

SUMMARY

of the doctoral thesis entitled:

RESEARCH REGARDING THE EFFICACY OF SOME NATURAL INGREDIENTS ON MORPHOLOGY AND PRESERVATION OF ARTISAN MEAT PRODUCTS

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KEYWORDS: natural ingredients, artisan meat products,
preservability, morphology

The main objective of the PhD thesis is to evaluate the impact of some natural ingredients, including essential oils, natural antioxidants, and different types of salt on the shelf life and histostucture of artisan meat products.

In this regard, the research conducted aimed at the following objectives:

A. Major objectives:

- Objective 1: Selection and characterisation of natural ingredients to be tested for further use in artisan meat products;
- Objective 2: Physico-chemical, microbiological, histological, histometric and sensory evaluation of the products obtained with the addition of the afore mentioned natural ingredients in order to determine their effectiveness on shelf life;
- Objective 3: Statistical interpretation of the data obtained and the determination of the natural ingredients with the highest efficacy according to the purpose.

B. Minor objectives:

Conducting a literature review regarding the following:

- The natural ingredients used in the meat industry for their preservative role;
- Future directions for using histological methods meat and meat products freshness assessment;
- Meat products freshness determination methods correlated to shelf-life.

The thesis is structured into two parts and eight chapters, according to the required writing rules. The first part comprises a literature review regarding the topics presented in the research objectives, while the second part presents the results of the personal research.

Part I

The bibliographical study presented in the first part is structured into three chapters.

Chapter I: RESEARCH ON THE USE OF NATURAL PRESERVATIVE INGREDIENTS IN THE MEAT INDUSTRY

KEYWORDS: *essential oils, natural antioxidants, natural non-iodized salt, iodized salt, salt mineral water*

In Chapter I are introduced the fundamental concepts that are critical for comprehending artisan meat preservation. The chapter begins by addressing the cultural and gastronomic importance and a general overview of artisan meat products. In addition, it discussed the main challenges associated with this type of meat products preservation without the use of synthetic additives. In this direction, considering the increasing consumer demand for healthier products, the chapter also addresses the significance of natural ingredients. Moreover, in accordance with the current consumers' preferences towards sustainable

and health-conscious food, it underscores the critical significance of natural ingredients in the meat preservation.

The stated objectives of the study encompass a review of artisanal preservation techniques as well as the potential application of natural antioxidants and essential oils within the artisanal meat processing sector (Subchapters 1.2 and 1.3). This chapter presents an exhaustive literature review that addresses various topics pertinent to natural ingredients and their impact on artisanal meat products. It then examines the scientific principles regulating salting and its effect on the qualities of animal products, beginning with an examination of historical and traditional salting techniques. Subsequently, the chapter discusses the use of natural antioxidants, such as herbs, spices, and plant extracts, for the purpose of meat preservation. Additionally, it explains how antioxidants work to reduce oxidative damage to meat, therefore preserving its quality and extending its shelf life.

The review subsequently proceeds to discuss essential oils obtained from plants, emphasising their capacity to inhibit pathogens and shield against damage. Moreover, a comprehensive examination of the essential oil extraction techniques and their practical applications in meat quality preservation is covered in this chapter.

Chapter II: RESEARCH ON THE USE OF HISTOLOGICAL METHODS TO DETERMINE THE FRESHNESS OF MEAT AND MEAT PRODUCTS

KEYWORDS: *meat, meat products, freshness, shelf life, muscle, histostructure*

In order to respond to the evolving landscape of food preservation, the development and use of innovative techniques for meat and meat products freshness assessment is imperative. In this respect, microscopic imaging stands out as a promising method, providing important informations beyond numerical analysis.

Sub-chapter 2.1 covers the historical background of this complementary method of integrity, quality and freshness determination in the meat industry and outlines current trends worldwide. In this respect, at its core, histological methods entail the meat and meat products meticulous examination, making possible the observation of the subtle structural and compositional changes as meat freshness decreases. These changes, which are most imperceptible in classical examination conditions, can be considered as markers for meat quality and freshness.

Subchapter 2.2, in addition to providing global perspectives, illustrates the challenges that the histological method inevitably faces. Some of the challenges are related to the need for specialised equipment along with expertise for sample preparation, resulting in a time-consuming and financially expensive analytical process. In addition, the results precision depends on several factors, such as specimen quality, operator expertise and staining techniques. Furthermore, although histological evaluation is informative, it may not cover all aspects of meat quality, such as taste and flavour.

Despite these challenges, histological analysis persists as a pragmatic and cost-effective tool for assessing meat freshness, especially when juxtaposed with alternative methods. Its worldwide adoption, endorsed by regulatory bodies in regions such as the European Union and the United States, underlines its essential role in modern meat preservation and quality control.

In the evolving landscape of the food industry, histological methods continue to provide a complex microscopic perspective on the world of meat and meat product freshness, consolidating their position as indispensable components of contemporary meat quality assessment.

In addition, subchapter 2.3 presents the methodology for obtaining and examining histological specimens and the usefulness of quantitative histological methods for assessing the degree of freshness of meat products and food products.

Chapter III: RESEARCH ON THE MEAT PRODUCTS FRESHNESS ASSESSMENT METHODS IN RELATION TO PRESERVABILITY

KEYWORDS: *Total Volatile Nitrogen Bases, pH, microbiological methods, NTG*

Meat and meat products freshness assessment, a complex approach involves both physico-chemical (Subchapter 3.1) and microbiological (Subchapter 3.2) evaluations. Among the physico-chemical assessments, the measurement of TVBN (Total Volatile Nitrogen Bases) plays a key role. High levels of

TVBN often signify increased protein degradation, a key precursor to meat spoilage, making interpretation essential for determining marketability. Moreover, TVBN levels show a significant correlation with the shelf life of meat products, highlighting their relevance in the domain.

Another important parameter used for meat and meat products freshness assessment is the pH measurement. pH influences the water holding capacity, colour stability and microbial safety, serving as a multiple-perspective source of informations. At the same time, microbiological evaluations play a key role in assessing the freshness of meat. The determination of NTG involves various methods, culture media and incubation conditions adapted to quantify bacterial populations. These meticulous approaches help to assess the microbial load in meat products and the results are interpreted to obtain quality information.

In this framework, the use of physico-chemical and microbiological assessments (NTG, *E-coli* and *Salmonella spp*), provide a strong foundation for determining the meat and meat products freshness and shelf-life. In conjunction, these methods act as essential instruments for quality and safety guaranteeing and for meeting the regulatory requirements.

Part II

The personal research is structured in four chapters.

Chapter IV: MATERIALS AND METHODS

KEYWORDS: *essential oils, natural antioxidants, salt, physicochemical methods, microbiological methods*

This chapter presents the research methodology, providing information on the selection and characterisation of natural ingredients, the production of artisanal meat products and the various methods used to evaluate the effects of the selected natural ingredients.

Subchapter 4.1 starts with the meticulous selection and characterisation of natural ingredients. *In vitro* microbiological examination of essential oils by the Agar Well Diffusion method revealed that *Cinnamomum zeylanicum* essential oil is an outstanding candidate, showing exceptional efficacy against common foodborne pathogens and spoilage agents. The chapter deepens the characterization by quantifying the polyphenolic content of the essential oil. At the same time, the methodology for obtaining lyophilised blueberry extract is presented, laying the foundation for a comprehensive characterization of the ingredient. The salt's and mineral-saline water mineral content and is accurately determined by the ICP-OES method.

The process of selecting artisan meat products (Subchapter 4.2) resulted in choosing artisan pork pastrami as the matrix for further research. Several compelling reasons, including the possibility that the matrix lends itself to efficient freshness monitoring by histological and histometric methods, underline this selection. The product was obtained following an artisan recipe obtained from a local producer in the Prahova County, ensuring the authenticity and fidelity of the research matrix.

Subchapter 4.3 takes an in-depth look at the methods used to assess the effects of selected natural ingredients. A wide range of physico-chemical and microbiological evaluations, including Total Volatile Nitrogen Bases, pH, NTG, *Listeria spp.* and *Salmonella spp.* highlight the impact of the ingredients on the meat products.

Furthermore, in order to complement the research by assessing product acceptability, a sensory evaluation was conducted. Additionally, the morphometric evaluations included measurements of nuclear surface area, perifibrillar space, muscle fibre diameter and adipocyte diameter, delving into the structural details of meat quality. All the histological and morphometric determinations, were analysed by using the Zeiss ZEN 3.4 software. The data collected was interpreted through statistical analysis methods using MedCalc V.22.007 software.

Chapter V: RESULTS AND DISCUSSION ON THE EFFECT OF CINNAMON ESSENTIAL OIL ON THE MORPHOLOGY AND PRESERVABILITY OF ARTISAN MEAT PRODUCTS - EXPERIMENT I

KEYWORDS: *essential oil, Cinnamomum zeylanicum, shelf life, morphology*

Chapter V presents the findings on the artisan meat products obtained with *Cinnamomum zeylanicum* essential oil addition.

In this regard, the research begins with the TVBN levels determination in artisan pork pastrami samples, revealing intriguing trends. In particular, the 0.5% UES (0.5% *Cinnamomum zeylanicum* Essential Oil) treatment consistently showed the lowest values of TVBN at all three time points: T0 (24 hours post-processing), T1 (30 days post-processing) and T2 (60 days post-processing). At the same time, throughout the research period, only the 0.5% UES treatment consistently kept the values of TVBN below the regulatory limit 45 mg NH₃/100g. This remarkable adherence to regulatory standards underlines the potential of this treatment in ensuring the safety and compliance of meat products.

Subsequently, the way in which different concentrations of UES interact with the pH levels is further explored, a distinct pattern emerging at all time points. The classification of pH levels reveals a consistent trend between treatments. At each time point, batch M (artisan pork pastrami with iodized salt addition) consistently shows the highest pH values, followed by UES 0.5%, UES 0.25% and UES 0.12%. This dynamic interaction between UES concentration and pH levels adds a level of complexity to the understanding of the physicochemical aspects of meat preservation.

In terms of microbiological determinations, at all-time points and across all treatment groups, the presence of *β-glucuronidase-positive Escherichia coli* remains at minimal levels (<9.1 cfu/g). Similarly, *Salmonella spp.* and *Listeria monocytogenes* are undetectable/25g. These findings highlight the effectiveness of the selected preservation strategies against common foodborne pathogens and spoilage agents.

At the same time, the histological perspective reveals significant variations in different aspects of muscle fibres and adipocytes. The appearance and number of muscle fibre nuclei, muscle fibre striations, Conheim fields and perifibrillar spaces show nuanced changes over the research period. These structural changes provide valuable informations regarding the evolution of meat products in time.

Moreover, the muscle fibre nuclei area shows a consistent decrease over time, with the 0.5% UES treatment showing the highest values after the M batch.

The evolution of muscle fibre diameter follows a similar trend, with a general decrease over time. The concentration of UES seems to influence this trend, with UES 0.5% having the highest values after batch M.

Examining the perifibrillar space between the sarcolemma and the endomysium, notable expansions were observed, especially with the 0.5% UES treatment. This expansion provides crucial information about the structural dynamics of meat products.

Finally, the research includes a sensory evaluation of the products obtained with the addition of UES in order to measure the consumer acceptability. Although no significant differences appear between treatments, the UES 0.25% treatment is perceived to be the juiciest and with the most intense flavour. In addition, consumer perceptions indicated the UES 0.5% treatment as having the most nonspecific odour and highest colour specificity.

Chapter VI: RESEARCH ON THE EFFECT OF LYOPHILISED BLUEBERRY FRUIT POWDER ON THE MORPHOLOGY AND PRESERVABILITY OF ARTISAN MEAT PRODUCTS - EXPERIMENT II

KEYWORDS: *lyophilised blueberry powder, Vaccinium myrtillus L., shelf life, morphology*

In Chapter VI a wide range of results and informations derived from the lyophilised blueberry powder (LAF) effect analysis on artisan meat products were presented. The research covers several aspects on the quality, shelf-life, and sensory attributes of meat products. The chapter begins by examining the TVBN levels in artisan pork pastrami, revealing a particular dynamic. In this regard, the LAF 2% treatment has the lowest values of TVBN at the initial time point (T0) and shows a remarkable efficiency at later time points (T1 and T2). However, the regulatory limits are exceeded at T2 moment.

The pH values classification exhibits distinct evident patterns at all time points, with batch M consistently displaying the highest pH values. In this regard, LAF 2% slightly exceeds the threshold value observed in the literature (5.3) at time T0, emphasising the essential role of acidity monitoring in meat preservation.

Microbiologically, all treatment groups maintain minimal levels of β -glucuronidase positive *Escherichia coli*, undetectable numbers of *Salmonella* spp. and absence of *Listeria monocytogenes* throughout the experiment.

On the other hand, the research reveals an opposite correlation between LAF concentration and NTG levels, with this parameter increasing over the research period. This result highlights the complex interaction between the preservation methods and microbial dynamics.

Regarding the histological examination, the research reveals variations in the appearance and number of muscle fibre nuclei, fibre striations, Conheim fields and perifibrillar spaces. These observations provide valuable information related to the structural transformations of meat products and the influence of LAF. Exploration of the muscle fibre nuclear area reveals a decrease over time, with the 2% LAF treatment showing the largest areas after the M group. The evolution of the muscle fibre diameter outlines a nuanced picture, with variability influenced by muscle type, genetic and pathological factors, these complex findings highlighting the challenge of applying the results beyond controlled experimental conditions. The perifibrillar spaces between sarcolemma and endomysium is highly noticeable for 2% LAF, this expansion being observed in all treatments over time. The results for the adipocyte areas showed a decrease observed over time, with the LAF 2% treatment showing the highest values. However, the intrinsic variability of adipocyte size highlights the need for cautious interpretation.

The sensory evaluations provide information on consumer acceptability. While consistency parameters do not show significant disparities, sensory attributes such as odour specificity (most non-specific odour - LAF 1.5% and LAF 2%), colour specificity (most non-specific - LAF 1.5% and LAF 2%), juiciness (lowest values - LAF 1.5% and 2%) and aroma intensity (most intense aroma - LAF 2%) show intriguing subtleties that provide information on product desirability.

Chapter VII: INVESTIGATIONS ON THE EFFECT OF IODISED SALT, NON-IODISED NATURAL SALT AND SALTED MINERAL WATER ON THE MORPHOLOGY AND PRESERVABILITY OF ARTISAN MEAT PRODUCTS - EXPERIMENT III

KEYWORDS: *iodized salt, natural non-iodized salt, salt mineral water, preservability, morphology*

Chapter VII presents an analysis of the findings and insights obtained from Experiment III, which examines the effects of various salting methods and types of salt on artisan pork pastrami.

In this direction, the study begins by analysing the levels of TVBN in artisan pork pastrami. At the initial time point of the research (T0), the treatment with natural non-iodized salt (SNN) recorded the lowest values, a trend that persisted throughout the study, including at T1 and T2 time points. However, it is important to point out that the TVBN values for the SNN treatment at T1 and T2 exceed the regulatory legislative limits.

At the same time, as far as the effect of different salting methods on pH levels is concerned, it becomes clear that they have a visible impact. The pH classification reveals distinct patterns at all time points of the research, with SI (iodised salt) consistently showing the highest pH values. Also, both SI and SNN treatments consistently exceed the threshold identified in the literature (5.3), highlighting the importance of pH monitoring in meat preservation.

Microbiologically, as all treatment groups showed minimal levels of β -glucuronidase positive *Escherichia coli* (below detection limit), undetectable numbers of *Salmonella* spp. and absence of *Listeria monocytogenes* throughout the experiment. The evolution of NTG shows intriguing dynamics, with SNN emerging as the most effective treatment, although NTG levels were above the threshold value (1×10^7 cfu/g) at T1 and T2.

In terms of histological examination, research shows fluctuations in the appearance and number of muscle fiber nuclei, muscle fiber striations, Conheim fields, perifibrillar spaces and adipocytes. The

aforementioned observations provide significant insights into the structural changes that take place in the meat products as well as the effects of various salting techniques.

Regarding the muscle fibres nuclear area analysis there is a progressive decrease, with AM treatment showing the highest values. Over time, a similar pattern is observed in muscle fibre diameter, with a high variability attributed to factors such as muscle type, genetic factors, and pathological conditions. Similar to other treatments, the complexity of this situation makes difficult the generalisation of the results beyond controlled laboratory settings.

In regard to peribrillary space between sarcolemma and endomysium, an enlargement was observed across all the treatments during the research period, with a particularly notable effect seen in the SI treatment. The evaluation of adipocyte areas demonstrates distinct patterns, the SNN treatment showing the highest values. Nevertheless, the inherent variability of adipocyte size emphasises the importance of careful interpretation.

The results of the sensory analysis provides further insights, showing no notable variations in the parameters of odour, colour, and consistency. On the other hand, there are variations in juiciness, with AM and SI receiving lower scores, while SNN stands out in this regard. The flavour intensity is also influenced by the treatment, and it has been observed that the AM and SNN treatments are perceived to have a more pronounced flavour.