallels the production of hydroperoxides, occurs in the early stages of lipid oxidation and conjugated hydroperoxides are expected to decompose to the secondary products (Choe et al., 2011). Our results agreement with previous studies, in which it was reported that the concentration of CD significantly increased in cooked pork patties (Juntachote et al., 2006; Lee et al., 2010), and in cooked chicken meat treated with antioxidants over the refrigeration period [32]. The results from the present study suggest that edible films added sage and bay leaves extracts applied to patties were able to increase the resistance to oxidation, in comparison with the C and F groups, as shown by the lower conjugated diene contents presented in antioxidant groups at day 4th. CD hydroperoxides are expected to decompose to secondary products that have not the ability of UV light absorption or cause reduction in absorptivity. In the present study, this was also observed.

4.4. para-Anisidine value (p-Av)

During lipid oxidation, hydroperoxides, the primary reaction products, decompose to produce secondary oxidation products (aliphatic aldehydes, ketones, alcohols, acids and hydrocarbons) which are more stable during the heating process, responsible for off-flavors and off-odors of edible oils [33]. In order to ensure a better monitoring of lipid oxidation process in the heating time, the simultaneous detection of primary and secondary lipid oxidation products is necessary. The effect of cooking treatments on the oxidation of lipids was evaluated using reliable oxidation parameters. . One of them is p-anisidine which is used for aldehydes (secondary products) generated by the breakdown of peroxides. Regarding the cooked samples, heat treatment caused a decrease in peroxide concentration, which were then transformed into secondary oxidation products. This resulted in an increase of the p-anisidine value.

4.5. TBA

This result indicates that lipid oxidation was effectively retarded by films and extract added films during and immediately after cooking. Chitosan and chitosan/sodium tripolyphosphate solutions containing β-carotene on ground beef patties had negligible TBA value as compared to controls [34] . Oussalah, Caillet [6] noted that milk protein-based films containing 1% EO of oregano and pimento applied on beef muscle slices resulted in either higher or statistically insignificant, which disagreed with our results. Their results of the TBARS analysis showed that the incorporation of EOs in films did not improve the protection of the meat samples against lipid oxidation. Nevertheless, it is known that pimento oil is rich in phenolic compounds such as eugenol, which are unstable and could generate phenolic aldehydes. These aldehydes could provoke a similar reaction to malonaldehyde that is determined by the TBARS method, and would probably increase the obtained values, i.e. mask the antioxidant effect of EOs [35].

A similar positive effect of sage or sage extract in meat systems has been reported by others. Sage reduced the TBARS values in raw and cooked pork patties [36] and also in patties which preapared from fresh and frozen pork meat [37] during storage at +4°C. Addition of 3% (w/w) sage EO efficiently inhibited lipid oxidation in raw pork and in cooked bovine meat [15]. Estévez, Ramírez [38] showed that 0.1% EO from sage was more effective in inhibiting the generation of MDA in liver pâté during 90 days of storage at 4 °C than 0.02% BHT. Also addition of 0.05, 0.10 and 0.15% (w/w) sage in Chinese sausage had lower TBA values as compared with controls [39]. Our results are in agreement with these previous results, indicating that sage and bay leaves can be used as a natural antioxidant addition for edible films which used for inhibition of oxidation in meat products. The compounds in sage showing the greatest antioxidant activity have been identified as carnosol, rosmarinic acid, and carnosic acid, followed by caffeic acid, rosmanol, rosmadial, genkwanin, and cirsimaritin [40]. Politeo, Jukić [41] identified eugenol and methyl as the main contributors to the antioxidant activity of L. nobilis.

4.6. Instrumental Color

As reported by Coskun, Calikoglu [31] the reason of this increasing might be lightening effect of edible films on cooked meatballs, also observed in the present study. Similar to our findings, Zhang, Lin [39] reported chinese sausage treated with 0.15% sage and stored at 4°C for 21 days had lower L* value compared with control samples at the end of storage period. The lower L* values of BL2-F, D4-EFand S4-EF groups may have resulted from the presence of pigment materials which sourced from the plant itself, such as chlorophylls [42].

Mc Carthy, Kerry [37] reported that SA extract resulted in lower a* values when applied to patties manufactured from previously frozen pork as compared with the control over 6-day refrigerated storage. Ganhão, Estévez [43] noted that the decrease of redness was significantly affected by the addition of the fruit extracts. Similar results were reported by Coskun, Calikoglu [31] who observed had lower a* values for ground beef patties wrapped in edible films incorporated with thyme and oregano EOs.

4.7. Conclusion

In the present study, D and S showed intense antioxidant activity as determined in vitro with total phenolic content and anti-radical activity against the DPPH. These two NAEs, when incorporated into whey protein based edible films, resulted in lower peroxide and CD value, particularly at later periods of refrigerated storage, with significant effect on TBARS and p-anisidine value, indicating that antioxidant F application on cooked beef patties surface may control lipid oxidation and its negative consequences on a ready-to-eat meat product. It is concluded that extracts of these plants could be successfully added to edible films to function as antioxidant. The antioxidant effect of phenolic compounds naturally present in the extracts may also be joined to the oxygen barrier effect and further antioxidant protection of the edible films. The results support the use of whey protein – bay leave and sage NAE films as packaging material may have potential to extent the shelf life of oxidation-susceptible products such as cooked beef patties in refrigerated storage time. As promising as these results are, additional research will be required to improve the present application to study the combined application of bay leave and sage in different meat products as well as the use of different quantities to those used in this study for optimization of the antioxidative effects.

5. Author Contributions

Tolga AKCAN: Designed and conducted the experiments and analyzed the data.

Fatma Meltem SERDAROĞLU: Drafted and finalized the manuscript.

6. Conflicts of Interest

The authors declare no conflict of interest and no need for an ethics committee approval in this paper.

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