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TESTING THE EFFECT OF *NIGELLA SATIVA* ESSENTIAL OIL SOLUTION ON CHICKEN BREAST pH AND TOTAL VOLATILE BASE NITROGEN DURING REFRIGERATION

Raluca-Aniela IRIMIA, Mara GEORGESCU, Liliana TUDOREANU, Manuella MILITARU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania

Corresponding author email: irimiaral@gmail.com

Abstract

Following consumers high demand for healthier food, the food industry is more than ever interested in replacing traditional chemical preservatives with natural preservatives such as plant extracts and oils. Therefore, we have tested the preservation potential of the 1% (T1%) and 2% (T2%) Nigella sativa oil solutions on chicken meat. Five chicken breasts (m. Pectoralis major) were purchased and each of them was divided in three aliquots, corresponding to Control, T1% and T2%. All chicken breast aliquots were refrigerated for 6 days. The pH and Total Volatile Base Nitrogen (TVB-N) was measured for Control and treatments on day 1, day 3 and day 6. After 6 days of refrigeration, the T2% treated meat had a pH = 6.062 ± 0.042 and a TVB-N = (26.100 ± 0.644) (*mg* NH_3*/100g meat), values which were significantly different compared to Control. The pH and TVB-N values measured for T1% treated meat were not significantly different from Control after 6 days of refrigeration. The T2% was found to be the most efficient treatment for preserving the meat during a refrigeration period of 6 days.*

Key words*: Nigella sativa, preservation, chicken breast, refrigeration, pH, TVB-N.*

INTRODUCTION

The use of essential oils for the meat products preservation is a healthy alternative to chemical preservatives, complying to consumers requirements (Hassanien, 2006; Marzieh et al., 2013; Georgescu, 2019). The latest scientific reports help producers, to identify the best alternative for improving the safety and quality of meat products by using natural oils and plant extracts instead of synthetic chemical derivatives (Osman et al., 2017; İlk et al., 2018).

One of the oils deeply studied, is that of *Nigella sativa* (black cumin). Following chemical analysis the *N. sativa* oil contains over 85% biological active compounds such as p-cymene, α-thujene, longifolene, α-pinene, β-pinene, thymoquinone and carvacrol (Karimi, 2019; Georgescu, 2018). Moreover the *N. sativa* extract demonstrated antimicrobial, anti-fungal and anti-oxidant properties which are highly useful characteristics for the current stringent needs of the food industry (Ramadan, 2016; Iwona et al., 2017). In addition, the *N. sativa* oil exhibited stronger radical dropping activity against 2,2-diphenyl-1-picryldiazyl radical compared to synthetic antioxidants (Ramadan, 2016; Kiralan et al., 2014).

Studies of the *N. sativa* oil effects were carried out on a multitude of foodstuffs (Osman et al., 2017) by using the essential oil in various forms, a great majority of the experiments being conducted for microbiological determinations. Thus, further studies in the field should also involve monitoring of the pH and Total Volatile Base Nitrogen in order to provide a broader view regarding the food safety and quality of the products treated with *N. sativa* essential oil.

MATERIALS AND METHODS

Five samples of chicken pectoral muscle (*m. Pectoralis major*) of approximately equal weight (200-250 g each) were purchased from a single producer and from different stores in order to randomize the process of sampling. The samples were transported in cooling bags with no refrigeration (approximately 15 minutes from the store to the laboratory, for each transport). Each chicken breast was divided into 3 aliquots of equal weight (Figure 1) and used for the "Control" (non-treated

meat), the 1% *N. sativa* oil solution treatment and the 2% *N. sativa* oil solution treatment (Figure 1). The Control and the two treatments contain each of them 5 aliquots of meat.

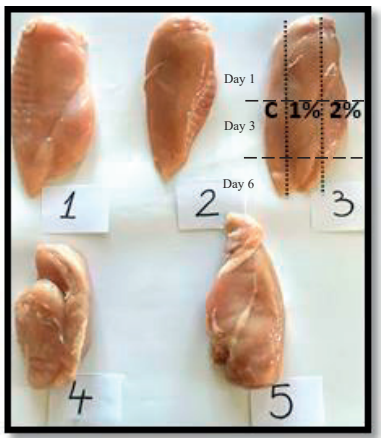

Figure 1. Breast samples collected - all aliquots were of equal weight. All parts (from the aliquots) were of equal weight

- Treatment solutions preparation

The treatment solutions were made by adding Black Caraway oil - cold pressed, 100% pure oil ("Ulei de negrilică", Carmita Classic SRL, Alba Iulia, Romania) to Deuterium Depleted Water (DDW). The solutions were: T1% - 1 ml of Black Caraway oil in 100 ml of solution and T2% - 2 ml of Black Caraway oil in 100 ml of solution.

- Meat samples preparation

Each of the five chicken meat breasts were sliced into three even aliquots. Thus, we had 5x3 meat samples which were used for the T1%, T2% and Control, as shown in Figure 1, for chicken breast number 3. The treatments T1% and T2% contained meat aliquots as described above. The treated aliquots were individually soaked into 20 ml of T1% and T2% solution respectively, and stored in polyethylene bags, for the whole duration of the experiment. Samples from the Control, T1% and T2% were analysed at 3 hours after the treatment (Day 1), after 3 days of refrigeration (Day 3) and after 6 days of refrigeration (Day 6), 45 samples in total. The refrigeration temperature was 2^0C.

- Measurement of pH and Total Volatile Base Nitrogen (TVB-N)

The TVB-N and pH (Figure 2) measurements were carried out according to SR ISO 2917:2007, SR 9065-7:2007, SR 9065-7:2007/C91:2009 and an AOAC method.

Prior to measurements, each collected part was grinded individually using a Braun MQ5020 grinder.

Figure 2. The pH determination

- Statistical data analysis

One-way ANOVA for independent samples was chosen to identify significant differences for pH and TVB-N respectively, between treatments in Day 1, Day 3 and Day 6. Our experiment has only n = 5 (very small sample size). Technically ANOVA can work if there is one value more than parameters to be estimated by the model (for k = 3 samples the minimum total sample size is n = k +1 = 4). However the rule of thumb for ANOVA is to have n=30 in order to generate a power of 80% of the analysis. Generally the accepted minimum of values per statistical sample is n = 7. When the sample size is very low (n = 5) the power of the analysis might be as low as 50%. Therefore, using 5 values per sample, for an ANOVA analysis there is a probability of approximately 50% to pick up on an effect that is present. Also we are aware of this situation and have decided to proceed with the experiment in order to acquire preliminary information we can use to develop a future larger scale experiment.

The data were tested for normality using the Shapiro-Wilk test. The Levene's test was used to test for equality of variances. When data were not normally distributed, we used the Kruskal-Wallis test.

The level of significance is 0.05.

When the data were normally distributed and the samples have unequal variances the choice for data analysis was Welch-ANOVA.
Dunnet test (with control) is the post-hoc test used for a significant ANOVA.
Following a significant Kruskal-Wallis test we used the post-hoc Conover test.
A two way ANOVA is not an option due to the fact that comparing the levels of pH and TVB-N between Day 1 and 6 has no practical meaning. Obviously, these values will be highly different.
The MedCalc Software version 18.10.2 and JMP 15 trial version were used for statistical data analysis.

RESULTS AND DISCUSSIONS

The pH and TVB-N values for all treatments during the 6 days refrigeration period are presented in Table 1.

Table 1. pH and TVB-N of chicken breast treated with solutions of *N. sativa* oil during 6 days of refrigeration at 2^0C

Treatments	DAY 1		DAY 2		DAY 3	
	pH Mean ± SD	TVB-N (mg NH_3/100g meat) Mean± SD	pH Mean ± SD	TVB-N (mg NH_3/100g meat) Mean± SD	pH Mean ± SD	TVB-N (mg NH_3/100g meat) Mean± SD
CONTROL	5.898 ± 0.131	24.980 ± 0.414	6.072 ± 0.051	26.000 ± 0.200	6.226 ± 0.055	27.200 ± 0.547
T1%	5.880 ± 0.129	24.860 ± 0.439	6.028 ± 0.071	25.620 ± 0.228	6.122 ± 0.055	26.620 ± 0.589
T2%	5.848 ± 0.135	24.700 ± 0.424	5.856 ± 0.225	25.300 ± 0.158	6.062 ± 0.042	26.100 ± 0.644

The statistical analyses of the treatment's results are presented in Table 2 to Table 5.
The Control pH values (Table 1) for the first day are similar to the data reported by Saláková (5.66 and 6.08) (Saláková et al., 2009) and to the data reported by Baston (5.82-6) (Baston et al., 2002), the mean value in our experiment being 5.898.
After three days of refrigeration the pH values of the samples treated with *N. sativa* oil solutions were not significantly different (Table 2) compared to the pH values measured for Control.
However the TVB-N values of treated chicken breasts were significantly different from the Control after three days of refrigeration (Table 3).
After six days of refrigeration only the samples of the T2% treatment were significantly different from Control (Table 5).

Table 2. Statistical analysis for pH and TVB-N values of chicken breast treated with *N. sativa* oil solution

Data	DAY 1 pH	DAY 1 TVB-N	DAY 3 pH	DAY 3 TVB-N	DAY 6 pH	DAY 6 TVB-N
Factor	Treatment	Treatment	Treatment	Treatment	Treatment	Treatment
Sample size	15	15	15	15	15	15
Levene statistic Significance level	0.00746 P = 0.993	0.0134 P = 0.987	3.805 P = 0.052	0.635 P = 0.547	0.724 P = 0.505	0.145 P = 0.867
Shapiro-Wilk test for Normal distribution	W = 0.8789 Reject normality (P = 0.0457)	W = 0.8780 Reject normality (P = 0.0443)	W = 0.8647 Reject normality (P = 0.0282)	W = 0.9120 Reject normality (P = 0.1451)	W = 0.8765 Reject normality (P = 0.0420)	W = 0.9076 Reject normality (P = 0.1243)
Kruskal-Wallis test Test statistic Significance level	1.2800 P = 0.527	1.6350 P = 0.435	5.3150 P = 0.069	Not applicable	9.1250 P = 0.0103	Not applicable
ANOVA Single factor F-ratio Significance level	Not applicable	Not applicable	Not applicable	12.878 P = 0.001	Not applicable	4.277 P = 0.040

Table 3. Dunnett's test (Comparisons with Control) for TVB-N values of chicken breast treated with *N. sativa* oil solutions after 3 days of refrigeration (Day 3) Control Group = CONTROL; α=0.05; |**d**|=2.50241. Positive values show pairs of means that are significantly different

LSD Threshold Matrix		
Level	Abs(Dif) - LSD	p-Value
CONTROL	-0.31	1.0000
T1%	0.067	0.0188
T2%	0.387	0.0002

Table 4. Post-hoc analysis (Conover test) for pH values for chicken breast treated with *N. sativa* oil solutions after 6 days of refrigeration (DAY 6)

Factor	n	Average Rank	Different (P < 0.05) from factor nr.
(1) T1%	5	7.50	(3)
(2) T2%	5	4.00	(3)
(3) CONTROL	5	12.50	(1)(2)

Table 5. Dunnett's test (comparisons with Control) for TVB-N values of chicken breast treated with *N. sativa* oil solution after 6 days of refrigeration (Day 6). Control Group=CONTROL; a=0.05; |d|=2.50241 Positive values show pairs of means that are significantly different

LSD Threshold Matrix		
Level	Abs(Dif) - LSD	p-Value
CONTROL	-0.94	1.0000
T1%	-0.36	0.2501
T2%	0.158	0.0234

These results suggest that the T2% is more efficient for longer refrigeration periods, however the power of the tests is around 50%, therefore, there is a probability of approximately 50% to pick up on an effect that is present. In these circumstances, it is recommended to develop an experiment with at least 20 samples per level (statistical group).
In Day 1 the values of TVB-N for the Control have a mean of 24.98 NH_3/100 g. These results are different from the ones reported by Baston (mean value 20.5 NH_3/100 g) (Baston et al., 2002). One of the main reasons for this difference could be that the meat samples were

not purchased directly from the producer, in the first day after slaughtering. However, our mean value are similar to the mean values found by Baston (22.2-24.9 mg NH_3/100 g) for refrigerated meat samples after 3 - 5 days of refrigeration (Baston et al., 2002).

The pH and TVB-N values for the treated chicken meat were not significantly different compared to Control, after 3 hours from the treatment in Day 1 (Table 2).

On the other hand, current studies that are correlating *N. sativa* oil effects with the possibility of shelf-life extension, are made particularly for fish products and according to Commision Regulation no. 2074/2005, the value of 25 mg NH_3/100 g should be considered adequate for human consumption, as reported by Georgescu (Georgescu, 2020). This value is similar to the value accepted for poultry. Therefore, as reported by Raeisi, the 2% and 4% *N. sativa* plant extract has an delaying effect on TVB-N formation on *Oncorhynchus mykiss* refrigerated fillets (Raeisi et al., 2015). It is noted that the treatment method in this situation, is different by the one used in our experiment, but the results are indicating the same conclusion over a similar period of refrigeration time (Raeisi et al., 2015). Also, Ozpolat noted that *N. sativa* oil has indeed an delaying effect regarding the TVB-N formation in fresh *Barbus grypus* fillets, during storage at 2 ± 1°C (Ozpolat et al., 2017).

CONCLUSIONS

The 2% concentration solution was more efficient compared to the 1% solution regarding the chicken breast preservation during a 6 days refrigeration period.

After 6 days the measurements for the T1% were not significantly different from Control (non-treated meat).

The 2% solution of *N. sativa* pure oil, could be used by the food industry as an alternative to chemical preservatives, however, further investigations are needed. One of the important area to be investigated could be to identify the possible organoleptic changes of the meat during cooking after treatments with *N. sativa* pure oil solutions.

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