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Research Article
Effect of Fenugreek, Coriander and Thyme Essential Oils Addition on Microbiology of Soybean Meal and Sunflower Meal in Different Storage Periods

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Abstract

Objective: This study was organized with the aim to determine the effects of fenugreek (*Trigonella foenum-graecum*), coriander (*Coriandrum sativum*) and thyme (*Thymus vulgaris*) essential oils added to stored soybean meal and sunflower meal on lactic acid bacteria (LAB), yeast, mould formation, nutrient and colour changes. **Methodology:** In order to explore the relationship between essential oil addition and feed microbiology under different times of storage, 4×2 factorial study design was used. Thus "absence of essential oils (control group)" versus "addition of essential oils" and "storage during 30 days" versus "storage during 60 days" at 22±2°C and 55% relative humidity (HR) were compared. **Results:** The addition of essential oils to vegetable protein sources has a positive impact on the counts of microorganisms and nutrient composition. The addition of essential oils to sunflower and soybean meal inhibited the growth of mould. **Conclusion:** Feed ingredients with essential oils addition demonstrated protective effect.

Key words: Essential oil, microbiology, soybean meal, sunflower meal, storage

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Soybean meal and sunflower meal are by-products of the edible oil industry and used as a valuable protein source feedstuffs in poultry diets. These feedstuffs used in mixed feed production are at the risk of microbial contamination.

Moulds are ubiquitous and thus unavoidable contaminants in all animal feeds. Many moulds are toxigenic and produce mycotoxins[1]. The growth of moulds and production of mycotoxins by these moulds in feed ingredients can cause significant economic losses[2-4].

The FAO estimates that 25% of the world food crops are affected by mycotoxins each year and constitute a loss at post-harvest[5]. Mycotoxins can be formed on crops in the field, during harvest, during storage, processing and feeding.

Residues of mycotoxins may also be found in food of animal origin (meat, milk, eggs and cheese) as the consequence of feed contamination. Mycotoxins are toxic compounds and some of them are also mutagenic, genotoxic, carcinogenic or teratogenic[6].

Recently, the use of spices and essential oils as antioxidants and antimicrobial agents in foods is becoming of increasing importance. Antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods[7-10]. Generally, the extent of the antioxidant and antimicrobial effects of the essential oil could be attributed to their phenolic compositions[11].

The microbial properties of spice and essential oils have been known for a long time and a number of studies of the antimicrobial effects of spices, essential oil and their components have been reported[7-10].

Essential oils are one of the most efficient feed additives for mould prevention. For example, in different parts of world, attention has been paid to exploiting plant products as novel chemotherapeutant and preservatives in plant protection and food storage[12-14].

Usage of essential oils in feedstuffs and feeds has been reported to reduce the growth of fungi, yeast and bacteria[15-21].

The purpose of this study was to investigate the effects of fenugreek (*Trigonella foenum-graecum*), coriander (*Coriandrum sativum*) and thyme (*Thymus vulgaris*) essential oils addition to common protein rich components of animal feeds on nutritional loss, color change, the growth of lactic acid bacteria (LAB), yeast and mould under varying storage times.

MATERIALS AND METHODS

Feed treatment and storage: The investigated commercial feedstuffs included soybean and sunflower meals. They were obtained from the regional feed market located in Thrace district of Turkey.

Essential oil of fenugreek (*Trigonella foenum-graecum*), coriander (*Coriandrum sativum*) and thyme (*Thymus vulgaris*) were obtained from a domestic shop. In the current study, 0.05 mL of essential oils were added kg^{-1} of feedstuffs.

In order to explore the relationship between essential oil addition and feed microbiology under different times of storage, 4×2 factorial study design was used. Thus "absence of essential oils (control group)" versus "addition of essential oils" and "storage during 30 days" versus "storage during 60 days" at $22\pm2°C$ and 55% relative humidity (HR) were compared. Each cell in the factorial design was represented by 3 samples of feedstuffs, stored in a room ($22\pm2°C$, 55% HR) in open polyethylene bags (24×38 cm).

Microbiological, chemical and colour analysis: Ten grams of each sample were added to 90 mL of peptone water 0.1% (10 g peptone L^{-1} of water) and kept at room temperature for 30 min. This mixture was shaken and diluted to the concentrations 10^{-2}, 10^{-3} and 10^{-4}. The concentration of lactic acid bacteria (LAB) was evaluated by means of the count of colony forming units (cfu) on Man-Rogosa and Sharp agar (MRS, 110660 MERCK-Germany) with an overlay of the same agar and incubated in anaerobic conditions for three days at $30\pm2°C$. Yeast and mould counts were determined on malt extract agar (1.05398 MERCK- Germany), after incubation at 30°C for 5 days. LAB, yeast and mould counts were obtained according to the methods reported by Seale *et al.*[22]. Aflatoxin B1 analysis was performed according to the methodology proposed by Trucksess *et al.*[23].

The dry matter (DM, Method 934.01) content of the feedstuffs was determined by drying the samples at 60°C for 72 h in an oven. Crude ash (CA) was obtained after drying at 600°C for 4 h (AOAC[2], Method 942.05). Crude protein (CP, Method 954.01), crude cellulose (CC, Method 978.10) and ether extract (EE, Method 920.39) were determined in accordance with the methods specified in AOAC[24].

The colour parameters of feedstuffs were determined in terms of stimulus colour parameters based on Commission International de I'Eclairage (CIE L*, a*, b*) using colour meter (Hunter-Lab tristimulus colorimeter, D25LT model).

Statistical analysis: Concentrations were transformed to the logarithmic scale. The data were investigated by two-way analysis of variance (ANOVA) with Duncan's multiple range test using the PASW statistics18 packed program for windows (SPSS[25]). The ANOVA was based on a general linear model which included the main effects such as storage time and essential oil addition and their two-way interactions.

RESULTS

No aflatoxins were detected in any of the investigated feedstuffs before or after storage. Table 1 shows the observed effects of addition of essential oils on microbiology of soybean meal and sunflower meal. The detection limit was 10 cfu g^{-1} and it is represented as <0.01.

The concentration of LAB in the control groups was found to be higher than the other groups after 30 days of storage in sunflower meal. The addition of thyme essential oil decreased the concentration of LAB after 30 days of storage. However, at the end of the 60th day there was a numerical increase in the concentration of LAB but the difference statistically was not significant (p>0.05).

The effect of storage time on yeast count was not statistically significant (p>0.05). But the effect of the addition of essential oils was statistically significant (p<0.001). It was observed that the effect of storage time and essential oils on the count of moulds was significant (p<0.001). The addition of thyme and coriander essential oils in stored sunflower meal reduced the count of moulds.

It was observed that the storage period was effective on the concentration of LAB in soybean meal (p<0.001). However, the effects of treatments on yeast and mould counts were not significant (p>0.05). The addition of essential oils to soybean meal was insignificant on the growth of mould (p>0.05). This is thought to be due to the destruction of negative factors as a result of heat treatment of the soybean meal.

The effect of essential oils added to sunflower meal and soybean meals at different periods on raw nutrients are presented Table 2 and 3.

As shown in Table 2, the effect of treatments on crude protein content of sunflower meal was statistically non significant (p>0.05). However, the effect on ether extract levels was statistically significant (p<0.001). The increase in ether extract levels in the sunflower meal is thought to be due to the preservation of microbial growth of essential oil.

As shown in Table 3, in soybean meal, the effect of treatment on crude protein was not significant (p>0.05). The effect of essential oil addition and storage period x essential oil interaction on ether extract level was found to be statistically significant (p<0.001).

The effect of essential oils on enhancing the ether extract level in soybean meal was similar to that in sunflower meal, probably due to inhabitation of microbial growth.

The values of L, a, b and yellow index of feedstuffs are shown in Table 4. As a result of 30 days of storage, the "L" colour value measured in the essential oil added sunflower meal ranged from 50.21-51.83. "L" colour level had the highest value in the coriander added group and the lowest in the control group. As a result of the statistically evaluation, the difference between treatments in terms of "L" value was found to be non significant.

In the sunflower meal at the end of 30 days of storage, the lowest value in terms of "a" value was determined in the coriander added group (3.20) and the highest value in the thyme added group (3.75).

The highest value (16.32) was found in fenugreek added group and the lowest value (14.26) in the coriander added group at the end of the 30 days of storage in terms of "b" value in sunflower meal. The effect on the colour changes in the sunflower meal of the treatments was not statistically significant.

The "L" colour value measured by adding essential oil to the soybean meal ranged from 57.98-63.45. As a result of 60 days of storage, the highest value (63.45) was found in the control group without added essential oil."L" value was the lowest (57.98) in the control group at the end of 30 days of

Table 1: Effect of essential oils addition on microbiology of soybean and sunflower meal

		Soybean meal			Sunflower meal		
Storage time (days)	Essential oils	LAB	Yeast	Mould	LAB	Yeast	Mould
Before storage		0.542	1.613	0.452	0	0.841	1.511
	Control	1.644^{ab}	1.223^{ab}	0.000	1.113^{a}	0.573^{b}	2.045^{b}
	Thyme	0.841^{bc}	0.841^{ab}	0.151	0.690^{b}	2.327^{a}	1.088^{c}
30	Fenugreek	0.389^{c}	1.000^{ab}	0.151	1.025^{ab}	0.000^{b}	2.029^{b}
	Coriander	1.762^{a}	1.600^{a}	0.000	0.841^{ab}	1.827^{a}	1.188^{c}
	Control	0.389^{c}	1.253^{ab}	0.000	0.699^{ab}	0.000^{b}	2.602^{a}
	Thyme	0.000^{c}	1.115^{ab}	0.000	1.000^{ab}	2.2840	1.980^{b}
60	Fenugreek	0.000^{c}	0.452^{b}	0.000	0.929^{ab}	0.000^{b}	2.514^{a}
	Coriander	0.151^{c}	0.389^{b}	0.000	1.073^{ab}	1.564^{a}	1.040^{c}
Standard error of mean		0.128	0.121	0.011	0.027	0.130	0.008
P-levels							
Storage time		<0.00.1	0.069	0.195	0.923	0.258	<0.001
Essential oil		0.030	0.298	0.596	0.687	<0.001	<0.001
Storage time×essential oil		0.170	0.064	0.590	0.050	0.675	<0.001

Values with different letters in the same column are statistically significantly different (p<0.05)

Table 2: Effect of essential oils added to sunflower meals on raw nutrients

Storage time (days)	Essential oils	DM (%)	CA (%)	CP (%)	EE (%)	CC (%)
Before storage		91.49	5.13	28.50	0.57	30.68
	Control			23.59^{b}	0.57^{f}	
	Thyme			25.62^{ab}	0.67^{d}	
30	Fenugreek			26.75a	0.74^{b}	
	Coriander			25.09^{ab}	0.71^{c}	
	Control			25.47^{ab}	0.61^{e}	
	Thyme			25.29^{ab}	0.63^{e}	
60	Fenugreek			25.27^{ab}	0.93^{a}	
	Coriander			25.49^{ab}	0.74^{b}	
Standard error of mean				1.118	0.0001	
P-levels						
Storage time		0.830	<0.001			
Essential oil		0.330	<0.001			
Storage time x essential oil		0.230	<0.001			

Values with different letters in the same column are statistically significantly different (p<0.05)

Table 3: Effect of essential oils added to soybean meals on raw nutrients

Storage time (days)	Essential oils	DM (%)	CA (%)	CP (%)	EE (%)	CC (%)
Before storage		87.92	5.42	47.93	1.44	5.63
	Control			48.03	1.16^{a}	
	Thyme			47.43	1.47^{d}	
30	Fenugreek			48.66	1.47^{d}	
	Coriander			46.55	1.25^{b}	
	Control			50.16	1.34^{c}	
	Thyme			48.13	1.34	
60	Fenugreek			49.59	1.45^{d}	
	Coriander			50.31	1.36^{c}	
Standard error of mean				2.763	0.001	
P-levels						
Storage time		0.054	0.087			
Essential oil		0.639	<0.001			
Storage time×essential oil		0.573	<0.001			

Values with different letters in the same column are statistically significantly different (p<0.05)

Table 4: Values of L, a, b and yellow index of feedstuffs

		Soybean meal			Sunflower meal		
Storage time (days)	Essential oils	L	a	b	L	a	b
Before storage		62.57	8.52	28.47	50.79	2.96	13.81
	Control	57.98	9.65	27.94^{b}	50.21^{b}	3.37^{ab}	14.74^{ab}
	Thyme	58.44	10.27	28.85^{ab}	50.96^{ab}	3.75^{a}	15.24^{ab}
30	Fenugreek	60.88	9.64	29.33^{a}	50.54^{ab}	3.67^{a}	16.32^{a}
	Coriander	61.28	9.36	28.87^{ab}	51.83^{a}	3.20^{b}	14.26^{b}
	Control	63.45	9.59	29.79a	50.56^{ab}	3.72^{a}	15.24^{ab}
	Thyme	61.19	9.60	28.80^{ab}	51.64^{a}	3.75^{a}	15.66^{ab}
60	Fenugreek	60.62	9.51	29.34^{a}	50.86^{ab}	3.41^{ab}	14.83^{ab}
	Coriander	58.63	9.84	28.28^{b}	49.70^{b}	3.44^{ab}	14.94^{ab}
Standard error of mean		6.956	0.698	0.160	0.288	0.028	0.593
P-levels							
Storage time		0.344	0.828	0.167	0.483	0.363	0.945
Essential oil		0.933	0.918	0.132	0.192	0.044	0.325
Storage time×essential oil		0.225	0.815	0.012	0.020	0.124	0.235

Values with different letters in the same column are statistically significantly different (p<0.05)

storage. The difference between treatments in terms of "L" value was statistically insignificant (p>0.05). The difference in "a" and "b" values was not statistically significant in soybean meal.

DISCUSSION

According to the results of the present research, the effects of adding essential oils to soybean meal and sunflower meal on LAB, yeast, mould, nutrient losses and colour changes were different and positive at different storage times. The addition of essential oils to soybean meal and sunflower meal did not cause any adverse effects on LAB, yeast and mould formation during storage. The raw nutrient contents of feedstuffs were also not adversely affected by the increase in storage time. Helander *et al.*[26] reported that essential oils exhibit antimicrobial activity on many microorganisms, including gram-negative and gram-positive bacteria.

The addition of essential oils to the feedstuffs was found to be mould inhibiting. Thyme essential oil is among the most commonly used and known essential oils due to its antimicrobial properties. Thyme essential oil is a phenolic volatile oil and contains carvacrol and thymol[27]. Depending on the storage period, nutrients losses were probably affected by the addition of essential oil. In particular, the addition of essential oils to stored soybean meal and sunflower meal had a positive effect on the ether extract in storage time.

The nutrients and microorganism contents of the feedstuffs are highly variable due to their origin and the difference in their production methods. The storage of different feedstuffs at different periods affects the nutrients and microorganism levels of the products in a very different way. The use of essential oils results in minimal loss of stored feedstuffs on adverse conditions. The addition of essential oil to the feedstuffs in this study prevented the formation of mould.

As a result of the research, it has been shown that the addition of essential oils can be stored in feedstuffs especially ether extracts and crude protein contents without any loss. According to these results, there is no significant difference between the pre- and post-storage periods in terms of nutrients content of essential oil added feedstuffs. There was an increase in the count of moulds in control groups after storage. This increase was prevented by the addition of essential oils. The addition of essential oil has a protective effect on the feedstuffs. However, it should be known that this affect is not at desired level in all kinds of storage conditions. It is necessary to know that the storage conditions and the properties of the feedstuffs are also related.

On the other hand, colour measurement has started to be used frequently in the classification and quality assessment of agricultural products in recent times. Colour is a genetic factor and is affected by environmental factors and especially oxidation. The colour of the feedstuffs is important both for the attractiveness to the animals and for the beneficial functions of the natural pigmentation in the body. In particular, chickens prefer bright yellow feeds to opaque feeds. In addition, pigment substances in feedstuffs provide high degree of pigmentation to the chicken's body and egg yolk. Such products are also preferred by consumer. This study showed that the addition of essential oil to feedstuffs could be stored without any change in colour parameters.

CONCLUSION

This study showed that besides the physical and chemical structure of the feedstuffs, hygienic quality is also important. The microbial structure of the feedstuffs not only affects animal and human health negatively but also causes economic loss. Therefore, more scientific research is needed to determine the natural aromatic plants and their extracts with antioxidant activity.

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