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STUDY CONCERNING THE EFFECT OF LAVENDER ESSENTIAL OIL ON THE QUALITY OF SMOKED SAUSAGES DURING STORAGE

STUDIU PRIVIND EFECTUL ULEIULUI ESENŢIAL DE LAVANDĂ ASUPRA CALITĂŢII CÂRNAŢILOR AFUMAŢI ÎN TIMPUL DEPOZITĂRII

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ABSTRACT | REZUMAT

Interest in the use of LEO (*lavender essential oil*) in the food industry has increased over the last 30 years due to its pleasant aroma and flavour, as well as its ability to control the development of infectious agents. In this paper, we investigated the possibility of using *lavender essential oil* in the manufacture of sausages, by observing the impact on the physicochemical and histological properties of smoked sausages during cold storage, over 9-days. Experimental sausages were made by mixing fresh pork with different concentrations of essential oils. Maintaining the pH of the followed-up samples is associated with lavender oil enrichment for most samples. It has been observed that the final pH of all supplemented samples is lower than that of the control sample; for higher concentrations, a decreasing pH is noted towards the end of the storage period. When it comes to pH, its preservative effect is obvious at all concentrations, but is more apparent at higher concentrations, as demonstrated by the fluctuations in the levels of TVN (total volatile nitrogen). In comparison to the control samples, samples containing high concentrations of oil demonstrate the integrity of the muscle fibres and the sarcolemma. To confirm the statistical significance of these conclusions, large batches of samples must be studied. A further study should investigate in more detail the role of lavender essential oil constituents as well as the mechanisms of synergy between these constituents. Further studies are also required for the standardization of investigation methods, validation of results, and assessment of new technologies.

Keywords: physicochemical and histological analysis, lavender essential oil, sausages

Interesul pentru utilizarea UEL (ulei esențial de lavandă) în industria alimentară a crescut în ultimii 30 de ani datorită aromei și aromei sale plăcute, precum și capacității sale de a controla dezvoltarea agenților infecțioși. În această lucrare, am investigat posibilitatea utilizării uleiului esențial de lavandă în fabricarea cârnaților, prin observarea impactului asupra proprietăților fizico-chimice și histologice în timpul depozitării la rece, timp de 9 zile. Cârnații experimentali au fost făcuți prin amestecarea cărnii de porc proaspete cu diferite concentrații de uleiuri esențiale. Menținerea pH-ului probelor urmărite este asociată cu îmbogățirea uleiului de lavandă pentru majoritatea probelor. S-a observat că pH-ul final al tuturor probelor îmbogățite cu ulei esențial de lavandă este mai mic decât cel al probei martor; pentru concentrații mai mari se observa o scădere a pH-ului spre sfârșitul perioadei de depozitare. Când vine vorba de pH, efectul său de conservare este evident la toate concentrațiile, dar este mai evident la concentrații mai mari, așa cum demonstrează fluctuațiile nivelurilor de TVN (azot ușor hidrolizabil). În comparație cu probele de control, probele care conțin concentrații mari de ulei demonstrează integritatea fibrelor musculare și a sarcolemei. Este necesar să se examineze loturi mai mari de probe pentru a verifica semnificația statistică a acestor concluzii. Modul de acțiune al constituenților uleiului esențial de lavandă și mecanismele de sinergie dintre constituenți necesită mai multe cercetări. Sunt necesare, de asemenea, standardizarea metodelor de investigare, experimente complementare pentru validarea rezultatelor și noi tehnologii.

Cuvinte cheie: analize fizico-chimice și histologice, ulei esențial de lavandă, cârnați

It has been known for some time that essential oils and their components have been used in food as preservatives and to extend the shelf life of food by inhibiting the growth of microorganisms. In the European Union, several essential oil components are approved for use as flavourings in food. In the United States, the FDA has registered flavours such as limonene, thymol, eugenol, carbonate, cinnamaldehyde, vanillin, carvacrol, and citral with the U.S. Food and Drug Administration (13, 14).

Lavender (Lamiaceae) is native to the Canary Islands and Cape Verde Islands, Madeira, Mediterranean

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Basin, North Africa, South Asia, Arabian Peninsula, and tropical NE Africa. Although the genus name is familiar, only a few species are known, such as *L. Angustifolia Miller* or *English lavender*, *L. latifolia* known as *spike lavende*r, and *L. stoechas* or *French lavender* (15). There are numerous varieties of *English lavender* grown for their essential oil, but they are also popular as garden plants. Natural antimicrobial compounds are prepared from different types of EOs and their constituents in order to minimize the effects of microbial activity in food products (2). The food safety issue is one of the current challenges for the meat industry. Foodborne pathogens and increasing antibiotic resistance are the major concerns of consumers. Plants produce several antimicrobial agents to protect against attack by plant pathogens. Herbal extracts fall under the term essential oils, although they are a complex mixture of aldehydes, ketones, alcohols, esters, phenols, and other constituents. (6, 20). Essential oils and their components have been used in food as preservatives and to extend the shelf life of food by inhibiting the growth of microorganisms (8, 9).

However, there are conflicting reports on the efficacy of essential oil supplementation in reducing pathogen transport, with at most negligible effect *in vivo* (16, 22). *Lavender (Lavandula angustifolia)* has been used in dry form or as an essential oil since ancient times and enjoys considerable attention due to its preservative, antimicrobial, and antioxidant properties, which are particularly due to its phenolic and polyphenolic substances (11). Both modern studies and the long-term use of lavender indicate that it can be considered a safe product in the food industry, where limited amounts of EO are generally used as part of complex blends (8). According to a study by Djenan and colleagues (2012) and Kueçuekyilmaz et al. (2017), the use of these ELOs instead of chemical-based antibacterial treatments could improve food safety and preservation (7, 18). It is imperative to be familiar with the physicochemical properties of essential oils, including, their range of target organisms, their mechanism of action, their minimal inhibitory concentration (MIC), and their effectiveness in preserving food (14). Despite this, little attention has been paid to their histological aspect against some of the most common pathogens found in meat and meat products.

In this study, we propose to examine the effects of lavender essential oil on the physicochemical and histological characteristics of smoked sausages during 9 days of cold storage and identify potential avenues for developing natural preservatives based on essential oils.

MATERIALS AND METHODS

The smoked sausage samples used for this study were traditionally prepared using 8 kg of pork trimmings (Fig. 1). Among the spices used in this experiment were (140g / kg) salt, (8g / kg) thyme, (8g/ kg) sugar, (12g / kg) pepper, granulated garlic (40g / kg) and drinking water (400ml / kg). The concentration of LEO used in the formulation of sausages was 0.2%, 0.5%, 1%, 1.5%, 2%, 2.5%, and 3%. The lavender essential oil (*Lavandula angustifolia*) was purchased from the Fares Company, Orăştie. Several steps are involved in the production of the sausages, including preparation of raw ingredients, weighing them, grin-

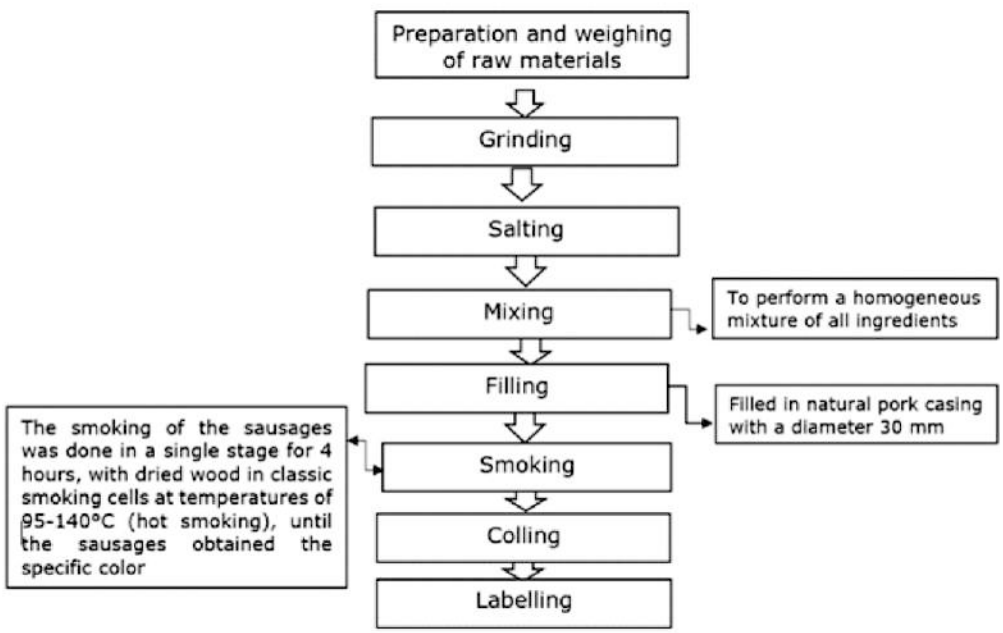

Fig. 1. Flow chart of Sausage samples enriched with lavender oil

ding, salting, adding LEO, and mixing for 15 minutes before filling in the pig membrane. The sausages were smoked for four hours, with dried beech wood, in classic smoking cells at temperatures between 95 and 140°C (hot smoking), until the desired colour was achieved (light red). Finally, they were cooled and labelled. A total of two batches were produced.

Preparation of the experimental batches

The samples were divided into two groups: treated samples and untreated samples = control samples. The treated group was divided into 7 groups (1000 g each) to which lavender oil was added at concentrations of 0.2%, (LEO1), 0.5%, (LEO2), 1% (LEO3), 1.5 (LEO4), 2%, (LEO5), 2.5% (LEO6), and 3% (LEO7). The freshness indicators were examined every 3 days and a microscopic examination was performed on the 3rd day of the experiment.

Methods

1. **Physical-chemical analysis**:

a. pH measurement,

b. Measurement of total volatile nitrogen (TVN)

The pH of the samples was measured using an AOAC method (Association of Official Analytical Chemists, 1990) (1). Total volatile nitrogen in meat was measured using the Romanian Standard, which provides for direct distilation of smoked sausage after the addition of magnesium oxide (MgO) (SR 9065-7: 2007) (2).

2. **Histological analysis**:

From the microscopic examination perspective, the samples were subjected to the routine method of paraffin embedding, using a histoprocessor, and sectioned and stained by the conventional haematoxylin – eosin (HE) technique. The sections were examined with the Olympus BX 41 Clinical Microscope (Deutschland GmbH, Hamburg, Germany) with an integrated computer photo shooting system

RESULTS AND DISCUSSIONS

Results of physical-chemical analysis:

a. pH trend

During different periods of storage, the pH values of samples containing lavender essential oil decreased more than in control samples. When the lavender concentration was increased to 3%, it had the greatest effect on lowering pH than lower concentrations (0,2 - 0,5%). Table 1 compares the pH values of various pork-smoked sausage formulations on the first day, third day, the sixth day, and the ninth day of storage. Based on the changes in pH during the storage of the samples for all batches, it can be concluded that lavender oil has a positive impact on the pH value of the samples on this indicator of freshness (Fig. 2).

Salem et al. (2010) found that thyme oil was added to minced beef, when stored at 4°C, with different concentrations of thyme oil (1.5%, 1%, and 0.5%), resulting in a significant reduction of pH values as compared with the untreated samples, and the highest concentration of the oil significantly reduced pH levels as compared to the lower concentrations (23). Those results are also in accordance with those reported by Brilliana et al. (2017), who reported that adding cinnamon essential oil to freshly ground beef can reduce pH values during refrigeration. This is due to the active components contained in cinnamon essential oils such as cinnamon aldehyde, linalool, and eugenol, which contain several aromatic compounds, similar to lavender (5). On the sixth day of the experiment, the pH value in the control samples decreased from 5.27 to 5.04, and then it increased to 5.34, on the last day of the experiment. The results suggested that the pH value in control samples declined from 5.27 to 5.04 over the six-day course. This result agrees with that of the Turkish researchers Kundakci (2007), Bozkurt and Erkmen (2004), and Kayaard and Gök (2004), who described how pH values started to decrease due to lactic acid production in Turkish sausages (sucuk) (4,17,19). The higher pH value of the control sample (pH=5,04) on the last day of the experiment indicates the level of meat spoilage caused by protein breakdown for free amino acid production, which is a precursor to NH3 and amines. Raw beef meat samples measured by Hashemi G., et al. (2017) had higher pH values in control samples than in samples treated with cinnamon oil, likely due to free amino acid production which produces NH3 and amines, which are alkaline compounds. Thus, the preserving effects of lavender essential oil can be seen at any concentration but are more pronounced at higher concentrations, as can be confirmed by the fact that TVN fluctuation indicates a preservative effect (12).

Table 1

pH values of different pork smoked sausage formulations as a function of storage time

	Day			
	1	3	6	9
Maximum admitted level in association with aw≤0.90 Canadian Federal Guidelines, Agriculture Canada	**5.4**	**5.4**	**5.4**	**5.4**
Control sample	5.27	5.06	5.04	5.34
Sausages with lavender oil 0,2%	5.15	5.26	5.28	5.26
Sausages with lavender oil 0,5%	5.41	5.25	5.26	5.27
Sausages with lavender oil 1%	5.14	5.25	5.25	5.28
Sausages with lavender oil 1,5%	5.21	5.24	5.23	5.26
Sausages with lavender oil 2%	5.26	5.21	5.2	5.11
Sausages with lavender oil 2,5%	5.29	5.2	5.14	5.08
Sausages with lavender oil 3%	5.34	5.19	5.13	5.01

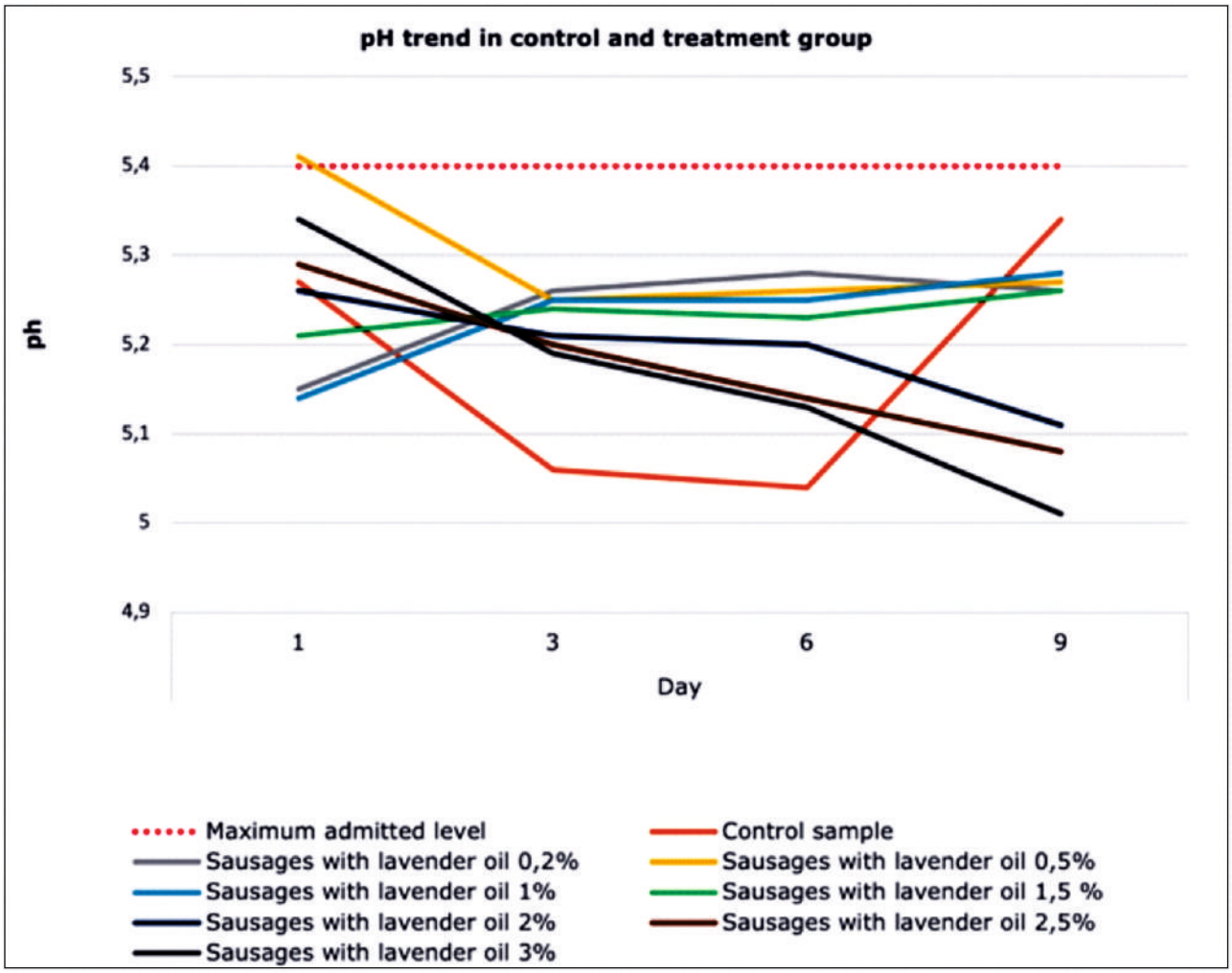

Fig. 2. pH trend in control and treatment group

b. TVN content

The samples treated with lavender oil at different concentrations showed a decrease in the values of total volatile nitrogen compared to those recorded in the control samples, especially on the 3rd and 6th day of the experiment (Fig. 3). The concentration of lavender oil (2.5 and 3%) was more effective in lowering the levels of total volatile nitrogen (TVN) than the rest of the concentrations. The more lavender essential oil is added to the sausage samples, the lower the observed TVN values. These lower TVN values were obtained because these plants contain preservative compounds. Generally, the TVN was a reliable indicator of autolysis and bacterial proteolysis in meat in relation to the amino acid concentrations, such as tyrosine and tryptophan, in a meat extract. These outcomes coincide with Liu, et al. (2009), who stated that TVN values and microbial numbers increased significantly during storage in control samples. In addition, the samples containing more essential oil had significantly lower TVN values (21). This trend can be seen in the findings of Biswas et al. (2011) and Shaltout, F et al. (2017) who found a reduction in TVN levels in meat treated with higher concentrations of cinnamon oil (1,5%) as compared with control samples (3, 24).

2. Histological analysis results

Observations of the control sample from a microscopic view show adipose tissue well attached to muscle tissue with both tissues showing a preserved morphology from a cellular perspective. Vegetable spices are evenly distributed throughout the product section. Adipose tissue and connective tissue are closely related (Fig. 4.) In the control samples, all components are well connected in the form of a single tissue conglomerate. There are sections of fragmented striated muscle tissue in the sausage specimens treated with low concentrations of oil, but most have preserved histological morphology, although others are interrupted by empty optical spaces, and have homogenous sarcoplasm and nuclei. The muscle tissue is surrounded by abundant adipose tissue with preserved cellular content and lysed nuclei. The presence of plant fragments and mineral salt aggregates is observed

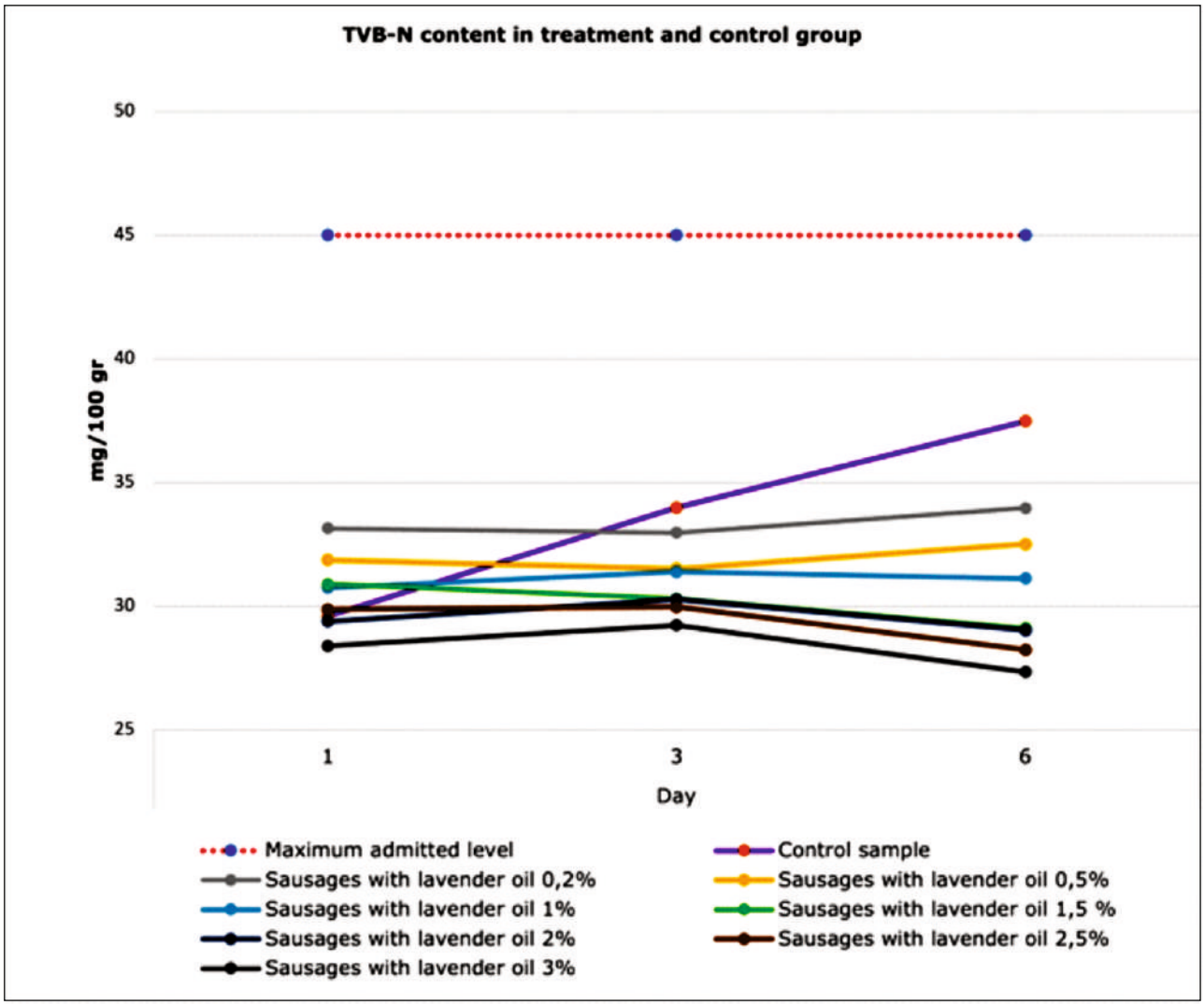

Fig. 3. TVB-N contents in treatment and control group

throughout the thickness of the histological preparation (Fig. 5). The tissue components are dispersed in the sections of the sausages treated with high concentrations of lavender oil and do not appear in the form of conglomerates (Fig. 7) as in the samples of sausages with very low concentrations of lavender oil. Smoking causes muscle fibers to lose their individuality, a change that is unrelated to lavender oil.

The results of other studies have shown that it is not possible to directly apply essential oils to food products. For example, in a study of fish meat, a biodegradable chitosan gelatine film was prepared, into which the EO was incorporated, and then applied directly to the fish (10). The development of new methods for using essential oils and a better understanding of their activity will lead to more mass use of products like lavender essential oil (safe and effective control agents). In the tissue structure of sausage samples, there was no significant difference between samples containing lower and higher levels of lavender essential oil. It is important to note that the tissue components of the control samples were more compact as a conglomerate than those of the treated samples, which were more dispersed.

CONCLUSIONS

The findings of our study revealed that lavender oil at different concentrations has a qualitatively demonstrated, effective preservative action based on the freshness parameters (pH and TVN) which agree with the literature and with our expectations. Microscopically, the integrity of the muscle tissue is preserved in the treated samples compared to the control sample. To confirm the statistical significance of these conclusions, experimental studies on large batches of samples are required. A further study should investigate in more detail the mode of action of lavender essential oil constituents as well as the mechanisms of synergy between these constituents. To understand how a mi-

croorganism works, standardization of investigation methods, complementary experiments to validate results, and new technologies are required.

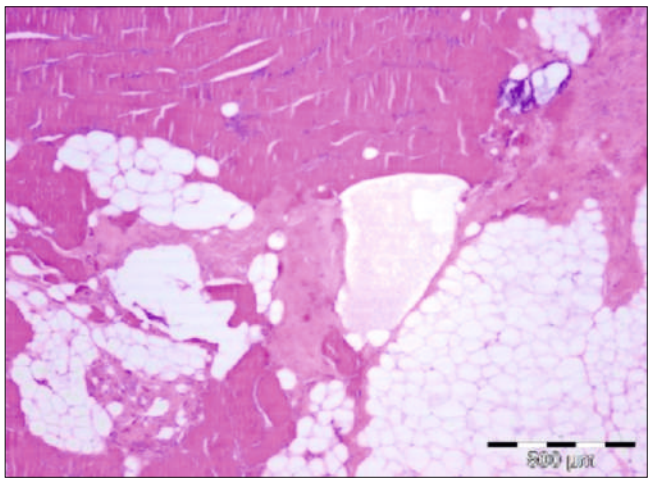

Fig. 4. Microscopic appearance of sausages control sample HE stains (ob. 10X)

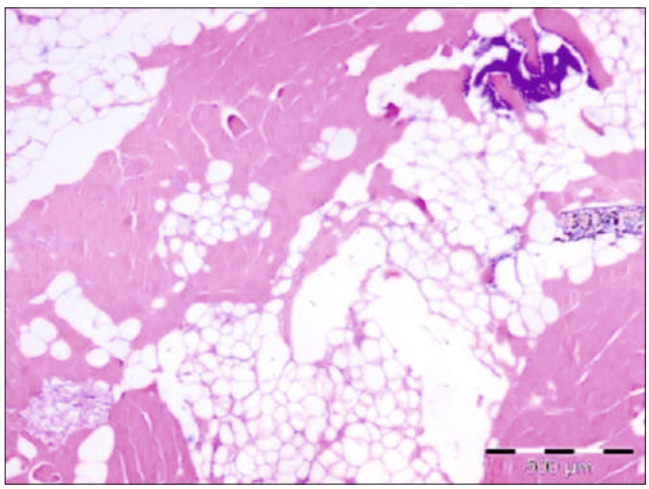

Fig. 5. Sausages enriched with lavender oil at a concentration of 1.5%, HE stains (ob. 4X)

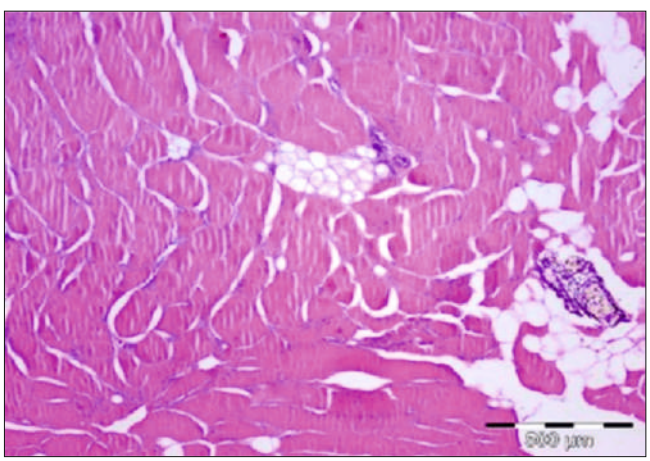

Fig. 6. Sausages enriched with lavender oil at a concentration of 3% HE stains (ob. 4X)

REFERENCES

1. *AOAC*, (1990), Official methods of analysis of the AOAC, 15th ed. Methods 932.06, 925.09, 985.29, 923.03. Association of official analytical chemists. Arlington, VA, USA
2. *Bhavaniramya S., Vishnupriya S., Al-Aboody M., Vijayakumar R., Baskaran D.*, (2019), Role of essential oils in food safety: Antimicrobial and antioxidant applications.Grain and 0il Science and Technology, 2(2):49-55
3. *Biswas S., Chakraborty A., Patra G., Dhargupta A.,* (2011), Quality and acceptability of duck patties stored at ambient and refrigeration temperature. Int J Livest Prod, 1(1):1-6
4. *Bozkurt H, Erkmen O.,* (2004), Effects of temperature, humidity and additives on the formation of biogenic amines in sucuk during ripening and storage periods. Food Science and Technology International,10(1):21-28
5. *Brilliana I., Manuhara G., Utami R., Khasanah L.*, (2017), The effect of cinnamon bark (*Cinnamomum burmani*) essential oil microcapsules on vacuumed ground beef quality. IOP Conference Series: Materials Science and Engineering, 193: 012057
6. *Dhifi W., Bellili S., Jazi S., Bahloul N., Mnif W.*, (2016). Essential Oils' chemical characterization, and investigation of some biological activities: A Critical Review. Medicines, 3(4):25
7. *Djenane D., Aïder M., Yangüela J., Idir L., Gómez D., Roncalés P.*, (2012), Antioxidant and antibacterial effects of Lavandula and Mentha essential oils in minced beef inoculated with E. coli O157: H7 and S. aureus during storage at abuse refrigeration temperature. Meat Science,92(4):667-674
8. *Erland L., Mahmoud S.,* (2016), Lavender (*Lavandula angustifolia*) Oils. Essential Oils In Food Preservation, Flavor And Safety. Ed. Preedy V.R., (ed.) Academic Press, London, UK, 501-508
9. *Georgescu M., Dobrea M., Tăpăloagă D., Raita S., Dobrea V.,* (2018), Functional evaluation of *Nigella sativa* seed oil effect on pathogen enriched Artisan cheese. Journal of Biotechnology, 280:S54
10. *Gómez-Estaca J., López de Lacey A., López-Caballero M., Gómez-Guillén M., Montero P.,* (2010), Biodegradable gelatin–chitosan films incorporated with essential oils as antimicrobial agents for fish preservation. Food Microbiology, 27(7):889-896
11. *Gulcin W.*, (2004), Comparison of antioxidant activity of clove (*Eugenia caryophylata* Thunb) buds and lavender (*Lavandula stoechas* L.). Food Chemistry, 87(3):393-400
12. *Hashemi Gahruie H., Hosseini S., Taghavifard M., Eskandari M., Golmakani M., Shad E.*, (2017), Li-

pid oxidation, color changes, and microbiological quality of frozen beef burgers incorporated with shirazi thyme, cinnamon, and rosemary extracts. Journal Of Food Quality, 2017:6350156
13. *Hashemi S., Davoodi H.*, (2010), Phytogenics as new class of feed additive in poultry industry. Journal Of Animal and Veterinary Advances, 9(17) :2295-2304
14. *Hyldgaard M., Mygind T., Meyer R.*, (2012), Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. Frontiers in Microbiology, 3:12
15. *Imelouane B., Elbachiri A., Ankit M., Benzeid H., Khedid K.,* (2009), Physico-Chemical Compositions and Antimicrobial Activity of Essential Oil of Eastern Moroccan Lavandula dentata. International Journal of Agriculture and Biology,11:113-118
16. *Inamuco J., Veenendaal A., Burt S., Post J., Tjeerdsma-van Bokhoven J., Haagsman H., Veldhuizen E.*, (2012), Sub-lethal levels of carvacrol reduce *Salmonella* Typhimurium motility and invasion of porcine epithelial cells. Veterinary Microbiology, 157(1-2):200-207
17. *Kayaardi S., Gök V.,* (2004), Effect of replacing beef fat with olive oil on quality characteristics of Turkish soudjouk (sucuk). Meat Science, 66(1): 249-257
18. *Küçükyilmaz K., Kiyma Z., Akdağ A., Çetinkaya M., Atalay H., Ateş A., Bozkurt M., Gürsel F.E.*, (2017), Effect of lavender (*Lavandula Stoechas*) essential oil on growth performance, carcass characteristics, meat quality and antioxidant status of broilers. South African Journal of Animal Science, 47(2):178
19. *Kundakci A., Kayacier A., Ergonul B.*, (2007), Effect of starter culture and packaging on the chemical, microbiological and sensory quality of turkish soudjouck (sucuk). International Journal of Food Properties, 10(3):537-547
20. *Laranjo M., Fernández-León A., Agulheiro-Santos A., Potes M., Elias M.*, (2019), Essential oils of aromatic and medicinal plants play a role in food safety. Journal Of Food Processing and Preservation, 46(8):e14278
21. *Liu D., Tsau R., Lin Y., Jan S., Tan F.*, (2009), Effect of various levels of rosemary or Chinese mahogany on the quality of fresh chicken sausage during refrigerated storage. Food Chemistry; 117 (1):106-113
22. *Michiels J., Missotten J., Rasschaert G., Dierick N., Heyndrickx M., De Smet S.,* (2012), Effect of organic acids on *Salmonella* colonization and shedding in weaned piglets in a seeder model. J Food Prot, 75:1974-1983
23. *Salem-Amany M., Amine-Reham A., Afifi S.A.,* (2010), Studies on antimicrobial and antioxidant efficiency of some essential oils in minced beef. Journal of American Science, 6 (12):691-202
24. *Shaltout F., Thabet M.G., Koura H.A.*, (2017), Impact of some essential oils on the quality aspect and shelf life of meat. BMVJ, 33(2):351-364
25. *** SR 9065-7:2007 Meat and meat products. Determination of total volatile /basic nitrogen content.