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RESEARCH ARTICLE

Investigation of the Possible Use of Black Cumin (*Nigella sativa* L.) Essential Oil on Butter Stability [1]

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[1] *Some results of this study was presented as a poster in "9th Euro Fed Lipid Congress, September 18-21, 2011, Rotterdam, Netherland"*

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Summary

The aim of this study was to investigate the effect of black cumin (*Nigella sativa* L.) essential oil on butter stability. For this aim, 0.05, 0.1 and 0.2 wt-% essential oil was added to the butter. Antioxidant activity of the essential oil was compared to that of synthetic antioxidant BHT (100 ppm). All samples were stored at 4±1°C for 90 days. Thiobarbituric acid and peroxide values of all samples containing essential oil were decreased depending on concentrations. The amount of 0.2% of essential oil had showed strong antioxidant activity, which was almost equal to that of BHT. The addition of essential oil reduced total aerobic mesophilic bacteria, lactic acid bacteria and coliform bacteria count during storage period but did not show remarkable antifungal activity. Samples containing essential oil were preferred by the panellists compared to Control sample. Present results indicate that black cumin essential oil may be considered as a new source of natural antioxidant.

Keywords: *Antioxidant, Antimicrobial effect, Butter stability, Essential oil, Nigella sativa* L.

Tereyağı Stabilitesi Üzerine Çörekotu (*Nigella sativa* L.) Uçucu Yağı Kullanılabilirliğinin Araştırılması

Özet

Bu araştırmanın amacı, çörekotu (*Nigella sativa* L.) uçucu yağının tereyağının stabilitesi üzerine etkini araştırmaktır. Bu amaç için üretimden hemen sonra tereyağına 0.05; 0.1 ve 0.2 (ağırlık/%) çörekotu uçucu yağı ilave edildi. Uçucu yağın antioksidan aktivitesi sentetik antioksidan BHT (100 ppm) ile karşılaştırıldı. Tüm örnekler 90 gün süresince 4±1°C 'de muhafaza edildi. Uçucu yağ içeren tüm örneklerin tiyobarbitürik asit ve peroksit değerleri, konsantrasyonlara bağlı olarak azaldı. %0.2 seviyesindeki uçucu yağ ilavesi, BHT ile hemen hemen eşit güçte antioksidan aktivite gösterdi. Uçucu yağ, toplam aerobik mezofilik bakteri, laktik asit bakteri ve koliform bakteri sayılarını depolama süresince azaltmış, ancak dikkate değer bir antifungal aktivite göstermemiştir. Uçucu yağ içeren örnekler kontrol örnek ile karşılaştırıldığında panelistlerce tercih edilmiştir. Sonuçlar, çörekotu uçucu yağının, yeni bir doğal antioksidan kaynağı olarak kabul edilebilir olduğunu göstermiştir.

Anahtar sözcükler: *Antioksidan, Antimikrobiyal etki, Tereyağı stabilitesi, Uçucu yağ, Nigella sativa* L.

INTRODUCTION

Butter, a food consumed all over the world, is used both directly and as an ingredient in a variety of dairy products [1]. Lipids, which are important macromolecules in foods, affect nutritional value, texture, flavour and shelf life of a product [2]. On the other hand, all fat and fat containing foods are vulnerable to oxidative deterioration, which reduce both nutritional quality and makes the food unacceptable for consumers [3]. It is reported that the oxidation of lipids is related to human diseases like cancer, heart diseases, membrane damage and ageing, so that antioxidants are used in foods to control and delay oxidation [4]. Use of natural antioxidants instead of synthetic

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ones has become popular as consumer awareness about chemical additives increased [5,6]. That is why researchers have been focusing on new natural sources that have high antioxidant activity and harmless for health to prolong shelf-life and to increase food stability [7-9].

Nigella sativa L. belongs to Ranunculaceae family, which is commonly known as "black cumin" in English and "çörekotu" in Turkish. The genus *Nigella* L. includes about 20 species [10] from the Mediterranean to Western Asia and 12 species in Turkey [11]. Black cumin (*Nigella sativa* L.) seeds have been used as a spice and the seeds or essential oils have been widely used in traditional medicinal applications [12,13]. Black cumin seed has over 100 different chemical constituents, including abundant sources of all the essential fatty acids [14]. The seeds contain essential oils, fixed oils, proteins, phospholipids, saponin and other some components [12] Essential oils, which are secondary metabolite products of aromatic plants, are strong odorous, volatile and natural complex compounds [15]. Many components are identified from the essential oil of *N. sativa* L. But the major ones are thymoquinone (27.8%-57.0%), *p*-cymene (7.1%-15.5%), carvacrol (5.8%-11.6%), *t*-anethole (0.25%-2.3%), 4-terpineol (2.0%-6.6%) and longifoline (1.0%-8.0%) [16].

Although several studies on the antimicrobial and antioxidant properties of members of *Nigella* L. genus are carried out [17-22] there are a few studies published on the use of *N. sativa* species in butter production. The aim of this study is to investigate the effects of *N. sativa* essential oils at three concentrations (0.05, 0.1 and 0.2 wt-%) on butter stability as an antioxidant according to Control and with BHT samples, and on sensorial properties as well.

MATERIAL and METHODS

Materials

Nigella sativa L. seeds were purchased from local markets in Erzurum province in the Eastern Anatolia region of Turkey. Sour raw cream used in the butter production was provided by the Leben Dairy Products Plant located in Erzurum, Turkey.

Methods

Isolation of the Essential Oils and GC-MS Analysis Conditions

The essential oils in mature seeds were subjected to steam distillation using a Clevenger-type apparatus for 4 h. The oils were extracted with $CHCl_3$ and then dried over anhydrous Na_2SO_4 and stored under N_2 atmosphere at 20°C in a sealed vial until use. The essential oil was chemically analyzed and identified by GC and GC-MS. The analysis of the essential oil was performed using a Thermofinnigan Trace GC/Trace DSQ/A1300 (EI quadrupole) equipped with an SGE-BPX5 MS capillary column (30 m x 0.25 mm i.d.. 0.25 mm). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium was the carrier gas, at a flow rate of 1 mL/min. Injector and MS transfer line temperatures were set at 220 and 290°C, respectively. The program used was 50 to 150°C at a rate of 3°C/min, held isothermal for 10 min, and finally raised to 250°C at 10°C/min. Diluted samples (1:100 v/v, in methylene chloride) of 1.0 mL were injected manually in splitless mode. The components were identified based on the comparison of their relative retention times and mass spectra with those of standards provided in Wiley7N library data of the GC-MS system, and data provided in the literature [23]. The results were also confirmed by comparison of the elution order of the compounds with their relative retention indices on non-polar phases reported in the literature.

Preparation of Butter Samples

The butter samples were manufactured from commercial cow milk cream under controlled condition in the Leben Dairy Plant (Erzurum, Turkey). The cream was not pasteurized to see antimicrobial effect. After butter samples were heated at 32°C, they were divided into five parts. *N. sativa* essential oil was added at 0.05, 0.1 and 0.2 wt-% levels to the first three samples, and coded NS1, NS2 and NS3, respectively. 100 ppm BHT was added to the fourth sample. The fifth sample was the Control sample which did not contain any essential oil or BHT. All samples were individually stirred carefully and homogeneously for 5 min at 40°C. Then each sample were divided into 250 g packages and stored at 4±1°C for 90 days until analysis. All experiments were performed in duplicate.

Chemical Analysis

The thiobarbituric acid (TBA) value was determined to a modified version method according to Rossell [24] of the method of Tarladgis et al.[25] and Ockerman [26]. The TBA value was calculated by using a standard curve [8]. The TBA value was the estimated as mg malonaldehyde/kg butter.

The peroxide value (PV) was determined according to the method described by Atamer [27] and Dagdemir et al.[8]. PV is expressed as milliequivalents (meq) of active oxygen per kg of butter.

Titratable acidity (lactic acid, %) was determined as suggested by Kurt et al.[28].

Microbiological Analysis

For microbiological analyses, 10 g of butter sample was weighed aseptically in 90 mL of 0.1% peptone plus 0.85% (wt/v) NaCl solution and homogenized in a sterile polyethylene bag by using a Stomacher (Mayo HG400 Stomacher, Italy) for 2 min. Serial dilutions were prepared from this solution [29]. The enumeration of total aerobic

mesophilic bacteria (TAMB) (Plate Count Agar, Merck, Darmstadt, Germany) at 30°C for 72 h [30], total coliforms (VRBA) (Violet Red Bile Agar, Merck, Darmstadt, Germany) at 35°C for 24 h [30], yeasts and moulds (Potato Dextrose Agar, Merck, Darmstadt, Germany; acidified 10% tartaric acid) at 25°C for 5 d [29], lactic acid bacteria (LAB) (de Man Rogosa Sharp Agar, Merck, Darmstadt, Germany) at 30°C for 72 h [31], were performed. All determinations were made in duplicate.

Sensory Analysis

The sensory properties of the butter samples were evaluated according to the method suggested by Bodyfelt et al.[32]. Five sensory attributes containing odour, taste, colour, texture, and general acceptability were evaluated according to a 1- (poor) to 9-point (excellent) scale by eight trained panellist. For this aim samples were removed from the refrigerator about 1 h prior to evaluation and kept at room temperature. Warm water and bread were also provided to the panellists to cleanse their palates between samples.

Statistical Analysis

The experimental design consisted of a completely randomized design in a factorial arrangement: Five treatments (butter samples: C, NS1, NS2, NS3, BHT), four storage periods (2, 30, 60, 90 days), and two replicates. Statistical analysis of the data was made using the analysis of variance (ANOVA) of the SPSS program, version 10.0.1. Means with a significant difference were compared by Duncan's multiple range tests. All analyses were made in duplicate.

RESULTS

The chemical composition of essential oil used in our study was *p*-cymene (17.40%), carvacrol (16.94%), thymoquinone (12.33%), borneol (11.55%), thymol (6.61%), linalool (5.21%), terpinen-4-ol (3.49%), β-caryophyllene (3.02%), ascoridole (2.61%), 4-terpineol (2.39%), thymol methyl ether (1.61%), α- thujene (0.56%).

TBA, peroxide and titratable acidity values of the butter samples are shown in *Table 1*. As PV and TBA values could not be determined on 2 d of storage, acidity values for the same period are not given as well. As the essential oil concentration is increased acidity values raised *(Table 1)* On the other hand, this increase was lower than the Control but higher than BHT containing sample. The primary lipid oxidation is widely measured with PV in fats, oils and fat containing foods [33]. As seen in *Table 1*, when oxidative stability of *N. sativa* L. essential oil at three concentrations were compared with the Control sample, especially, NS2 and NS3 essential oil concentrations were effective on reducing the oxidation level of butter samples. TBA values of butter samples are shown in *Table 1*. Generally, TBA values of all samples increased during the storage.

The effects of antioxidant addition and storage periods on some microbiological properties of butter samples are shown in *Table 2*. The TAMB counts decreased in all samples during storage period. TAMB counts of all samples were similar statistically on 2 and 30 days of storage. But the differences between samples were started to seen on 60 day of storage. The LAB counts of butter samples decreased during the storage period. While until 60 day of storage there were no statistical differences between samples, essential oil and BHT containing samples were lower than control sample at the end of the storage. As seen *Table 2*, while the coliform bacteria count decreased, yeast-mould count increased during the storage periods in all samples. Arici et al.[19] tested the antibacterial activity of five *N. sativa* essential oils from different regions of Turkey on some spoilage and/or pathogenic bacteria and LAB. Researchers reported that the antibacterial effects of the samples may be closely related to their high percentage

Table 1. *Effect of treatments and storage periods on acidity (lactic acid %), PV (meq O_2/kg) and TBA (mg malonaldehyde/kg) in samples of butter*
Tablo 1. *Tereyağı örneklerinde TBA (mg malonaldehyde/kg), PV (meq O_2/kg) ve asitlik (% laktik asit) üzerine depolama periyodu ve muamelelerin etkisi*

Variants	Storage Period (days)	Butters[1]				
		C	NS1	NS2	NS3	BHT
Acidity	30	0.44 ± 0.01^{aB}	0.41 ± 0.00^{abB}	0.43 ± 0.02^{aB}	0.45 ± 0.01^{aB}	0.39 ± 0.00^{bB}
	60	0.52 ± 0.03^{bB}	0.48 ± 0.01^{bAB}	0.52 ± 0.06^{bAB}	0.63 ± 0.02^{aA}	0.48 ± 0.03^{bA}
	90	0.82 ± 0.05^{aA}	0.56 ± 0.07^{bcA}	0.60 ± 0.00^{bcA}	0.67 ± 0.02^{bA}	0.55 ± 0.02^{cA}
PV	30	0.22 ± 0.03^{aC}	0.13 ± 0.02^{bC}	0.13 ± 0.01^{bC}	0.09 ± 0.01^{bC}	0.14 ± 0.04^{bC}
	60	0.72 ± 0.05^{aB}	0.57 ± 0.04^{abB}	0.35 ± 0.01^{bB}	0.33 ± 0.01^{bB}	0.65 ± 0.23^{aB}
	90	1.70 ± 0.12^{aA}	1.45 ± 0.09^{abA}	1.32 ± 0.11^{bA}	1.29 ± 0.07^{bA}	1.23 ± 0.13^{bA}
TBA	30	0.15 ± 0.01^{aC}	0.13 ± 0.01^{abB}	0.12 ± 0.01^{abB}	0.12 ± 0.00^{abB}	0.12 ± 0.01^{abB}
	60	0.25 ± 0.00^{aB}	0.22 ± 0.01^{bA}	0.20 ± 0.02^{bA}	0.22 ± 0.00^{bA}	0.18 ± 0.01^{bA}
	90	0.28 ± 0.01^{aA}	0.24 ± 0.01^{bA}	0.23 ± 0.01^{bA}	0.22 ± 0.01^{bA}	0.22 ± 0.00^{bA}

[1] ***C:*** *Control (no additives); NS1, NS2, NS3 butters with respectively 0.05, 0.1 and 0.2% of N. sativa essential oil;* ***BHT:*** *butters with 100 ppm BHT;* $^{a-c}$ *Means within a row with no common superscript differ (P<0.05);* $^{A-C}$ *Means within each column of each category followed by the different letters are significantly differ (P<0.05)*

Table 2. *Effect of treatments and storage periods on some microbiological properties of butter samples (log cfu/g)*
Tablo 2. *Tereyağı örneklerinin bazı mikrobiyolojik özellikleri üzerine depolama periyodu ve muamelelerin etkisi (log kob/g)*

Variants	Storage Period (days)	Butters[1]				
		C	NS1	NS2	NS3	BHT
TAMB	2	6.83±0.21^{aA}	6.84±0.07^{aA}	6.80±0.15^{aA}	6.84±0.14^{aA}	6.73±0.15^{aA}
	30	6.70±0.18^{aAB}	6.72±0.06^{aA}	6.65±0.08^{aA}	6.71±0.29^{aA}	6.75±0.17^{aA}
	60	6.60±0.04^{aAB}	6.36±0.09^{abcB}	6.17±0.00^{bcB}	6.13±0.01^{cB}	6.41±0.17^{abAB}
	90	6.32±0.12^{aB}	5.88±0.02^{bcC}	5.71±0.16^{cC}	5.81±0.04^{cB}	6.06±0.02^{bB}
LAB	2	6.47±0.12^{aA}	6.40±0.39^{aAB}	6.62±0.17^{aA}	6.66±0.26^{aA}	6.60±0.09^{aA}
	30	6.22±0.25^{aA}	6.62±0.09^{aA}	6.31±0.33^{aAB}	6.15±0.41^{aAB}	6.62±0.09^{aA}
	60	6.32±0.32^{aA}	6.26±0.31^{aAB}	5.97±0.01^{aBC}	5.79±0.03^{aB}	5.98±0.07^{aB}
	90	6.20±0.14^{aA}	5.75±0.02^{bB}	5.69±0.00^{bC}	5.65±0.07^{bB}	5.73±0.05^{bC}
Coliform bacteria	2	4.83±0.09^{aA}	4.54±0.10^{abA}	4.48±0.12^{bA}	4.45±0.13^{bA}	4.71±0.13^{abA}
	30	4.67±0.06^{aA}	4.42±0.03^{aA}	3.78±0.16^{bAB}	3.26±0.18^{cB}	4.43±0.36^{aAB}
	60	4.64±0.18^{aA}	4.04±0.17^{abB}	3.05±0.90^{bB}	2.95±0.41^{bB}	3.38±0.44^{abB}
	90	3.55±0.12^{aB}	2.25±0.07^{bC}	<1^{dC}	<1^{dC}	1.65±0.49^{cC}
Yeast-mould	2	4.03±0.91^{aA}	3.30±0.89^{aA}	3.57±0.27^{aB}	3.20±1.03^{aA}	3.51±0.48^{aA}
	30	4.61±0.07^{aA}	4.27±0.21^{abA}	4.18±0.14^{abAB}	3.64±0.29^{bcA}	3.49±0.38^{cA}
	60	5.37±0.33^{aA}	4.69±0.26^{abA}	4.69±0.36^{abA}	3.74±0.94^{bA}	4.18±0.09^{abA}
	90	4.69±0.34^{aA}	4.68±0.30^{aA}	4.43±0.11^{aA}	4.52±0.31^{aA}	4.16±0.19^{aA}

[1] ***C:*** *Control (no additives); NS1, NS2, NS3 butters with respectively 0.05, 0.1 and 0.2% of N. sativa essential oil;* ***BHT:*** *butters with100 ppm BHT;* $^{a-d}$ *Means within a row with no common superscript differ (P<0.05);* $^{A-C}$ *Means within each column of each category followed by the different letters are significantly differ (P<0.05)*

of thymoquinone, *p*-cymene and carvacrol which have antibacterial effect [18]. Viuda-Martos et al.[34] suggested that bacteria type is important factor on antibacterial activity because of their cell membrane differences. As seen *Table 2*, the coliform bacteria counts of the butter samples were generally lower when compared to the Control samples. The samples containing 0.1% and 0.2% essential oil had the lowest coliform bacteria count. Yeast and mould counts were increased until the 60 days of storage period but then a slight decrease was seen for the 90 days, except NS3 sample *(Table 2)*.

Sensory properties are the main measurement criteria to detect food quality and consumer enjoying. The effects of treatments and storage periods on the sensory properties of butter samples are shown *Table 3*.

DISCUSSION

Antioxidant activity of essential oils could be attributed to the high contents of some components present in the oils. One of the examples is represented by Viuda-Martus et al.[34] who found the composition as *p*-cymene (33.03%), thymoquinone (32.18%) and α-thujene (13.01%). In another study, the contents of *N. sativa* essential oil were found as thymoquionone (23.25%), *p*-cymene (32.02%), carvacrol (10.38%), α- thujene (2.40%), and thymol (2.32%) [35]. Same researchers reported that composition of black cumin could change depending on the geographic distribution, time of harvest and agronomic practices. In our study, the contents were found some differences.

In this study, at the end of the storage the acidity (%) were in this order: BHT < NS1 < NS2 < NS3 < C. *N. sativa* essential oil is mostly composed of fatty acids including linoleic (58-65%), oleic (22-24%), palmitic (13-20%) and stearic acid [36]. Our result was in agreement with Jasinska and Wasik [37], who revealed that savoury added to the butter resulted in significantly higher lipid acidity during the cold storage. Increase of acidity depending on the concentration can be due to high unsaturated fatty acid content.

Essential oil containing samples showed better anti-oxidant activity than the Control and BHT containing sample especially on 30 and 60 days of storage and there was no differences between concentrations. However, at the end of the storage, there were no statistically significant differences between essential oil containing and BHT samples but lower than Control sample. It can also be concluded that there was a negative correlation between essential oil concentrations; as the essential oil concentration increased, PV values were decreased. Among the samples, Control showed the highest PV values in all storage periods. However, PV increased statistically significantly during the storage periods in all samples. In this study 0.2% level showed the most antioxidant activity. In another study by Ozkan et al.[38] who found that 2% *Satureja clicica* essential oils showed

Table 3. *Effect of treatments and storage periods on sensory properties of butter samples*
Tablo 3. *Tereyağı örneklerinin duyusal özellikleri üzerine depolama süresi ve muamelelerin etkisi*

Variants	Storage Period (days)	Butters[1]				
		C	NS1	NS2	NS3	BHT
Colour	2	8.40±0.14^{a,A}	8.45±0.07^{aA}	8.38±0.18^{aA}	8.25±0.07^{aA}	8.40±0.14^{aA}
	30	8.15±0.21^{a,A}	8.35±0.21^{aAB}	8.35±0.21^{aA}	8.40±0.14^{aA}	8.05±0.35^{aA}
	60	8.05±0.35^{aA}	7.90±0.14^{abC}	8.00±0.28^{abA}	8.10±0.14^{aA}	7.40±0.14^{bB}
	90	7.90±0.14^{aA}	8.00±0.00^{aBC}	7.88±0.18^{aA}	7.73±0.10^{aB}	7.30±0.00^{bB}
Texture	2	8.38±0.18^{aA}	8.25±0.00^{aA}	8.13±0.18^{aA}	8.15±0.21^{aA}	8.25±0.35^{aA}
	30	8.13±0.18^{aAB}	8.25±0.35^{aA}	7.90±0.14^{aA}	7.75±0.35^{aA}	7.94±0.26^{aA}
	60	7.88±0.18^{aAB}	7.65±0.21^{aA}	7.63±0.18^{a}A	7.70±0.42^{aA}	7.75±0.35^{aA}
	90	7.63±0.18^{aB}	7.75±0.35^{aA}	7.69±0.27^{a}A	7.69±0.27^{aA}	7.60±0.57^{aA}
Odour	2	8.38±0.53^{aA}	8.50±0.00^{aA}	8.38±0.18^{aA}	8.25±0.35^{aA}	8.50±0.35^{aA}
	30	8.00±0.35^{aAB}	8.00±0.28^{aB}	8.00±0.00^{aAB}	8.00±0.28^{aA}	8.00±0.00^{aB}
	60	7.63±0.18^{aAB}	7.63±0.17^{aB}	7.69±0.27^{aB}	7.69±0.27^{aA}	7.50±0.00^{aB}
	90	7.38±0.18^{bB}	7.58±0.11^{abB}	7.63±0.18^{abB}	7.88±0.18^{aA}	7.50±0.00^{abB}
Flavour	2	8.25±0.35^{aA}	8.38±0.18^{aA}	8.38±0.18^{aA}	8.33±0.11^{aA}	8.38±0.18^{aA}
	30	7.38±0.53^{aA}	8.13±0.18^{aA}	8.15±0.21^{aA}	8.20±0.28^{aA}	8.13±0.18^{aA}
	60	8.13±0.18^{aA}	8.00±0.17^{aA}	8.00±0.28^{aA}	8.10±0.14^{aA}	8.25±0.35^{aA}
	90	7.38±0.18^{bA}	7.83±0.24^{abA}	8.00±0.00^{aA}	8.04±0.23^{aA}	7.88±0.18^{aA}
Overall Acceptability	2	8.50±0.00^{aA}	8.38±0.18^{aA}	8.50±0.00^{aA}	8.53±0.32^{aA}	8.40±0.14^{aA}
	30	7.90±0.57^{aA}	7.94±0.08^{aAB}	8.04±0.23^{aB}	7.94±0.26^{aA}	8.00±0.00^{aAB}
	60	7.75±0.35^{aA}	7.83±0.24^{aB}	7.94±0.08^{aB}	7.75±0.35^{aA}	8.05±0.35^{aAB}
	90	7.55±0.35^{aA}	7.77±0.15^{aB}	8.00±0.17^{aB}	7.94±0.08^{aA}	7.50±0.00^{aB}

[1] ***C:*** *Control (no additives); NS1, NS2, NS3 butters with respectively 0.05, 0.1 and 0.2% of N. sativa essential oil;* ***BHT:*** *butters with 100 ppm BHT;* $^{a-b}$ *Means within a row with no common superscript differ (P<0.05);* $^{A-C}$*Means within each column of each category followed by the different letters are significantly differ (P<0.05)*

more effective antioxidant activity than 0.5 and 1% level, it was reported that antiradical and antioxidant activity could vary depending on the concentration and content of essential oil. Another study revealed that both aqueous and methanolic extracts of cumin varieties containing bitter cumin, cumin and black cumin inhibited lipid peroxidation [20]. Mariod et al.[21] revealed that while black cumin seed-cake showed antioxidant activity on corn oil by decreasing PV after 72 h when compared to Control without antioxidant.

TBA values of samples containing essential oil and BHT were lower than that of Control sample. No significant differences were found between all essential oil and BHT containing samples at the end of the storage. Control sample showed the highest value *(Table 1)*. Our results were in agreement with Dagdemir et al.[8] who indicated that there were no significant differences between *Thymus haussknechtii*, *Origanum acutidens* 0.2 and 0.1%, BHT and control butter samples on the 60th day of storage. Antioxidant activity of *N. sativa* L., using two TLC screening methods showed that thymoquinone, carvacrol, *t*-anethole and 4-terpineol demonstrated important radical scavenging property [17]. It was reported that the antioxidant activity were positively correlated with phenolic content of black cumin and antioxidant properties of black cumin extracts either equal to or higher than BHA and BHT [4]. It was observed that black cumin essential oil inhibited lipid peroxidation 92.56% and DPPH radical formation at a rate of 80.25% [35].

Essential oil containing samples were statistically lower than the sample with BHT and Control on 60 and 90 days of the storage in terms of TAMB counts *(Table 2)*. A study demonstrated that TAMB counts of soft white cheeses 0.3% and 1% essential oil of *N. sativa* decreased from 7.6×10^6 to 1.34×10^6 log cfu/g and 6.2×10^6 to 9.4×10^5 log cfu/g, respectively, after 6 days of storage [40]. LAB counts were decreased depending on the concentration of essential oil. The lowest LAB count was found 5.65±0.07 in NS3 sample (0.2% essential oil) at the end of the storage period *(Table 2)*. This result was in agreement with studies by Ozkan et al.[38] and Dagdemir et al.[8] who indicated, respectively, that the LAB counts of butter samples containing *Saturaja cilicica* essential oils and *T. haussknechtii* and *O. acuditens* were decreased. It can be concluded that the concentration of essential oil was an important factor of the growth of coliform bacteria. At the end of the storage, coliform bacteria counts were in the order of NS3 = NS2 < BHT < NS1 < Control *(Table 2)*. A study conducted by Arici et al.[19]

who showed that generally, the fixed oils of the black cumin samples had higher antibacterial activity against spoilage and pathogenic bacteria than LAB. Another study showed that 0.3% and 1% essential oil of *N. sativa* decreased the *S. aureus*, *Brucella melitensis* and *Escherichia coli* in cheese samples stored for 0, 2, 4 days of storage period at refrigerator temperature [39]. On the other hand, it was reported that when essential oils were added to complex food systems, antibacterial effectiveness is diminished and sensory properties could be affected negatively [34]. Although the yeast-mould counts of essential oil and BHT containing samples were lower than the Control, there were no statistical differences in all samples at the end of the storage. A similar result was determined by Dagdemir et al.[8] who studied *T. haussknechtii* and *O. acuditens* as antioxidants. Another study has shown that while 2% black cumin added to butter, it exhibited antiyeast activity against *Candida zeylanoides* and *Candida lambica* but *Candida kefyr* [40]. Variable results from the literature on effect of essential oils on different microorganisms kinds can be attributed to kind of seed selected, essential oil composition and concentration.

The addition of the essential oils significantly affected the sensory properties of the butter samples. The colour scores of the butter samples were similar during the 90 days of storage, except for BHT which had the lowest scores. All butter samples were same statistically in terms of texture scores. There were no significant difference in the flavour scores of all butter samples during the storage period, and scores were found between 7.38 and 8.38 *(Table 3)*. The overall acceptability scores of all butter samples were same statistically ($P<0.05$). But at end of the storage, the order was BHT (7.50) < C (7.55) < NSI (7.77) < NS3 (7.94) < NS2 (8.0). Generally flavour and overall acceptability scores of samples containing essential oil were higher than those of Control. Essential oil containing samples received higher scores for each parameter. As to the general evaluation of sensory scores, all butter samples were within acceptability limits changing from 7.30 to 8.53.

N. sativa L. essential oils and its concentrations used in this study showed a higher antioxidant activity than the respective Controls. Especially the PV and TBA values of the samples containing 0.2% essential oil were similar to the BHT containing samples. It was also effective on TAMB, LAB and coliform bacteria. The yeast and mould counts of the samples containing essential oils were lower than Control sample and BHT containing sample. Besides microbiological and chemical properties, sensorial properties of foods are much more important criteria for consumer acceptance. From this point the essential oil concentration must be taken into consideration in choosing the antioxidant. That is why *N. sativa* L. and its concentration used in our study were suitable from all points. From all the evaluated studies, it is clear that natural antioxidant sources can be preferred over synthetic ones to enhance not only the shelf life of product but also for the protection of nutritional properties. Thus, the results indicate that *N. sativa* L. essential oil may be considered as new source of natural antioxidant. However, there is still need for more studies to determine the effective essential oils dose.

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