THESIS OF THE DOCTORAL DISSERTATION

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THE USE OF BIOACTIVE COMPOUNDS AND HIGH HYDROSTATIC PRESSURE IN CHICKEN MEAT PRESERVATION

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1. INTRODUCTION AND OBJECTIVES

Chicken meat is vulnerable to quality deterioration; microbial contamination, oxidation, and organoleptic changes together with autolytic enzymatic spoilage. Lipid oxidation (LO) the most common form of chemical, non-microbial cause of quality deterioration in meat during processing. LO is mainly responsible for limiting the shelf life, increasing toxicity, and decreases the market value of meat. Moreover, microbial growth and contamination in meat are another major concern causing quality defects and possess potentiality to cause food-borne illness. Range of factors can cooperate in accelerating the spoilage process in meat products and growth of yeast, mold, and pathogenic microorganisms. These pathogens need to be controlled in the meat industry and the best strategy to improve the safety of meat products throughout the stages of preharvest, postharvest, processing, storage, distribution, and consumption is providing the adequate hygiene and the application of antimicrobial intervention technologies Various method has been applied for many years to control the growth of microorganisms and preserve the meat and food products including conventional thermal treatment and new strategies such as high hydrostatic pressure (HHP) processing, ultrasound processing, MAP (modified atmosphere packaging) and vacuum packaging, and pulsed electric fields (PEF) processing.

The consequences of these detrimental factors affecting meat and meat products can also be limited or inhibited using antioxidants/antimicrobials consequently extending the shelf-life and improving product quality. The antioxidants and antimicrobials can be of synthetic or natural origin. The demand for these synthetic antioxidant/antimicrobials has been decreased in recent years initiated the growing concern among consumers due to safety of synthetic chemicals and their potential toxicological and carcinogenic effects. Many natural BACs are receiving worthy attention for a number of a wide range of antimicrobial, flavouring, and antioxidant activities in preserving and improving the nutritional quality of food and meat products. More specifically, has benefits to eliminate undesirable food-borne pathogens, controlling spoilage microorganisms, reducing lipids/protein oxidation and preventing the secondary products from oxidation process (that lead to oxidative rancidity issues). Many EOs and their BACs are documented and considered to be ‘Generally Recognized as Safe’ to be applied in different food systems and approved by the Food and Drug Administration. Accordingly, they can participate intensifying the manufacture of heather meat and meat products with providing antimicrobial, antioxidant, antiulcer, insecticidal, antidiabetic and antinociceptive. However, only low concentrations of BACs can be applied in meat preservation, due to the serious flavour properties. The application of BACs and some EOs as meat preservatives are under investigation, not yet exploited commercially and requires detailed

knowledge about the minimum acceptable concentration and mechanism of action related to the mentioned properties in food preservation. There is a tendency in the meat production industry toward using the high-pressure processing (HHP) technique. HHP is a nonthermal pasteurization technology that effectively inactivates foodborne pathogens in meat and foods through applying proper pressure levels to keep nutritional values, and the sensorial (textural) characteristics almost intact. Yet, the integration of HHP with natural additives like EOs and BACs could achieve synergistical or additive effects to improve the preservative effects of HHP in maintaining the quality of food.

1.1. OBJECTIVES

Chicken meat is prone to quality deterioration during refrigeration storage, making serious issues to both consumers and producers. Based on the literature oxidation, physicochemical, microbial spoilage, and organoleptic changes are the major quality attributes that correlate with the decreased shelf life of fresh chicken meat. The overall objective of this study was to illustrate the application of natural bioactive compounds with mild preservation technology (HHP) in extending the shelf-life and improving the quality of fresh vacuumed chicken meat in refrigerated conditions. The specific aims of the study were:

- To investigate the antioxidant, antimicrobial and preserving activity against physicochemical properties of natural bioactive compounds in fresh ground chicken meat. For this aim allyl isothiocyanate, carvacrol, linalool, and piperine were proposed.
- To compare the *in vitro* antimicrobial activity of various natural BACs against six bacterial strains, three Gram-positive and three Gram-negative bacteria. In order to select the most BACs for further investigations in meat model.
- To apply the suggested BACs (α-Terpineol and allyl isothiocyanate) at different concentrations with vacuum packaging in preserving the qualitative attributes of chicken meat during refrigerated storage.
- The preservative effect of α-Terpineol and allyl isothiocyanate was examined separately. But what about if they are combined and used in combination with different levels of high hydrostatic pressure (300 and 600 MPa)? Do they have a synergistic effect to enhance the shelf-life of ground chicken meat at 4 °C in 3 weeks storage period?

2. RESULTS

The use of bioactive compounds from plant materials as natural antioxidants/antimicrobials has a great potential to preserve meat from deterioration. The application of natural BACs is presumed necessary to preserve the functionality, reduce the foodborne illness, control the quality losses in meat and meat products. However, the high concentrations can be restricted to avoid unacceptable levels of flavours and odours. The technologies like HHP have already been proved to extend the shelf life of various species of food. The combination of both BACs and HHP is gaining researchers' attraction to persist and preserve the nutritional quality of various species of food.

2.1. Use of allyl-isothiocyanate, carvacrol, linalool, and piperine to preserve fresh chicken meat during chilling storage.

In the first experiment, meat samples were mixed with 500 and 1000 ppm of AITC (allyl-isothiocyanate), CARV (carvacrol), LIN (linalool), and PIP (piperine) (dissolved in 5 % sunflower oil); while in control, no BACs were added (only sunflower oil). The samples for each parameter were then placed in polyethylene bags vacuum packaged and stored at 4 ± 0.5 °C for up to 8 days. Samples were taken at different time intervals for different analyses on days 0, 3, 6, and 8. The physicochemical properties of chicken meat (pH, colour, WHC), lipid oxidation (thiobarbituric acid reactive substances-TBARS), odour detection (E-nose based smell detection), and microbiological (aerobic mesophilic counts-AMCs) properties were monitored. Agar well diffusion was applied for CARV, AITC, LIN, and PIP to study the *in vitro* antimicrobial effect of these BACs.

The use of AITC, CARV, LIN, and PIP in chicken meat had shown a protective effect against some colour parameters and a nonsignificant reduction in LO compared to untreated meat. AITC particularly 1000 ppm of AITC showed considerably higher effect compared to other BACs in increasing L^*, b^* and h^*, decreasing a^* values, and caused a reduction in the numbers of AMCs. Compared to untreated samples an increased a^* value was perceived in meat treated with CARV, LIN, and PIP and has a great contribution towards the final colour intensity of the meat. Based on the storage time only significant WHC was detected with LIN-1000 compared to an increased drip loss in control, while PIP-500 exhibited almost stabilized efficiency in WHC. BACs mainly CARV and LIN had a clear protective effect against LO by keeping TBARS scores lower than 2 mg MDA/kg with a smaller flavour impact. Prolongation of the lag phase of the growth of AMCs was observed, except with PIP. Therefore *in vitro* antimicrobial effect of these BACs was studied. The AITC particularly 1000 ppm of AITC showed a considerably higher effect compared to CARV in

reducing the growth of *P. lundensis, St. aureus,* and *B. cereus*. However, CARV was more active in reducing the growth of *E. coli, L. monocytogenes,* and *S.* Typhimurium. LIN showed *in vitro* inhibitory effect against G+veB and G-veB bacteria except for *P. lundensis*. No inhibition activity was noticed for PIP. E-nose was able to classify the samples and detected odour accumulation of BACs in meat. The findings of the present study highlight the potential of BACs (CARV, AITC, LIN, and PIP) to enhance the quality of meat and meat products.

2.2. Evaluation of the in-vitro antimicrobial activity of bioactive compounds

In the second experiment, two methods: disc diffusion assay and MIC method were applied to evaluate the *in vitro* antimicrobial effect of selected BACs against six bacterial strains (three G+veB and three G-veB). The disc diffusion assay applied for the whole selected BACs including CARV, AITC, LIN, and PIP. Based on the *in vitro* (agar well diffusion and disc diffusion assay) the MIC method was determined for all selected BACs used in this studying. MICs for the BACs identified and the most active BACs were used in further experiments.

Applying the disc method, the components with the widest spectrum of antibacterial activity against the studied bacteria were found to be CARV, followed by thymol, eugenol. While. using MIC method AITC showed the best activity among all the BACs followed by geraniol, citronellol, CARV, αTPN (α-Terpineol), thymol, eugenol, LIN, and cuminaldehyde. The lowest MIC was found with AITC at 0.004 μl/ml against both *St. aureus* and *S.* Typhimurium. While α-Pinene and γ-Terpinene found to be less active to show MIC. Both AITC and αTPN were chosen as the most effective BACs in liquid form against AMCs, *L. monocytogenes, S.* Typhimurium, and *P. lundensis.*

2.3. *Effect of α-Terpineol on chicken meat quality during refrigerated conditions*.

In the third experiment, based on the *in vitro* antimicrobial activity and the MIC αTPN was used in MIC-1, MIC-2, and MIC-4. For the meat treatment the proportion of 5 % of a mixture of 0.25 + 4.825 + 1.5 g of BAC + DW + ethanol, respectively, in MIC-1 was used in 1000 g meat in MIC-2 these rates twofold and in MIC-4 these ratios were fourfold. The meat stored at 4 ± 0.5 °C for up to 14 days. Samples were taken at different time intervals for different analyses on days 0, 3, 7, 10, and 14. Later, the physicochemical properties (pH, colour, WHC), meat pigments, lipid oxidation (TBARS), odour detection (e-nose based smell detection), sensory properties, microbiological properties (AMCs, *L. monocytogenes, S.* Typhimurium, and *P. lundensis*) and myoglobin content of chicken meat were monitored.

The different concentrations of αTPN were able to alter the physicochemical attributes of chicken meat during 14-day storage. At the end of the storage period, αTPN MIC-2 and MIC-4 compared to control significantly increased pH and lightness, while MIC-1 was active in keeping the *L** values close to the initial *L** values, and *a** values decreased in meat containing higher-level αTPN. Increasing trends of *b** value and *C** colour intensity were detected at day 14 in samples containing a higher rate of αTPN compared to a slight decrease with no significant rate in MIC-1 and control. Moreover, different levels αTPN particularly MIC-2 and MIC-4 were able to show a significant effect on decreasing WHC. Besides, αTPN decreased MetMb, DeoMb, and increased OxyMb pigments. Additionally, the control group showed higher TBARS values compared to the rest of the samples, whereas the meat containing αTPN showed a reduction in TBARS values with no significant variation**.** This result indicates that the E-nose can classify the chicken meat as either fresh or spoiled with rancid flavour. The αTPN particularly higher level (MIC-4) showed antimicrobial activity against AMCs, *L. Monocytogenes*, caused total inhibition to the *P. lundessis, L. Monocytogenes, and S.* Typhimurium, while MIC-1 and MIC-2 kept the numbers of *P. lundessis* below 3.0 log CFU/g on day 14.

2.4. Effect of allyl isothiocyanate on chicken meat quality during refrigerated conditions

In the fourth experiment, based on the *in vitro* antimicrobial activity and the MIC AITC was used in MIC-1, MIC-2, and MIC-4. For the meat treatment the proportion of 5 % of a mixture of 0.008 + 49.944 + 0.096 g of BAC + DW + ethanol, respectively, in MIC-1 was used in 1000 g meat in MIC-2 these rates twofold and in MIC-4 these ratios were fourfold. The meat stored at 4 ± 0.5 °C for up to 14 days. Samples were taken at different time intervals for different analyses on days 0, 3, 7, 10, and 14. Later, the physicochemical properties (pH, colour, WHC), meat pigments, lipid oxidation (TBARS), odour detection (e-nose based smell detection), sensory properties, microbiological properties (AMCs, *L. monocytogenes*, *S.* Typhimurium, and *P. lundensis*) myoglobin content of chicken meat were monitored

AITC especially a high level of (MIC-2 and MIC-4) showed a significant decline in pH, an increase in *L** and folded *b** value and significantly decreased *a** values at the end of storage compared to control, while the addition of a low level of AITC (MIC-1) was effective in maintaining the *L** value. Similar to yellowness, increasing trends of *C** colour intensity were detected. At the end of the storage in contrast to MIC-1 showed an increase in WHC, while no significant effect was noticed in independent meat groups throughout the storage period. AITC decreased MetMb and DeoMb and increased OxyMb in chicken meat. The meat containing AITC showed a reduction in TBARS values visibly in meat treated with MIC-2 and MIC-4 with no

significant effect compared a significant increase in control. During storage, the least cell count of AMCs recorded in meat treated with AITC MIC-4. The cell counts of *L. monocytogenes* in all meat samples increased except MIC-4 which reduced the cell count by 2.3 log reduction. Regarding *S.* Typhimurium, the highest cell numbers were observed in inoculated control. However, *S.* Typhimurium numbers were decreased in meat treated with AITC. Moreover, *P. lundensis* increased in all meat groups except in meat contained MIC-4 of AITC did not exhibit the growth at day 10 onward. The E-nose showed that the concentration of AITC and the days of storage had overlapping between the control, meat treated with MIC-1 and MIC-2, while MIC-4 on different days exhibited a clear tendency to the opposite direction.

2.5. Combined effect of bioactive compounds with high hydrostatic pressure on quality attributes of chicken meat in refrigerated conditions

In the fifth experiment, based on the *in vitro* MIC of αTPN and AITC, the value of MIC-1 of BACs from experiment three and four was selected and combined with HHP at 300 and 600 MPa. Samples were taken at different time intervals for different analyses on days 0, 5, 10, 15, and 21. Later, physicochemical properties (pH, colour, WHC, water activity), meat pigments, lipid oxidation, E-nose based smell detection, spreadability-TPA, sensory properties, and microbiological properties (AMCs, *L. monocytogenes, S.* Typhimurium, and *P. lundensis*) of chicken meat were monitored.

The less concentration of BACs (MIC-1) applied with HHP. At the end of storage the pH in control and meat treated with αTPN were decreased, in contrast to HHP600 treated samples that showed a significant increase in pH values, and the αTPN was able to control the pH of αTPN+HHP600 and αTPN+AITC+HHP600. At the end of storage L^* of almost all treated meat with BACs and HHP were increased compared to decreased values in control, and the highest L^* value was recorded in meat treated with αTPN+HHP600. At the end of storage the a^* values were increased only in control and meat treated with αTPN+AITC, whereas the b^* values were increased in all meat samples but the significant level of increase was seen in meat treated with AITC+HHP300, AITC+HHP600, αTPN+AITC+HHP300 and αTPN+AITC+HHP600, and the highest rate of b^* values recorded in meat treated with αTPN+AITC+HHP600. At the end of storage, the WHC was decreased in all treated meat samples while the significant decrease only notices in meat treated with AITC+HHP600 compared increased WHC in untreated meat. No major changes were witnessed in the a_w in treated meat. Similar to the previous experiment the αTPN and AITC decreased the % of MetMb and DeoMb in meat αTPN+AITC, while OxyMb increased in meat contain BACs. Whereas the control and meat treated with BAC+HHP exhibited

the decreased in MetMb and OxyMb and increase in DeoMb for 21 days storage. The meat treated with HHP exhibited a higher rate of LO particularly HHP600 that was surpassed the control meat on day 21. The lowest TBARS value was noticed in meat treated with αTPN+AITC that was 0.171 mg MDA/kg, indicating that the activity of αTPN+AITC in reducing the LO was higher than either using BACs and/or HHP alone and even the combination of both BACs and HHP. At day 21 the lowest AMCs was seen in meat treated with αTPN+AITC+HHP600 that showed 1.9 log CFU/g and compared to control it caused 6.3 log reduction in AMCs. Throughout the storage period αTPN+AITC+HHP600, AITC+HHP600, and αTPN+HHP600 were efficient to inhibit the growth of *L. monocytogenes.* Additionally, comparing to inoculated samples the αTPN+AITC+HHP300, AITC+HHP300, and αTPN+HHP300 caused 4.9, 4.6, and 5.8 log reduction in *L. monocytogenes.* These findings indicate that the BACs only or low level of HHP (300 MPa) was not effective in reducing the growth of *S.* Typhimurium, while the BACs αTPN and AITC combined with a high level of pressure HHP600 had enhanced antimicrobial effect against the growth of *S.* Typhimurium. Moreover, the meat pressurised and treated with both BACs showed no growth of *P. lundensis*. The BACs (αTPN and AITC) with HHP decreased counts of AMCs and *P. lundessis* to less than 7 log CFU/g. Indicating that these BACs with HPP and particularly 300 MPs was sufficient to extend the shelf of chicken meat to 3 weeks at 4 ± 0.5 °C storage.

BACs combined with HHP exhibited softness enhances specimen to spreadability. This finding proved the previous result, the E-nose separated the pressurized meat either that treated with or without αTPN and AITC. Regarding the sensory properties, as the storage intervals increased, the sensory scores designed for the different sensory attributes in control decreased considerably, while in treated meat with BACs and HHP has increased particularly for odour, appearance, and acceptability to buy. In samples treated with αTPN+HHP300, αTPN+HHP600, AITC+HHP300, AITC+HHP600, αTPN+AITC+HHP300, and αTPN+AITC+HHP600 the sensory score were increased with storage intervals for appearance, and acceptability to buy, however, less than 5.0 were recorded, except for odour in AITC+HHP300, AITC+HHP600, αTPN+AITC+HHP300, and αTPN+AITC+HHP600 that showed score higher than 5.0 on day 21.

2.6. NEW SCIENTIFIC RESULTS

1- In this study, I proved that using the *in vitro* microbiological analysis (agar well diffusion, paper disc, and minimum inhibitory concentration assay), the selected bioactive compounds showed a wide range of inhibitory effects against *Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus, Escherichia coli, Salmonella* Typhimurium, and *Pseudomonas lundensis*. Applying the minimum inhibitory concentration method, the widest spectrum of antibacterial activity against these bacteria were found to be with allyl-isothiocyanate followed by geraniol, citronellol, carvacrol, α-Terpineol, and thymol with the average of 0.008, 0.063, 0.063, 0.25, 0.25 and 0.25 µl/ml, respectively.

2- In meat model I found that the combination of both α-Terpineol (0.008 µl/ml), and allyl-isothiocyanate (0.25 µl/ml) with low levels of high hydrostatic pressure such as 300 MPa was effective in reducing the growth of aerobic mesophilic counts and *P. lundessis* to less than 7 log CFU/g, indicating the shelf life of vacuum packaged ground chicken meat stored at 4 °C increased to up to 3 weeks.

3- I found that 600 MPa and especially α-Terpineol+allyl-isothiocyanate+600 MPa caused about 6 log reduction in aerobic mesophilic counts. Besides α-Terpineol+600 MPa, allyl-isothiocyanate+600 MPa, and α-Terpineol+allyl-isothiocyanate+600 MPa were efficient to exhibit less than the detection level of 1.7 log CFU/g for *P. lundensis, L. monocytogenes,* and *S.* Typhimurium in vacuum packaged ground chicken meat stored at 4 °C for 21 days.

4- Despite the extensive use of piperine in food preservation on industrial level, I found that piperine in powder from at 500 and 1000 ppm had no microbiological protection in vacuum packaged ground chicken meat stored up to 8 days at 4 °C. Besides using agar well diffusion and paper disc assay the piperine did not showed inhibitory activity against *P. lundensis, E. coli* O157:H7, *St. aureus, L. monocytogenes, S.* Typhimurium, and *B. cereus*.

5- I found that the selected bioactive compounds and mainly the most studied (carvacrol, linalool), and less studied (allyl-isothiocyanate, α-Terpineol) had a clear protective effect against lipid oxidation by keeping thiobarbituric acid-reactive substances scores lower than 2 mg MDA/kg in vacuum packaged ground chicken meat stored at 4 °C for 8, 14 and 21 days.

6- I observed that a higher level of pressure (600 MPa) increased lipid oxidation, and increased the hardness of meat, while the activity of α-Terpineol+allyl-isothiocyanate in reducing the lipid oxidation and making the meat softer was higher than either using bioactive compounds (allyl-isothiocyanate, α-Terpineol) and/or pressure alone.

7- I demonstrated that the electronic-nose was able to classify the meat samples and detected odour accumulation of bioactive compounds (allyl-isothiocyanate, carvacrol, α-Terpineol, linalool, and piperine) in meat depending upon their concentration, storage time, and levels of pressure.
8- I observed that a higher level of pressure (600 MPa) caused an increase in CIELab; *L**, *b** value, decrease in *a** value, and decreased water holding capacity with changes in meat pigments were noticed in pressurized meat regardless of the contents of α-Terpineol, and allyl-isothiocyanate. However, less amount of these bioactive compounds; allyl-isothiocyanate, and α-Terpineol, carvacrol, and linalool were active in keeping the colour values close to the initial values. Additionally, the presence of α-Terpineol and allyl-isothiocyanate decreased metmyoglobin and deoxymyoglobin and increased oxymyoglobin in chicken meat. Whereas bioactive compounds+pressure exhibited a decrease in metmyoglobin and oxymyoglobin and increased deoxymyoglobin.

3. CONCLUSIONS AND RECOMMENDATIONS

Overall, the *in-vitro* trial showed the strong antimicrobial effect of BACs and particularly αTPN+AITC by keeping the low numbers of both G+veB and G-veB bacteria. This antimicrobial effect is confirmed in the meat model with both αTPN+AITC. The BACs and HHP especially αTPN+AITC+HHP had a very strong antimicrobial activity against AMCs, *L. Monocytogenes*, *S.* Typhimurium, and *P. lundessis*. These BACs are in potential to improve the sensory attributes of meat. Likewise, high level of BACs and HHP increase colour values in meat, while BACs at MIC-1 was active in keeping the *L**, *a** and *b** values close to the initial values, αTPN alone was more effective in increasing *C** value of meat, while AITC was more effective with HHP in increasing *C** value of meat. αTPN and AITC decreased MetMb and DeoMb and increased OxyMb in chicken meat. Whereas BAC+HHP exhibited a decrease in MetMb and OxyMb and increased DeoMb in 21 days storage. It is known that due to the high binding capacity of BACs to proteins and fats in meat followed by a decrease in efficacy and the physical stability BACs, this study, the BACs at low concentration was still effective monitoring the quality attributes of chicken meat.

In the future, αTPN and AITC such ingredients come from natural sources ought to attract the interest for further research as a food additive that can culminate to its consideration as a functional preservative can yet contribute favourably and holistically to the promotion of consumer's health and well-being. Futures studies can be carried out on the combination of the BACs to evaluate their synergistic effect with high potential preservation activity in food. Moreover, a combination of BACs and technologies such as HHP, ultrasound and MAP in chicken and other species of meat to justify their application in meat and meat products. More studies needed to quantify the minimum concentration of both αTPN and AITC that shows preservative effect against food spoilage pathogens and exhibit the minimum sensorial and nutritional impact on meat. However, research on the effect of these BACs with HHP on the safety of meat and meat products in association to pathogens *Listeria* and *Salmonella* using the presence/absence detection test are required. Furthermore, more studies needed to use such instruments like an electron microscope to investigate the destructive effect of HHP with αTPN and AITC and other BACs on the morpho-structure of meat such as an increase in lightness and the mechanism of bacterial cell inhibitions.

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