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INVESTIGATION OF MICROBACTERIAL ACTIVITY OF LAMIACEAE PLANTS IN MEAT PRODUCTS

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1. INTRODUCTION AND AIM

The globalisation of food trade has led to an increase in the prevalence of food-borne diseases caused by bacteria, fungi, viruses and parasites, which have become a significant global health concern. It is estimated that around 600 million food-borne infections and 450,000 deaths occur annually due to the presence of microorganisms in food. Microorganisms associated with food safety hazards are mostly responsible for self-limiting illnesses, including nausea, vomiting, abdominal cramps, diarrhoea and headaches.

Meat is considered one of the most perishable foods due to the microbial and oxidative deterioration processes that occur in the product. The microorganisms most commonly found in raw meat include *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, *Penicillium*, *Aspergillus*, *Fusarium* and *Alternaria* species, which can produce secondary metabolites that are toxic to humans.

In order to minimise the risk of contamination with food-bome pathogens, advanced processing and preservation techniques have been developed within the food industry. Since the mid-20th century, the use of artificial preservatives in food processing has been a common practice. However, consumer perceptions of processed foods have generally become negative, with a preference for additive-free alternatives over synthetic substances.

The following section outlines the objectives of this study:

- The objective is to investigate the potential uses of various medicinal and aromatic plants, their extracts and active ingredients in the meat industry.

- The investigation will also examine the effect of different medicinal and aromatic plants (thyme, sage, oregano, rosemary, basil) and their essential oils on the microbiology of raw sausage.
- The minimum inhibitory concentration (MIC) of the essential oils included in the study was determined, and 2-2 essential oils were selected per bacterium.
- The objective is to add essential oils to baking sausages and pork liver pâté that have been contaminated with pathogenic bacteria at MIC levels and to study their short-term effect on the bacteria used for contamination.

2. MATERIALS AND METHODS

The following experiments were designed, conducted and analysed, depending on the results obtained.

The initial stage of the measurement design involved the execution of preliminary experiments. In these experiments, the sausage mixture to be tested was prepared by combining commercially available meat ingredients (pork leg, pork fat) with the basic seasoning spices (black pepper, white pepper, cumin, red pepper powder, nutmeg, salt). The dried sage (*Salvia officinalis* L.) supplement selected for the initial study was sourced from the Food Science Department's own herb garden. The sage was incorporated into the product at a rate of 0.5%, 1%, 1.5% and 2%, respectively, while the control product was devoid of any herbs. Additionally, an extract of sage was prepared by alcohol extraction and its antimicrobial activity was evaluated by agar diffusion well test.

In light of the conclusions from the preliminary experiment, the experiment was continued using a premixed, ready-to-use sausage mix,

which more closely resembles the conditions typically encountered in an industrial setting than the product produced in a sterile laboratory. The selected herbs were medical sage, common thyme, basil, oregano and rosemary. The herbs were sourced from a commercial supplier. The dose concentrations of the dried herbs were determined based on the concentrations that had been previously described in the preliminary experiment.

In the third stage of the experiments, the essential oils of the herbs listed above were added to the sausage samples in equal concentrations, thus forming the basis of the subsequent analysis.

Additionally, an experiment was conducted to assess the impact of the essential oils on the cell count in the short term. To investigate this, products were artificially infected with pathogenic bacteria of known magnitude and essential oils were added. One of the products was the sausage paste that was already in use, while the other was liver pâté. The minimum inhibitory concentrations of the essential oils were determined for these experiments, and the lowest concentrations were selected and added to the products.

2.1. Raw and additive materials, chemicals, equipments and sausage production technology used in the preliminary experiment

2.1.1. Raw and additive materials

The meat used in the preliminary experiment (5000 g) was purchased from a nearby butcher's shop, and the spices used were commercially available (Kotányi Hungária Kft., Törökbálint, Hungary; Lacikonyha Magyarország Kft., Budapest, Hungary).

The sage used in the preliminary experiment came from the plot behind the Department of Food Science, Faculty of Agriculture and Food Science, Széchenyi István University.

The natural pig small intestine used in the production was also commercially available (Böllérbolt, Kőrös József Károly EV., Pécs, Hungary).

The vacuum bags used for the vacuum packaging of the products were purchased from the Győr site of Gasztronauta Kft.

2.1.2. Sausage production technology

Spices were added to the raw pork minced in a mincer to the appropriate particle size and to the minced lard, according to the recipe: sweet ground red pepper, salt, ground black pepper, ground white pepper, ground cumin, ground nutmeg, ground garlic. They were mixed in a blender until they were sufficiently homogeneous and had a particle size of 4-6 mm, then divided into 1-1 kg portions, to which the dried, grinded herbs or, in later experiments, the essential oils were added in appropriate quantities. The control product contained no added herbs or essential oils. The mass was filled into natural pig small intestine (Böllérbolt, Kőrös József Károly EV., Pécs, Hungary) and vacuum-packed in 150x200 mm vacuum bags (Gasztronauta Kft., Győr, Hungary).

2.1.3. Microbiological testing of sausage samples

The product was tested on days 0, 7, 14 and 21. A vacuum-packed raw sausage has a shelf life of approximately 14-21 days, due to the microbiological activity and enzymes that are still active.

The products have been tested in accordance with Decree 4/1998 (XI. 11.) of the Ministry of the Health, which establishes the permissible levels of microbiological contamination in foodstuffs.

Staphylococcus aureus was tested on Baird-Parker medium supplemented with egg yolk tellurite emulsion at 37°C for 24-48 hours. The Staphytect plus test was used for confirmation.

Yeasts and moulds were tested on Yeast Glucose Chloramphenicol agar at 25 °C and evaluated after 120 hours by counting yeast and mould colonies separately.

For *Clostridium perfringens*, Tryptose Sulfite Cycloserine agar was used at 37 °C for 24 hours and then supplemented with DRCM medium after incubation at 37 °C for 44 to 48 hours.

Coliforms and *Escherichia coli* were detected on ChromoCult Coliform Agar, evaluated at 37 °C after 48 hours. *E. coli* were confirmed using Kovács indole reagent (Biolab Ltd., Budapest, Hungary).

Total colony forming units were determined using Plate Count agar and plates were evaluated at 30 °C after 72 hours.

Salmonella were first enriched in buffered peptone water at 37 °C, after 24 h 0.1 mL were pipetted into selective Rappaport-Vassiliadis medium and incubated for 24 h at 41 °C. In addition to the RV, 1 mL was pipetted into Müller-Kauffmann selective medium and incubated for 24 hours at 37°C. Finally, Xylose Lysine Deoxycholate and Brilliant Green Phenol red Lactose Sucrose agar were selectively incubated for 24 h at 37 °C. *Salmonella* antigens were used for confirmation.

2.1.4. Making an extract from the sage

In the preliminary experiments, the method of Durling et al. (2007) was used, which consists of extracting the grinded sage after drying to constant weight in a drying oven at 35 °C for 24 h in a mixture of 81 v/v% ethanol:water at a ratio of 6:1 extractant:sample weight for 4 h in a 40 °C shaking water bath. After filtration, the extract was evaporated at 78°C to constant weight to remove the alcohol. The extract was stored in a refrigerator at +4±1 °C until the inhibition test was performed.

2.1.5. Agar diffusion well test

Prepare a solution of the material to be tested and pipette this into the pre-drilled holes in the Petri dish. The 10 mm wells will hold approximately 20 microlitres of solution and at the end of the incubation period, if there is an inhibitory effect, no bacterial activity will be observed around the wells. The pipetted solution diffuses into the agar plate to exert its effect.

The sage extract was tested against Salmonella, E. coli, St. aureus and Enterococcus faecium on Triptone Soy Agar after 24 hours at 37°C. At the end of the incubation period, the inhibition zones formed were measured.

2.2. Raw and additive materials, chemicals, equipments and sausage production technology used in the experiment with dried herbs

2.2.1. Raw and additive materials

For further experiments, the sausage mix was purchased pre-mixed, as we wanted to use the microflora found under industrial conditions as the basis for the tests.

The herbs used were also commercially available, again to model industrial conditions.

2.2.2. Microbiological testing of sausage samples

Sausage samples supplemented with selected thyme, sage, rosemary, basil, oregano at 0.5%, 1%, 1.5% and 2% were tested according to the methods described in section 2.1.4. The control product did not contain any dried herbs.

2.3. Raw and additive materials, chemicals, equipments and sausage production technology used in the experiment with essential oils

2.3.1. Raw and additive materials

For the further experiments, the sausage mix was purchased premixed, as we wanted to use the microflora found under industrial conditions as the basis for the tests.

The essential oils used in the experiments (Rosmarinus officinalis, Thymus vulgaris, Origanum vulgare L., Ocimum basilicum L., Salvia

officinalis L.) were purchased from NHR Organic Oils Ltd. (Brighton, United Kingdom) and NSH Organics Ltd. (Budapest, Hungary).

2.3.2. Microbiological testing of sausage samples

Using the methods described in section 2.1.5., samples of sausages supplemented with selected essential oils of thyme, medical sage, rosemary, basil, oregano at 0.5%, 1%, 1.5% and 2% were tested. The control sample did not contain any essential oils.

2.4. Raw and additive materials, chemicals, equipment and descriptions of microbiological tests for contaminated sausages with added essential oils

2.4.1.Raw and additive materials

For the further experiments, the baking sausage mix was purchased premixed, as we wanted to use the microflora found in industrial conditions as a basis for the tests.

The essential oils used were also commercially available, again to model industrial conditions.

2.4.2.Determination of the minimum inhibitory concentration (MIC) of bacterial strains and essential oils used for testing

Staphylococcus aureus ATCC 6538, Salmonella enterica subspecies enterica serovar Typhimurium ATCC 17028 and Escherichia coli ATCC 25922 from the Department of Food Science collection were used for the study.

For the study, clean cultures were used and inoculated onto TSA plates 16-24 h prior to inoculation to infect samples with fresh cultures.

The minimum inhibitory concentration (MIC) of the essential oils was determined using the macrodilution method recommended by the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2012). Essential oils were dissolved in 5% dimethyl sulfoxide solution (Molar Chemicals, Halásztelek, Hungary) containing 0.1% TWEEN-80 (Biolab Zrt, Budapest, Hungary) (Cazella et al., 2019) and pipetted into Mueller-Hinton broth (Biolab Zrt, Budapest, Hungary) at half dilution to obtain final concentrations ranging from 0.39-200 µL/mL (Perdana et al., 2021). Bacterial suspensions of Staphylococcus aureus ATCC 6538, Salmonella enterica enterica subspecies enterica serovar Typhimurium ATCC 17028 and Escherichia coli ATCC 25922, which were cell counted using a McFarland densitometer (Grant Instruments Ltd, Cambridge, United Kingdom) adjusted to 5 × 105 CFU/mL, were added to the tubes and mixed (Figure 6). After incubation (at 37°C for 16 h), 100 µL of resazurin solution (0.01%) (Germed, German Democratic Republic) was added to each tube and incubated for a further 1 h at 37°C. The MIC was defined as the lowest concentration of essential oil.

2.4.3.Microbiological analysis of the sausage samples

Samples for microbiological tests were taken immediately after preparation of the products (0 h) and after 16, 24, 48 and 72 h. The general microbiological status of the products was also evaluated (according to the Ministry of Health Regulation 4/1998) and the infestation tests were performed after evaluation of the results.

The control products contained only essential oil and no bacterial suspension was added. Samples were collected at 10 g using a sterile scalpel, weighed with 90 mL of sterile saline (0.85%) and homogenised

using a Stomacher 400 (Seward, Worthing, UK) shaker. Samples were serially diluted to 10-6 and 0.1 mL was pipetted onto the surface of TSA medium (Biolab Zrt., Budapest, Hungary). Samples were spotted onto the surface of the medium using a sterile disposable streamer and plates containing 10–300 colonies were counted after incubation (37 ± 2 °C for 24 h).

2.5. Raw and additive materials, chemicals, equipment and descriptions of microbiological tests for contaminated liver pâtés with added essential oils

2.5.1. Production of the liver pâté

To produce liver pâté, the raw materials must first be heat treated. During the heat treatment, the core temperature of the liver was over 81°C for 60 minutes. The heat treatment is partly necessary to reduce cooking losses during the subsequent cooking of the product. In addition, the product was inoculated to test the effect of essential oils on the added bacteria, presumably in a sterile product. The microbiological status of the finished product was checked before inoculation.

The cooked raw materials were minced in a cutter, then poured into a collagen artificial intestine (Böllér-Ker Kft., Pápa, Hungary) and cooked at 81°C core heat with 60 min heat reduction. After heat treatment, it was cooled to below 6°C in ice water. The products were stored in a refrigerator at 4 °C until inoculation and vaccination.

No additives or spices were added to the product as these could have inhibited the bacteria used during inoculation.

2.5.2. Contamination and microbiological analysis of liver pâtés

The control pâtés were also tested for Staphylococcus aureus, Clotridium perfringens, Enterococcus faecalis, Salmonella spp, Escherichia coli, coliforms and colony forming units according to Ministry of Health Regulation 4/1998.

Bacterial suspensions were prepared using a McFarland apparatus (Grant Instruments Ltd, Cambridge, UK) and the cell count was adjusted to 0.5, diluted and added to the product so that the product contained 105 TKE/g of bacterial cells each. Suspensions were prepared from sterile water (121°C, 15 min) and contained only the added bacteria. For control purposes, the suspensions were plated on Mueller-Hinton agar (Biolab Zrt, Budapest, Hungary) and the plates were counted after 24 h to be included in the evaluation.

The predetermined amounts of essential oil were first pipetted into the pre-measured paste and then homogenised. After sufficient uniform mixing, bacterial suspensions were added to the liver pâté samples, 1 mL suspension per 75 g sample, to achieve a baseline cell count of 10^5 TKE/g.

2.6. Statistical analysis of the results

SPSS statistical software package version 27 (IBM, New York, USA) and Microsoft Office Excel 2019 (Microsoft Corporation, Redmond, USA) were used for statistical analysis of the results.

In the statistical analysis, the results of the doped samples were compared with the control sample. In brackets, the p-value after the values is the two-tailed p-value obtained in the two-sample t-test, which indicates a significant difference below 0.05.

The results are presented as mean ± standard deviation.

3. RESULTS AND DISCUSSION

3.1. Preliminary test

In the preliminary test, *E. coli*, coliforms and total colony forming units also decreased from day 14, but the concentrations that resulted in the lowest cell counts were different (1.5% for *E. coli*, 0.5% for coliforms and 1% for total colony forming units). For *Salmonella*, all samples were positive by day 14, indicating that there was no effect of the addition of dried sage. There was no effect on yeast in either case. Mould counts also showed a decrease in the control sample, again with no effect of the added dried sage on the cell count. After a preliminary experiment, we decided to use industrial sausage mix and herbs and spices from the shops as they better model the microflora found in industrial conditions.

3.2. Industrial conditions

In the sausage samples, from industrial source, supplemented with dried sage, *E. coli* counts increased until the end of the experiment, with the control product having the lowest cell count. Coliform counts decreased from baseline as early as day 7 of sampling, were lowest in the 2% sample by day 14, and decreased most in the 2% sample. For total colony forming units, there was a decrease in cell counts from day 14, with the 2% sample having the lowest cell counts. *Salmonella* was only detected in samples up to day 7.

When dried oregano was added, *Cl. perfringens* decreased in the samples from day 7, with the concentrations resulting in the lowest cell counts varying on each sampling day (day 7: 1%; day 14: 1.5%; day 21: 0.5%). For yeast, the cell count decreased from day 14, with the lowest

cell count varying from day to day (day 7: 1.5%; day 14: control; day 21: 1.5%). For total colony forming units, the cell count increased steadily from day 14, with the 1.5% sample having the lowest cell count.

In the case of dried thyme, a slight decrease in *Cl. perfringens* was observed by day 7 and a great decrease by day 21. The lowest cell count was found in the 1% sample. In the coliform test, a decrease was observed in all cases by day 7, with the 2% dried thyme sample having the lowest cell count at the end of the study. In the yeast test, cell numbers had already decreased by day 7, resulting in the lowest cell counts changing with each inoculation, with the 2% sample having the lowest cell count on day 21. When all colony forming units were tested, cell counts increased on day 7, decreased on day 14 and increased again on day 21, when the control sample had the lowest cell count. *St. aureus* was only present in the samples from the first inoculation and was not detected in any of the subsequent inoculations. *Salmonella* was positive in the 0.5% sample on day 14 and in none of the other samples.

When dried rosemary was added, *Cl. perfringens* counts decreased from day 14, when the cell count was lowest in the 2% sample, and on day 21 in the 0.5% sample. For coliforms, a decrease in cell counts was observed from day 7, with the lowest cell counts in the 2% samples on days 7 and 14 and 1% on day 21. For yeast, the cell count increased gradually, with 1% on day 7, 2% on day 14 and control products having the lowest cell count on day 21. St. aureus was also detected in samples containing this herb only at the first inoculation. *Salmonella* testing showed positive results on day 14 only in the control and 1% samples.

Samples containing dried basil showed a decrease in *Cl. perfringens* cell counts from day 7, with the concentrations responsible for

the lowest cell counts varying (day 7: 1.5%; day 14: 1%). No typical colonies were found on day 21. For coliforms, a decrease in cell counts was observed from day 7, with the 0.5% supplemented sample having the lowest cell count on day 21. For yeast, the lowest cell count was observed on day 7 at a concentration of 1%. However, low cell counts were observed on days 14 and 21, with the 2% addition on day 14 and the 1% addition on day 21 giving the values obtained on day 14. For the fortified samples, the highest increase in total colony forming units was obtained with 1% fortification. Overall, the 2% sample showed the lowest cell count from day 14 onwards. As in the previous studies, *St. aureus* was only detected in the samples at the first day. *Salmonella* was only detectable in the 1%, 1.5% and 2% samples up to day 7, with the control and 0.5% samples still containing *Salmonella* on day 14.

The addition of dried herbs resulted in a notable impact at higher concentrations in some instances; however, this observed effect was not consistently pronounced. It can be stated that the results demonstrated that when rosemary was added in a dried form, *Salmonella* was not detectable in the added samples on day 7, with the exception of the 1% sample, which suggested an inhibitory effect. This was also observed in the case of coliforms, with the 1% sample exhibiting the lowest cell count. Dried thyme also demonstrated an inhibitory effect against *Salmonella*, with 1%, 1.5%, and 2% samples from day 7.

3.3. Essential oil addition

The samples with sage essential oil resulted in a reduction in the *Clostridium perfringens* count from day 7, with the 2% concentration exhibiting the lowest cell count of all samples by day 14. On day 21, no

typical colonies were observed on the plates. With regard to coliform, a reduction in cell count was observed from day 7 in all samples, with the 2% sample exhibiting the lowest concentration from day 14. In the case of yeast, the cell count exhibited an increase with the progression of the inoculation days in the samples that had been supplemented with essential oil. In contrast, a slight decrease was observed in the control sample from day 7. On day 21, the 0.5% sample exhibited the highest cell count. The detection of mould resulted in a slight decrease in cell count by day 14, with the absence of typical mould colonies in the samples by day 21. With regard to total cell counts, cell numbers increased gradually in all samples. However, in a considerable number of cases, there were notable discrepancies between samples. *Staphylococcus aureus* and *Salmonella* were only detectable in the samples at the initial inoculation.

The addition of oregano essential oil resulted in a decrease in *Clostridium perfringens* cell counts on day 7. The 2% sample exhibited the lowest cell counts on days 7 and 14, while no typical colonies were detected on day 21. In the coliform assay, cell counts exhibited a decline from day 7, with the 2% sample displaying the lowest cell counts. On day 21, no typical coliform colonies were observed on the plates belonging to the 2% sample, whereas typical coliform colonies were identified in the other samples. In the case of the total colony forming unit, cell counts exhibited an initial increase in all samples up to day 14, followed by a subsequent decline on day 21. From day 7 onwards, the 2% sample exhibited the lowest cell count in comparison to the other samples. Typical colonies of *St. aureus* were observed exclusively in the control sample by day 7. The 0.5% sample yielded positive results for *Salmonella* on day 7, the control sample on day 14, and the other samples at the initial test.

In the case of thyme essential oil, a decline in cell count was observed from day 14 onwards, coinciding with the detection of *Cl. perfringens*. The 2% sample exhibited the lowest cell count from the initial inoculation. On day 21, no typical colonies were observed, with the exception of the 2% sample. In the case of *St. aureus*, cell counts exhibited an increase from day 7 until day 14. At this point, only the control sample demonstrated the presence of typical colonies, which were no longer detectable on day 21. In the case of coliforms, cell counts exhibited a decline from the initial inoculation on day 7, with the control sample being the sole exception, displaying coliforms on day 14. By day 21, no colonies were present in the control sample. On day 7, the 2% sample exhibited the lowest cell count. The yeast cell counts in the samples exhibited slight fluctuations by day 7, followed by a decline by day 14, resulting in the absence of typical yeast colonies on the plates by day 21. The 2% sample exhibited the lowest cell count at all four sampling occasions. In all instances, total cell numbers increased by day 21, with the exception of the 1.5% and 2% samples, which exhibited a decrease. The presence of *Salmonella* was only observed in the control sample on day 7, with the 0.5% and 1.5% samples remaining *Salmonella*-free.

The addition of rosemary essential oil resulted in a notable reduction in *Cl. perfringens* by day 7, with a slight decline observed by day 14. However, by day 21, the control product exhibited the presence of typical colonies. A gradual decrease in coliform cell counts was also observed, with the 2% sample exhibiting the lowest cell count on day 7 and the 1.5% sample on day 14. In the case of yeast, no typical colonies were detected in the 1.5% sample on day 14, and in the other samples on day 21. When total colony-forming units were tested, there was an increase

in cell counts on day 14 and a subsequent decrease on day 21 in samples that had been supplemented with 1.5% and 2% essential oils. On day 7, the 2% sample exhibited no evidence of *St. aureus* colonies, and on day 14, neither sample did. With regard to the presence of *Salmonella*, the 0.5% sample exhibited positive results on day 7, while the control sample did so on day 14.

The addition of basil essential oil to the samples resulted in a notable reduction in cell counts when *Cl. perfringens* infection was tested. This was observed in both the 1% and 2% samples, which exhibited the absence of typical colonies on day 21. In the case of coliform, a reduction in cell count was observed on day 7, with the control and 0.5% samples exhibiting the presence of typical colonies on day 21. A reduction in yeast cell count was noted as the inoculation proceeded. On day 14, typical colonies were observed in the control, 0.5%, and 1% samples. On day 21, however, only the control sample exhibited typical colonies. For all colony-forming units, the cell count exhibited a gradual increase, with the 1% sample demonstrating the smallest increment. *St. aureus* was identified at the initial inoculation stage, occurring exclusively in the 0.5% and control samples. The *Salmonella* sample yielded a positive result on day 7, exclusively in the control sample.

The efficacy of essential oils in reducing the number of microorganisms has been demonstrated in multiple studies. In some instances, no discernible colonies were observed in products by day 14 or 21. However, the potential for microbial survival persists when consumed between days 0 and 14. Inappropriate kitchen preparation has the potential to result in illness among consumers. It has been demonstrated that the incorporation of oregano essential oil into meat products results in a

decline in cell counts of coliform and yeast. Similarly, essential oils of thyme, basil and rosemary can be incorporated into a variety of meat products. Nevertheless, the incorporation of essential oils at such elevated concentrations, even 0.5%, may already influence the organoleptic characteristics of the product, contingent on individual consumer preferences.

3.4. Contaminated samples, essential oil addition

A reduction in the number of *E. coli* bacteria present in the sausage samples was noted from the 16-hour mark onwards. The lowest cell count was observed in the oregano volatile oil sample, with a concentration of 0.78 µL/mL. In the samples containing *Salmonella*, a decrease in cell count was also observed from hour 16. The lowest cell count was produced by basil volatile oil (5.21 µL/mL) until hour 72, while the lowest cell count was observed from hour 72. In the case of *St. aureus*, a decrease in cell counts was observed from hour 48 onwards. However, the rate of decrease was negligible, even in the sample containing sage essential oil (5.21 µL/mL).

3.5. Minimal inhibitory concentration in liver pâtés

In liver pâté samples supplemented with a single MIC of essential oil, a reduction in the number of *E. coli*-infected samples was observed from hour 16 onwards in all cases. From hour 24 onwards, the sample supplemented with thyme essential oil (0.52 µL/mL) exhibited the lowest cell count, while there was minimal discrepancy between the cell count of the sample supplemented with oregano essential oil (0.78 µL/mL) and the control. With regard to *Salmonella*, a general decrease was observed from

hour 16 onwards, with basil essential oil (5.21 µL/mL) demonstrating the lowest cell count throughout. The reduction in cell count for both samples that had been supplemented with essential oil was insignificant in comparison to the anticipated level. The reduction in cell count was more pronounced in the control samples than in those supplemented with sage essential oil (5.21 µL/mL).

In liver pâtés, with *E. coli* administered at twice the minimum inhibitory concentration, a decrease was observed from hour 16 in samples supplemented with oregano (1.56 µL/mL) and thyme (1.04 µL/mL) essential oils. In contrast, the control sample demonstrated a steady increase in cell counts until the end of the study. The sample containing oregano essential oil exhibited the lowest cell count from hour 24 onwards. With regard to the *Salmonella* strain, the control sample also demonstrated a progressive increase in cell count until the conclusion of the study. In the samples containing essential oils, a decrease in cell count was observed from hour 16 onwards. The sample containing thyme essential oil (7.3 µL/mL) exhibited the lowest cell count until the conclusion of the inoculations. In the control sample, the cell count exhibited a gradual increase, even in the presence of *St. aureus* inoculation. Conversely, in samples containing twice the amount of sage essential oil (10.42 µL/mL), a decline in cell count was observed from the 24 th hour onwards.

In the case of the inoculated liver pâté samples, the reduction in cell count observed with twice the minimum inhibitory concentration (MIC) of essential oils was slightly more significant; however, this reduction did not yield the anticipated results. The essential oils were present in the liver pâtés at a concentration hundred thousandths of a percent. As evidenced in the literature, the antimicrobial effect of essential

oils is significantly influenced by the protein, carbohydrate, fat and other contents of the products in question. Nevertheless, even at such elevated concentrations, the organoleptic properties of the products are affected. A sensory evaluation was conducted with a concentration twice the minimum inhibitory concentration, which was not included in the dissertation due to the limited number of tasters (n=10). Nevertheless, it can be stated that even at this dosage level, on average, 32% of the tasters were able to identify the essential oil present in the sample. However, the results of the sensory tests indicated that when thyme essential oil was added at a concentration of 7.30 µL/mL, only 20% of the tasters were able to identify the essential oil of the plant in the product.

4. NEW RESEARCH RESULTS

1. I have confirmed that the addition of 0.5-2.0% of thyme, sage, oregano, rosemary and basil essential oils to sausage does not affect the viable counts of aerobic mesophilic microbes, yeasts, moulds and *Staphylococcus aureus*, nor the viable counts of *Salmonella* spp. At 2% essential oil concentration, *Clostridium perfringens* and coliforms were not detectable in the products at the time of final sampling, but these microbes may still be present in the products during the first 14 days. The inhibition observed is herb dependent: sage, oregano, thyme and rosemary can reduce *Cl. perfringens*, while basil can reduce *Cl. perfringens* and coliforms by 2-4 orders of magnitude.
2. In artificially inoculated liver pâtés, I demonstrated that basil and thyme essential oils applied at minimum inhibitory concentrations (1 × MIC) inhibited *Escherichia coli* from the 16th hour of

essential oil application and that thyme had a greater microbicidal effect compared to the control product at the end of the experiment. Basil had the greatest inhibitory effect on *Salmonella* species, while *Staphylococcus aureus* cell counts were not significantly reduced compared to the control product.

3. In artificially inoculated liver pâté, I demonstrated that oregano and thyme essential oils applied at minimum inhibitory concentrations (2 × MIC) inhibited *Escherichia coli* from 16 h after essential oil addition, and from 24 h of the experiment oregano had a greater inhibitory effect compared to the control sample, with *E. coli* cell counts showing an increasing trend throughout the experiment. For *Salmonella* species, oregano and thyme also had an inhibitory effect, while for *St. aureus*, sage had an inhibitory effect from hour 24 compared to the control sample.
4. I have shown that the MIC determined under laboratory conditions when used in a sample matrix can be influenced by a number of factors such as the composition of the raw materials and additives (protein, fat and carbohydrate content, pH) and the artificial environment in the laboratory (acid hydrolysed casein, beef extract, water soluble starch, pH of the culture medium).

5. PUBLICATIONS

5.1. Publications on which the thesis is based

Posgay, M.; Greff, B.; Lakatos, E.; Kapcsándi, K. (2023): Evaluation of Antibacterial Properties of Commercial Essential Oils on Foodbome Pathogens in a Liver Pâté-Type Product, Chemical Engineering Transaction, 107 pp. 253-258. (IF: 0,26)

Posgay, M.; Greff, B.; Kapcsándi, V.; Lakatos, E. (2022): Effect of Thymus vulgaris L. essential oil and thymol on the microbiological properties of meat and meat products: a review. HELIYON 8 : 10 Paper: e.10812. (IF: 3,776)

Posgay, M.; Kapcsándi, V.; Lakatos, Erika (2021): Antimicrobial effect of dried sage on the microbiological state of fresh Hungarian sausage. Acta Agraria Debreceniensis/Agrártudományi Közlemények, 189-192.

Posgay, M.; Lakatos, E.; Kapcsándi, V. (2020): The effect of herbs on the microbiological stability and nutritional quality of pariser. Acta Agraria Debreceniensis/Agrártudományi Közlemények, 101-104.

5.2. Papers published at a conference in Hungarian

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5.3. Other publications on other topics

Kapcsándi, V.; Lakatos, E.; Walcz, L.; Posgay, M. (2022): Gyógynövény drogok, valamint gyógynövény illóolajok antimikrobiális hatásának vizsgálata Escherichia coli, Salmonella valamint a Staphylococcus aureus baktériumok tekintetében, ACTA AGRONOMICA ÓVÁRIENSIS 63 : 1 pp. 33-53., 21 p.