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Nigella sativa SEED OIL ANTIMICROBIAL ACTIVITY AGAINST *Staphylococcus* spp. IN A FOOD MATRIX

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Abstract

The antimicrobial effects of Nigella sativa have been studied in vitro and in vivo, against various microorganisms such as Enterobacteriaceae, Staphylococcus, Streptococcus, Salmonella etc. Recent studies proved antimicrobial effects of Nigella sativa seed oil (NSSO) against various contaminant bacteria in cheese. Due to promising results concerning NSSO effect against bacteria in brined cheese, this study tested NSSO effect against naturally occurring Staphylococcus spp. which contaminates kneaded, sheep´s milk Romanian cheese. Three batches of traditionally manufactured raw milk kneaded cheese were considered: control cheese without NSSO and cheese samples enriched with 0.1 and 0.2 w/w NSSO. Staphylococcus spp. enumeration revealed a descending trend in CFU/g throughout the ripening period, for all batches of cheese. The counts were lower for the 0.1% w/w NSSO cheeses than for the control batch, but no statistical significance could be attributed to this difference (p-value - 0.57). However, for the 0.2% w/w NSSO batch of cheeses, Staphylococcus count registered noticeable decrease, and the results were statistically significant (p-value - 0.048), and no colonies were obtained by the end of the ripening period.

Key words: *kneaded cheese, Nigella sativa seed oil, ripening, Staphylococcus spp.*

INTRODUCTION

Raw milk traditional cheeses have been often associated with food borne infections or intoxications (Öner, 2006; Little, 2008; Choi et al., 2016; Prates, 2017; Bintsis, 2002; Gao, 2017). If most hard or semi-hard raw milk cheeses are usually ripened long enough to be safe for consumption, traditional soft cheeses continue to be a real hazard for public safety, as they provide appropriate environment for pathogens survival and development (Eck et al., 2000; Fox et al., 2000; Tăpăloagă, 2017; Ilie (a,b), 2017). A continuous search of antimicrobial solutions, adequate as natural additives in foods in general and in cheeses, in particular, has increasingly been reported by recent studies (Fadavi, 2015; Amatiste, 2014; El-Dahma, 2017; Wahba, 2010; Gouvea Fabiola dos Santos, 2017; Darwish, 2017).

Black cumin (*Nigella sativa*) seeds have been extensively used as spices in a wide range of foods and beverages especially in Middle and Far East countries, being appreciated for a wide range of pharmacological actions, such as anti-diabetic, anti-cancerous, immunity modulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, antioxidant etc. (Ahmad, 2013; Gholamnezhad, 2016). Most of *Nigella sativa* therapeutic properties have been attributed to thymoquinone, considered the most significant bioactive component of the essential oil (Ijaz, 2017). *Nigella sativa* is commonly added to food, as seeds or essential oil (Hassanien, 2015; Ramadan, 2016; Abedi, 2017), for various benefficial effects.

Recent literature provides studies of *Nigella sativa* seeds or cold pressed oil effects on the overall quality of cheeses (Hassanien, 2014; Hassanien, 2015; Mahgoub, 2013; Cakir, 2016).

The antimicrobial effects of *Nigella sativa* have extensively been studied *in vitro* (Muhammet, 2005; Utami, 2016; Forouzanfar, 2014; Bakal, 2017) and *in vivo* (Rafati, 2014) against various microorganisms such as *Enterobacteriaceae, Staphylococcus, Streptococcus, Salmonella, Helicobacter, Listeria, Pseudomonas, Klebsiella, Proteus* etc.

In this context, the paper attempted an assessment of *Nigella sativa* seed oil (NSSO) antimicrobial activity, against *Staphylococcus spp.* contaminating traditionally manufactured raw milk kneaded cheese.

MATERIALS AND METHODS

The experiment included three batches of cheese: control cheese without NSSO, 0.1% w/w NSSO enriched cheeses and 0.2% w/w NSSO enriched cheeses. Commercially available NSSO was purchased from Aghoras Invent SRL company, of Bucharest.

The NSSO was added to the mildly heated milk in the respective concentrations, before renneting. The content of the Ideal® rennet dose (8 g) was diluted in 250 mL warm distilled water and 25 mL solution were added to 10 l warm milk (30-35°C), under continuous manual mixing for 10 minutes.

Coagulation time was 30-45 minutes. The soft curd was left for further solidification needed for processing, for 15-30 minutes. Further stages of the technological process were followed according to the usual, traditional Burduf cheese manufacturing and included pressing, wriggling, bursting, resting, ripening in fir woodandfilling (fig.1) (Tăpăloagă, 2013; Tăpăloagă, 2018).

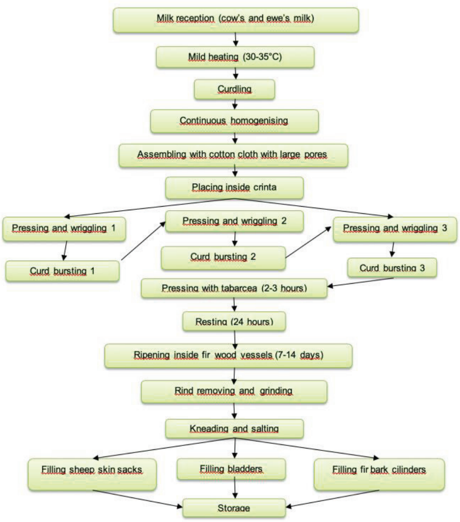

Figure 1. Soft kneaded raw milk cheese manufacture diagram

For this experiment, fir bark cylinders were filled with cheese and kept at dark, cold environment (8-10°C), according to traditional custom, throughout the experiment (for 42 days).

Experimental kneaded cheese samples were analyzed in duplicates for *Staphylococcus* spp. count, at 0, 14, 21, 28, 35 and 42 days of ripening. *Staphylococcus* spp. enumeration was performed using 3M™ Petrifilm™ Staph Express Count System (St. Paul, Minnesota, USA) and Petrifilm Staph Express disk (AOACOfficial Method of Analysis 2003.08, for dairy foods) (www.eoma.aoac.org).

Data analysis was performed by One way analysis of variance (ANOVA) using SAS(ANOVA version 9.1. SAS institute Inc., Cary, USA, 2003) (53). The threshold of significance level was $p<0.05$. The repetitions (duplicates) of determinations were not considered in statistical significance calculations.

RESULTS AND DISCUSSIONS

Staphylococcus spp. enumeration revealed lower counts for the 0.1% w/w NSSO cheeses than for the control batch, but no statistical significance could be attributed to this difference (p-value - 0.57) (Table 1). However, for the 0.2% w/w NSSO batch of cheeses, *Staphylococcus* count registered noticeable decrease, with strong statistical significance (p-value - 0.04), no colonies being obtained by the end of the ripening period (Table 2).

The antimicrobial effect of *Nigella sativa* oil and extracts on *Staphylococcus* spp. is extensively cited throughout literature, both in vitro (Uzair, 2017; Emeka, 2015; Forouzanfar, 2014) and in vivo experiments (Hannan, 2008; Rafati, 2014; Bakathir, 2011). *Staphylococcus* spp. is a commonly found contaminant of raw milk cheeses, especially those processed through traditional methods, as revealed in recent studies (Taban, 2017; Kav, 2011) As staphylococci can grow at high sodium chloride concentrations, brined cheeses are commonly associated with *Staphylococcus* contamination from milking and processing environment and personnel, staphylococcal toxins being a frequent cause of food borne intoxications (Bianchi, 2014). Therefore it was expected to find natural staphylococcal contamination of raw milk kneaded cheese (Ilie, 2018), but the revealed counts were not high enough for enterotoxin production (Fig. 2). For all cheese samples, *Staphylococcus* counts dropped starting with the 7th day of ripening, which may also be correlated with the dropping trend of pH values in all cheeses, as staphylococcal growth is limited at pH values below 5.8-6 (Delbes, 2006).

NSSO was significantly associated with lower staphylococcal counts than the ones noticed for control samples, throughout ripening: the *f*-ratio value was 0.32 for 0.1% w/w NSSO batch and 4.84 for 0.2% w/w NSSO cheese batch, compared with control, while the *p*-value was 0.57 for 0.1% w/w NSSO batch and 0.04 for 0.2% w/w NSSO cheese batch, compared with control (Fig. 2).

These findings are in agreement with other studies which report significant reducing effect against the proliferation of *S. aureus* by addition of NSSO (Hassanien, 2014). Other similar studies reveal significant antibacterial activity against *S. aureus* only at doses of 0.2% NSSO and not at lower levels.

Table 1. Data analysis for 0.1% w/w NSSO cheeses compared to control

k	**Treatments**		
	Control	**0.1% w/w NSSO**	
N	7	7	
∑X	15.29	13.74	
Mean	2.18	1.96	
∑X2	35.68	31.01	
St.dev.	0.61	0.82	
	Result details		
	SS	**Df**	**MS**
Between treatments	0.176	1	0.1716
Within treatments	6.3385	12	0.5282
Total	6.5101	13	
f-ratio value	0.32489		
p-value	0,579202		

Another opinion phrased by similar research states that both 0.1% and 0.2% NSSO supplementation induce significantly reduced counts in *S. aureus* and *E. coli,* but 0.2% concentration showed the most intense effect (Mahgoub, 2013).

Most authors consider a decrease of 1.3-1.5 log $CFUg^{-1}$, by the 21^{st} day of ripening, as being significant (Hassanien, 2014).

This study analyzed the degree of significance in terms of difference in count dynamics between treatment groups and control, throughout ripening, as this comparison was considered useful for assessing the impact of NSSO on natural contaminating microflora in regular ripening conditions and not the antimicrobial capacity of *Nigella sativa* seed oil on its own.

Table 2. Data analysis for 0.2% w/w NSSO cheeses compared to control

Data analysis item	**Treatments**		
	Control	**0.1% w/w NSSO**	
N	7	7	
∑X	15.29	8.48	
Mean	2.18	1.21	
∑X2	35.68	16.18	
St.dev.	0.61	0.993	
	Result details		
	SS	**Df**	**MS**
Between treatments	3.3126	1	3.312
Within treatments	8.2079	12	0.684
Total	11.5204	13	
f-ratio value	4.84304		
p-value	0.048077		

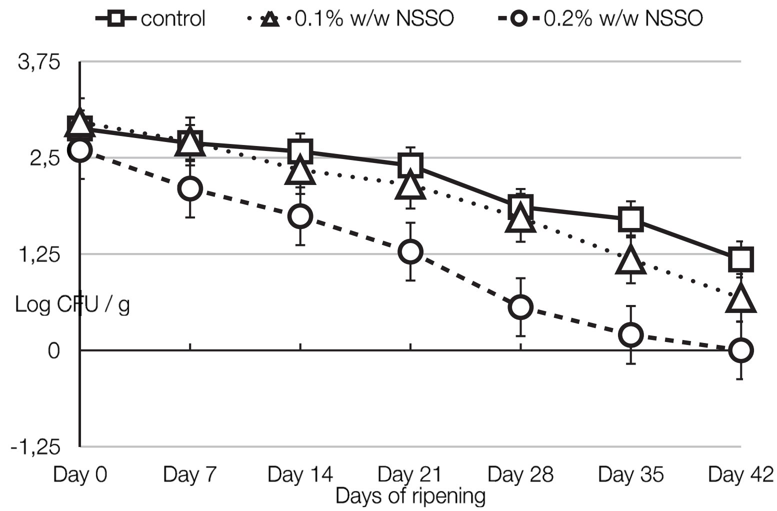

Figure 2. *Staphylococcus* spp. population fluctuation in NSSO enriched cheeses, compared to control batch, over ripening

CONCLUSIONS

Staphylococcus spp. enumeration revealed a descending trend in CFU/g throughout the ripening period, for all batches of cheese. The counts were lower for the 0.1% w/w NSSO cheeses than for the control batch, but no statistical significance could be attributed to this difference (p-value - 0.57).

However, for the 0.2% w/w NSSO batch of cheeses, *Staphylococcus* count registered noticeable decrease, and the results were statistically significant (p-value - 0.048), and no colonies were obtained by the end of the ripening period. Thus, NSSO could be a good option as additional measure for the hygiene control of traditionally manufactured raw milk cheeses.

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