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The use of bioactive compounds and high hydrostatic pressure in chicken meat preservation

Doctoral (Ph.D.) dissertation by

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AITC	Allyl-isothiocyanate
ANOVA	Analysis of Variance
AMC	Aerobic Mesophilic Count
ATP	Adenosine Triphosphate
AAs	Amino acids
αTPN	α-Terpineol
*a**	Redness
a_w	Water Activity
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
BAC	Bioactive compounds
B.	*Bacillus*
*b**	Yellowness
CI	Complete inhibition
CARV	Carvacrol
CFU	Colony Forming Unit
CDA	Canonical Discrimination Analysis
CIE	Commission Internationale de l'Eclairage
COMb	Carboxymyoglobin
DSC	Differential Scanning Calorimetry
DW	Distillate Water
DFD	Drak Firm Dry
DG	Dodecyl Gallate
DeoxyMb	Deoxymyoglobin
DMSO	Dimethylsulfoxide
EO	Essential Oil
E.	Escherichia
FOX	Ferric-xylenol Orange
FDA	Food and Drug Administrations
FAO	Food and Agriculture Organization
FRAP	Ferric Reducing Antioxidant Power
FIC	Fractional Inhibitory Concentration

GRAS	Generally Recognized as Safe’
GHPs	Good Hygienic Practices
GMPs	Good Manufacturing Practice
G-veB	Gram-negative Bacteria
G+veB	Gram-positive bacteria
GC	Gas Chromatographic
HACCP	Hazard Analysis and Critical Control Point
HHP	High Hydrostatic Pressure
HPLC	High-Performance Liquid Chromatography
*L**	Lightness
L.	*Listeria*
LO	Lipid Oxidation
]LC–MS	liquid Chromatographic-mass Spectrophotometer
LIN	Linalool
Mb	Myoglobin
MetMb	Metmyoglobin
MIC	Minimal Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
MAP	Modified Atmosphere Packaging
MDA	Malonaldehyde
MOSFET	Metal Oxid Semiconductor Field-Effect Transistor
NADH	Nicotinamide adenine dinucleotide
ORAC	Oxygen Radical Absorbance Capacity
OD	Optical Density
OG	Octyl gallate
OxyMb	Oxymyoglobin
PIP	Piperine
PTM	Pressure-Transferring Medium
PUFA	Polyunsaturated Fatty Acid
PSE	Pale Soft Exudative
PV	Peroxide Value
PCA	Principal Component Analysis
PG	Propyl gallate
R*	Lipid Free Radicals

ROOH	Lipid peroxide
RVM	Relevance Vector Machine
RM	Rigor mortis
RTE	Ready to eat
S.	*Salmonella*
St.	*Staphylococcus*
SFP	Staphylococcal Food Poisoning
SVM	Support Vector Machine
SEM	Scanning Electron Microscopy
TBHQ	Tert-butylhydroquinone
TSB	Tryptic Soy Broth
TSA	Tryptic Soy Agar
TBA	Thio Barbituric Acid
TPA	Texture Profile Analysis
TGE	Tryptic Glucose Extract
TBARS	Thio Barbituric Acid Reactive Substances
UFAs	Unsaturated fatty acids
WHO	World Health Organization
WHC	Water Holding Capacity
WBS	Warner-Bratzler shear force
WOF	warmed-over flavour
XLD	Xylose Lysine Deoxycholate
ΔE*	Total difference, Delta E

1. INTRODUCTION

Meat and meat products are perishable by nature and susceptible to quality deterioration by various sources causing spoilage, mainly during the preparation, storage, and distribution. Chicken meat is vulnerable to quality deterioration; microbial contamination, oxidation, and organoleptic changes together with autolytic enzymatic spoilage (Lucera et al., 2012). Which effects quality characteristics and can lead to undesirable reactions that deteriorate sensorial properties (e.g. flavour, odour, colour, and texture) of meat and meat products (Karabagias et al., 2011; Šojić et al., 2017). Therefore, if the meat and meat products not preserved and handled properly it could be a common vehicle for foodborne diseases and compromises the nutritional quality. Eventually, influencing product acceptance by consumers and potential public health issues, causing food insecurity and economic loss (Sant'Ana et al., 2012). It has been estimated that nearly 50 % of the total meat produced globally is spoiled and wasted at the level of household consumption as a result of poor preservative techniques and facilities (FAO, 2011).

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 (Sampels, 2013). As a result of the rapid depletion of endogenous antioxidants in meat after slaughter, oxidative damage can easily effect on lipids and proteins (Xiao et al., 2011). Oxidation of lipid is a complex process; depends on the chemical composition of meat, light, and oxygen access and storage temperature (Kanner, 1994). Moreover, microbial growth and contamination in meat are another major concern causing quality defects and possess potentiality to cause food-borne illness. Range of intrinsic and extrinsic factors can cooperate in accelerating the spoilage process in meat products and growth of yeast, mold, and pathogenic microorganisms. Major pathogenic bacteria including; *Listeria monocytogenes,* enterohemorrhagic *Escherichiacoli* O157:H7, *Salmonella* spp*, Staphylococcus aureus, Bacillus cereus, Campylobacter* spp*, Clostridium perfringens,* and *Aspergillusniger* (Dave & Ghaly, 2011). 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 (Gutiérrez et al., 2012). Concurrently with LO, microbial spoilage leads to significant sensory abnormalities in meat and meat products, therefore, exceptional protection required to offer extended shelf life. 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, microwave heating, irradiation, ozonation, cold plasma, and pulsed electric fields (PEF) processing (Figure 1) (Bahrami et al., 2020).

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. Synthetic additives currently permitted for use in foods are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), propyl gallate (PG), octyl gallate (OG) and dodecyl gallate (DG) has been widely used in meat and poultry products but with side effects. 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 (Fasseas et al., 2008; Jayathilakan et al., 2007; Karabagias et al., 2011; Shahidi & Ambigaipalan, 2015; Šojić et al., 2017). On the other hand, in recent years the use of natural and bioactive compounds (BACs) as preservatives, especially of plant origin that is known as medicinal plants are gaining a wide interest and has attracted the attention of researchers.

Many natural derivatives used in meat and food products that have potentiality as decontaminating agents are essential oils (EOs); rosemary, thyme, oregano, clove, grape seed extract, and BACs of fruits and plants; carvacrol, thymol, allyl-isocyanate, eugenol, linalool, piperine. These EOs and BACs could be imitative from plant segments and organs (for example buds, bark, seeds, leaves, fruits, twigs, wood, root, herbs, and flowers). The common methods of obtaining these BACs are; phytochemical screening assays, chromatographic techniques (HPLC), and non-chromatographic techniques (immunoassay and Fourier Transform Infra-Red) (Baker et al., 2017; Burt, 2004; Jridi et al., 2015; Naveena et al., 2006; Piñon et al., 2015; Preedy, 2015; Sasidharan et al., 2010). Several of these BACs are receiving worthy attention for a number of a wide range of antimicrobial, flavouring, antioxidant and organoleptic 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, preventing discolouration in food, reducing the need for antibiotics, reducing lipids/protein oxidation and preventing the secondary products from oxidation process (that lead to oxidative rancidity issues), shelf-life extension and strengthening immune cells in humans without leaving residues in the product or in the environment (Camo et al., 2008; Dufour et al., 2015; Fasseas et al., 2008; Fisher & Phillips, 2006; Gutierrez et al., 2008, 2009; Rokaityte et al., 2016; Tajkarimi et al., 2010; Yadav & Kamble, 2009; Zinoviadou et al., 2009). Whereas, some properties may lead to the reduction in the antimicrobial activity of BACs and limit their applications in meat and foods, including poor

aqueous solubility, pungent odour, and flavour, reaction with constituents of meat (Chacon et al., 2006; Nadarajah et al., 2005). Many EOs and their BACs are documented and considered to be 'Generally Recognized as Safe' (GRAS) to be applied in different food systems and approved by the Food and Drug Administration (FDA), European Union, Council Directive No. 95/2/EC of 20 February 1995 regulation on food additives and European Commission (2002/113/EC, 2002). Accordingly, they can participate intensifying the manufacture of heather meat and meat products (FDA, 2015; Karre et al., 2013; Preedy, 2015; Šojić et al., 2017) with providing antimicrobial (Burt, 2004; Chuang et al., 2020; Piñon et al., 2015), antifungal (Pattnaik et al., 1997), therapeutic potential (Gorgani et al., 2017), antioxidant (Hernández-Ochoa et al., 2014), anti-inflammatory, anticancer (Peana et al., 2002; Tasleem et al., 2014), antiulcer (Bai & Xu, 2000), antiviral (Astani et al., 2010), anesthetic (Ghelardini et al., 2001), ovicidal effects (Pandey et al., 2011), insecticidal (Pandey et al., 2013), antidiabetic and antioxidant activities in animal model (Arcaro et al., 2014), antinociceptive (Sousa et al., 2007). 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 and meat products preservation (Hintz et al., 2015; Tajkarimi et al., 2010; Tiwari et al., 2009). In Europe, the use of BACs such as AITC as a food additive, flavouring, anti-spoilage agent in food is under revision (EFSA, 2010).

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 in order to keep nutritional values, the sensorial (textural) characteristics almost intact, and provide a larger commercial shelf life of the product (García-Gimeno & Izquierdo, 2020). Several reports can be seen on the inactivation effects of HHP on foodborne pathogens in poultry meat such as for *Campylobacter jejuni* (Gunther et al., 2015), pathogenic *E. coli* (Sheen et al., 2018; Sommers et al., 2016), *Salmonella* and *L. monocytogenes* (Chuang et al., 2020; Li & Gänzle, 2016; Sheen et al., 2015). Despite the effectiveness of HHP in the inactivation of the targeted pathogens, the treatment of products at higher pressures (> 400 MPa) could cause a negative impact on the food quality (Chuang et al., 2020). 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. Limited research has been carried out on the preservation of meat using HHP and BACs as an effective hurdle approach to enhance the quality of the meat.

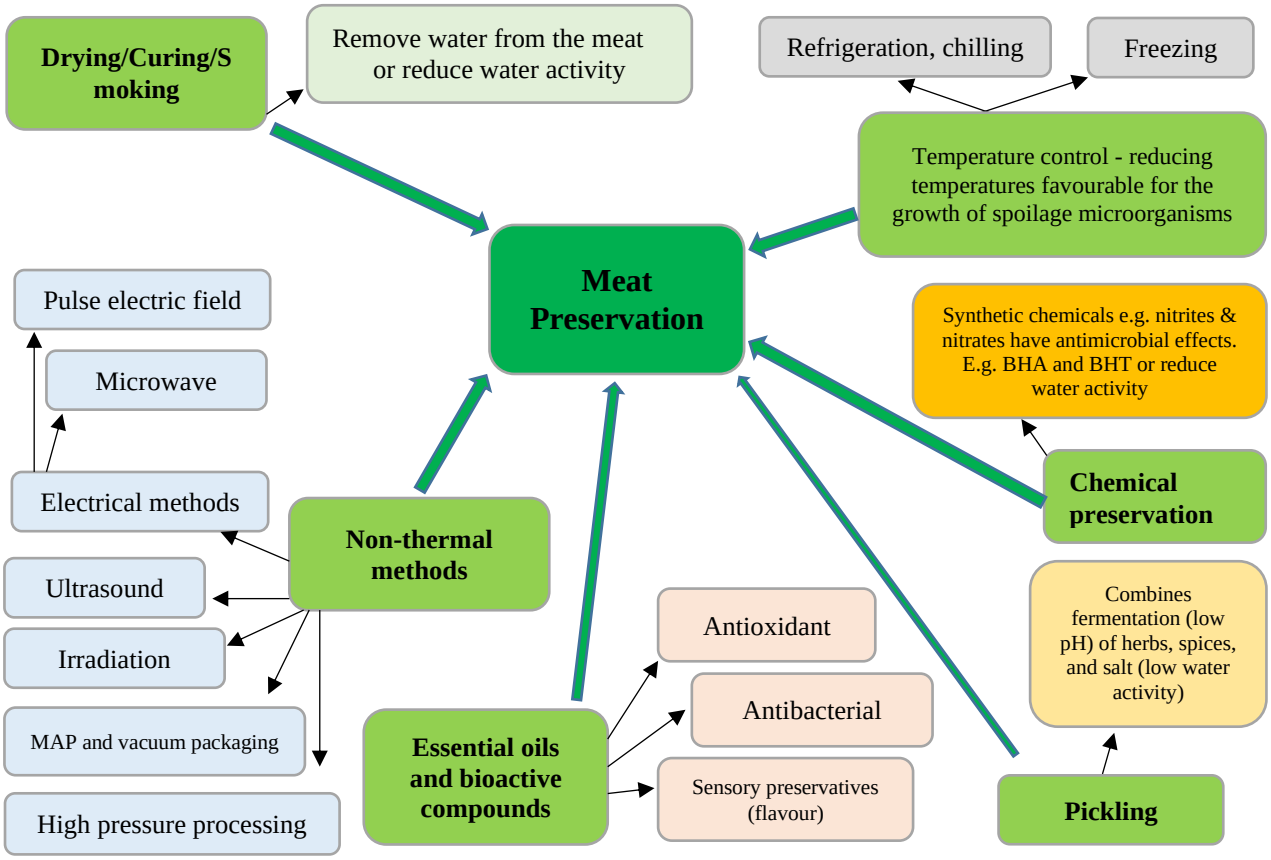


Figure 1: Various method used for meat preservation

1.1. Hypothesis:

- *In vitro* application of natural BACs can perform similar or better antimicrobial activity to the synthetic preservatives.
- The application of natural BACs can reduce the growth of a variety of spoilage microorganisms compared to the documented synthetic antimicrobials such as BHT and BHA.
- The use of combined BACs could have a synergistic or additive effect on the quality attribute of chicken meat during chilling storage.
- The use of HHP can increase the lipid oxidation, however, the use of HHP in combination with BACs can limit the oxidative rancidity and improve the sensory properties of meat.

1.2. 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 (High hydrostatic pressure) 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 17 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 physicochemical properties and sensory properties of ground chicken meat?

- Are the α-Terpineol and allyl isothiocyanate can empower the efficacy of HHP to control the lipid oxidation, and challenging the growth of aerobic mesophilic counts, *Listeria monocytogenes*, *Salmonella* Typhimurium and *Pseudomonas lundensis* to enhance the shelf-life of ground chicken meat in refrigerated conditions?

2. LITERATURE REVIEW

2.1. Meat quality

"Meat quality" is a term used to describe the overall meat quality elements including characteristics such as its physical, chemical, satisfaction (sensory quality), morphological, biochemical, microbial, technological (ability to be processed), security (hygienic quality), ethical, serviceability (ease of use, culinary), and healthiness (nutritional quality) properties (Mir et al., 2017; Northcutt, 2009). Both producers and consumers are interested in protecting the quality attributes of chicken meat. These attributes are often evaluated through assessing of physical properties, including colour, pH, water-holding capacity (WHC), drip loss, cook yield, water activity (a_w), juiciness, flavour, and texture (Warner–Bratzler shear force value), and microbial spoilage (Listrat et al., 2016; Nelson, 2015). With these claims the following sections are set to discuss the chicken meat quality attributes, methods implemented to assess them, and various factors affecting them.

2.2. Physicochemical properties of meat

2.2.1. Meat pH

Meat pH has been shown primarily to be associated with the muscle biochemical state at the time of slaughter and following the development of rigor mortis (RM). At the starting of the resolution of RM, the pH of the muscle drops due to the build-up of lactic acid and the occurrence of glycolysis. The pH of the chicken muscle normally at 24 hours post-mortem is 6.0-6.2 and it is equated with a multitude quality attributes such as colour, tenderness, WHC, cook loss and juiciness and directly affects the profit and shelf-life of the product (Barbut, 2015; Fletcher, 2002; Keeton & Osburn, 2010). The link between meat pH and biochemical or biophysical state and chemical reactions of the myoglobin (Mb) of the muscle indicates that a low-pH and/or high-temperature causes increased light reflectance properties. This might be explained by some proposed mechanisms such as high refraction in myofibrils, sarcoplasmic proteins denaturation, and high myofibrils surface reflectance (Swatland, 2004, 2008). Low pH value ($pH < 5.6$) near the isoelectric point can be characterized as watery refer to the light colour meat that often leads to pale, soft, and exudative (PSE), while high pH ($pH > 5.9$) higher than the isoelectric point lead to dry meat and refer to the dark colour meat that often characterized as being dark, firm, and dry (DFD) (Braden, 2013; Fletcher, 1999; Garcia et al., 2010). Low pH values and high temperature stimulate oxidation of meat pigments (Mb oxyhemoglobin) and protein denaturation and that lead

to lowering the WHC which results in increased cook-loss, drip loss, and decreased shelf life (Barbut, 1993). Additionally, low level of pH is an important hurdle for the growth of various foodborne pathogens nevertheless, some microorganisms like *L. monocytogenes* or *St. aureus* were found to have an increase in resistance to low levels of pH (~2.5) under anaerobic conditions during product manufacturing (Castellini et al., 2008; Roberts et al., 2020).

2.2.2. Water holding capacity

Water holding capacity (WHC) is one of the most important qualitative and functional parameters that affect the quality attributes and economic value of meat and meat products. WHC is the ability of meat to hold all or part of its water which plays a key role in defining the properties of both fresh and cooked meat (Bertram et al., 2002). WHC is closely related to sensory characteristics such as colour, texture, and firmness of raw meat, it is also related to the eating properties of cooked meat (Hughes et al., 2014). Besides, WHC affects the weight change during transport and storage, drip loss during thawing, weight loss due to purge and shrinkage during cooking, juiciness, and tenderness of the meat (Gault, 1985; Lawrie, 1985). Meat muscles are generally made up of protein structure and functionality that dominate the WHC (Puolanne & Halonen, 2010). Intercellular water (water between muscle fibers) occupies 5-12 % of the total water in meat, and the remaining is intracellular (within the muscle cells). It has been known that during storage the internal muscle fibers shrink laterally while expelling intracellular water to extracellular spaces that lead to increases in size (Guignot et al., 1993). Additionally, during post-mortem metabolism the rate and extent by which pH decrease is strongly influenced the WHC. High extent of pH decrease (acid meat) combined with a high temperature prior to slaughter can cause denaturation of muscle proteins, increased exudation and increased cooking loss and subsequently cause decrease in WHC of poultry meat (Huff-Lonergan & Lonergan, 2005). Moreover, pH influences the structure of myofibrils, and subsequently the WHC, tenderness, and colour features of meat (Castellini et al., 2008; Hughes et al., 2014). Meat with pale colour, softness, and poor WHC may develop in the muscle as a consequence of extensive protein denaturation and loss of protein functionality (McKee et al., 1998). Therefore, maintaining high-level WHC plays a key role to enhance the appearance, tenderness, toughness, juiciness, eating quality, yields and economical value of meat and meat products (Offer & Knight, 1988; Pedersen et al., 2003).

2.2.3. Water activity (a_w)

Water activity (a_w) is the water available in the food for biological reactions representing the water that not bound to molecules of food. The a_w could be defined as the ratio of the partial pressure of water vapour in the food to the partial pressure of water vapour above pure water at the same temperature (Belitz et al., 2009). The a_w determines the growth ability of micro-organisms, the reduction in a_w reduces the growth ability of micro-organisms. The a_w values above 0.98 are considered to be optimal for growth for the most spoilage microorganisms in meat (Hutkins, 2006). The reduction of a_w could be obtained either by drying fermented meat products or by adding additives and solutes such as salt or sugar.

2.3. Organoleptic properties of meat and meat products

Organoleptic (sensory) quality can include properties of meat such as texture, flavour (taste and aroma), and appearance (visual colour and instrumental colour) aspect. These are important quality attributes by which the consumers can easily determine the appropriateness of meat and meat products. Many factors have been studied that influence meat sensory attributes including, (a) *ante-mortem* factors such as; the age of bird at slaughter (e.g. flavour intensity is higher in meats from older animals), sex, strain, nutritional status, stocking density, stress level during handling and transportation, muscle type, fat composition and level, environmental conditions (litter, ventilation) and method of slaughtering. And (b) *post-mortem* factors such as; slaughter method, scalding temperatures, stunning techniques, carcass handing, ageing, cooking, product packaging, preservation and storage condition after cooking (Caballero et al., 2003; Farmer, 1999; Kyarisiima et al., 2011; Northcutt, 2009). Additionally, other factors such as microbiological deterioration of meat and rapid onset of LO are main problems that restrict the meat products acceptability and aids meat deterioration and casing reduced shelf life, decreasing nutritional value and functionality, drip losses, off-colour and off-flavour of the meat (Morrissey et al., 1998).

2.3.1. Methods used for assessing organoleptic properties of meat

The organoleptic characteristics of meat can be measured in different ways by either sensory panel or instrumental approaches or a combination of both. Each method has its advantages and disadvantages. Organoleptic quality attributes such as raw meat, ready to cook meat, ready to eat meat, cooked meat and dried sausage are preferred to be measured by a sensory panel. Standard sensory evaluation techniques for the organoleptic assessment include ranking or scaling, descriptive analysis, discrimination or scoring that carrying out by trained panelists

professionals/technicians/postgraduate students/consumers. In some cases, the panelists that involve in the sensory evaluation are qualified at different training sessions as due to ISO 8586 (2012) (Wood et al., 2004).

To find the highest organoleptically accepted concentration of EOs or a combination of EOs and their BACs in food and meat, both method panelists and man-made (electronic nose, electronic tongue) could be used. The panelist method is commonly used (Ghabraie et al., 2015; Rocío Teruel et al., 2015; Sharma et al., 2017), for this aim mostly meat products incorporated with different treatments and sensory evaluation of samples implemented to determine the samples that could pass the minimum accepted concentration. For cooking samples meat samples required inside product temperature about 72 – 80 ºC then it could be served with randomly coded identifications to panelists at (40-60ºC). Between tasting of each sample, plain water can be served to rinse the mouth. Such test could be carried out using individual booths constructed with air-conditioned and free of disturbing factors. For raw meat products, the sample should be kept in a container to preserve the sensory attribute e.g. odour prior to being assessed. For this type of product, the panelists evaluate different attributes such as general appearance (crust colour, mass colour), flavour, odour (odour intensity, rancid odour), for cooked meat; binding, texture (crispness, juiciness, firmness, and cohesiveness), taste (taste intensity, rancid taste) and overall acceptability of the product (Rocío Teruel et al., 2015; Sharma et al., 2017; Zhang et al., 2019). Usually, a panel of 8 to 10 trained tasters per session are involved carrying out the sensory analyses. The evaluation score by panelists of the sensory attributes for meat could be done using; a 9-point-point hedonic scale, where 9 scores represent an excellent product and 1 is giving for extremely poor meat products (Ghabraie et al., 2015).

The evaluation of organoleptic attributes can be rather laborious and expensive hence instrumental methods can be used to various extents. In Man-made instrumental methods including electronic noses (E-noses) and electronic tongues (E-tongues) could be used. The most instrumental sensory analysis aims to provide a method that will correlate to sensory evaluations, simply mimic the interaction of tested item with the human brain using smell and taste sensors (using gas and liquid sensors) (Baldwin et al., 2011). During the physical or chemical change, the volatile molecules are produced that interact with non-selective sensors by E-noses. These signals then could be sent to the computer that can make the grouping of sensors in several options; unique signal combinations, patterns or fingerprints, grouping based on a calibration and training process leading to the detection of the pattern (Baldwin et al., 2011). In the meat industry, the advantage of E-nose is most common in the assessment of the volatile changes that associated with organoleptic quality, spoilage shelf life, off-flavour production, taints and authenticity (Ghasemi-

Varnamkhasti et al., 2009). Rajamäki et al (2006) applied E-nose for evaluation of sensory quality changes in broiler chicken meat in MAP packages, they observed that E-nose could distinguish the fresh quality from deteriorated that was stable with certain microbial counts. The most common method classify from E-nose is through using the principal components analysis (PCA), and to use classifiers like algorithms suing the input such as support vector machine (SVM) and a relevance vector machine (RVM). E-nose and E-tongue each used in its own software package and their data are not integrated but the data from both E-nose and E-tongue could be imported into one program and then integrated (Wang et al., 2009). Despite using widely in food sensory evaluation, the two types of sensory measurement often span a very wide range of the correlation coefficients. Compared to human sensors the E-nose and E-tongue systems have disadvantages as they are influenced by the environment including temperature for both E-nose and E-tongue. E-nose affected by humidity and it causes sensor drift, even if calibration systems and built-in algorithms assist to compensate and reduce this effect (Baldwin et al., 2011).

2.3.2. Meat flavour

The flavour of meat is contributed by odour (smell) and taste of the meat and in general it is difficult to differentiate between them during consumption such as mouthfeel and juiciness (Calkins & Hodgen, 2007; Northcutt, 2009). The basic flavours that have been observed in meat include; sweet (sucrose), salty (NaCl), sour (citric acid), bitter (quinine sulfate), the flavours such as; metallic taste (ferrous sulfate) and umami (monosodium glutamate) has also been identified. Normally raw meat has little or no aroma properties the only taste could be like a blood-like taste. While the flavouring properties of meat develop during thermal treatment (cooking), however, the flavour precursors exist in the raw muscle such precursors are derivatives from the minor contents exist in muscle including carbohydrates, lipid, compounds such as amino acids (AAs), peptides, reducing sugars, vitamins, fatty acids, nucleotides and volatile components (Varnam & Sutherland, 1995). The meaty flavour and savoury, roast, and boiled properties of meat comes from the Maillard reaction between AAs, peptides, carbohydrates, and reducing sugars such as ribose in the meat while fatty aromas of cooked meat are obtained from the degradation of presented lipid (Kerry & Ledward, 2009). It has been reported that the flavour of raw poultry meat is attributed to unsaturated aldehydes that resulted from the oxidation of linoleic acid which can be made via triacylglycerol at high concentration (Coronado et al., 2002; Varnam & Sutherland, 1995). Addiotnally, Konopka et al. (1995) noticed that the main compounds that contribute to warmed-over flavour (WOF) in cooked beef, pork and chicken were; n-hexanal and *trans*-4,5- epoxy-(*E*)-2-decenal, WOF can be seen in reheated meat which resulted from LO and cause rancid

characteristics. It has been observed that the umami taste of food, especially in meat, can be enhanced through a combination of glutamate and 5-nucleotides (Kawai et al., 2002). The volatile compounds that produced from the Maillard reaction and LO have considered as major sources of chicken meat flavour these compounds are trans-2,4-decadienal, 2-furfurylthiol, methionol, nonanol, 2- trans-nonenal, ɣ-decalactone, β-ionone, p-cresol and 2- methyl-3-furanthiol. Various method and technology have been applied to preserve the meat flavour. Generally, when applying preservative techniques including the use of EOs and BACs the great amount of volatile compounds from plants, fruits, and vegetable sources should be considered to not change the original flavour of the meat (Lucera et al., 2012). During the process of eating, conditions such as anatomical and physiological characteristics of the person that eating the food and type of food matrix can affect the manner by which flavour is released from the product. Plant extracts, EOs and BACs have the antioxidant ability and they are effective in minimizing LO products such as pentanal and hexanal from chicken meat (Rababah et al., 2006). Moreover, electron beam irradiation has been applied to preheated chicken breast meat and showed a very little unfavourable effect on the flavour (Rababah et al., 2006). Additionally, MAP, freezing before irradiation, and the addition of some additives could decrease the odours associated with irradiation (Jayasena & Jo, 2013). High-pressure treatment has also been used in various meat products with showing no negative effects on the sensory quality (Hayman et al., 2004), whereas it has been stated that exposure of meat to a 300 MPa produce a better flavour and taste than the pressure of 450 MPa (Kruk et al., 2011).

2.3.3. Appearance and colour of the meat

Appearance, typically refers to the surface colour of meat, as a visual characteristic can play a crucial role and create the first impression that affects the perception consumers' about the product quality and affect their decision to purchase product (Mancini & Hunt, 2005). Colour is important meat sensory parameter that influenced by the addition of antioxidants and antimicrobials. Meat colour is frequently used in the poultry industry as an important indicator of freshness, wholesomeness, spoilage, and shelf-life of the meat. Thus, more than any other quality factor meat colour has influences on retail purchasing decisions. Enhancing the stability of meat colour via innovative techniques has been taken into consideration by the meat industry (Djenane & Roncalés, 2018). The colour of meat mainly depends on the concentration of myoglobin (Mb) in muscle and its chemical and physical state (mainly the concentration of O_2 and the oxidation state of Mb). The relative proportions of available Mb can determine the colour of the meat from purple-red, bright red and oxidized brown colour (Bak et al., 2019). The bright red colour is

universally used as a quality indicator of meat freshness and is preferred by consumers while brown colour (oxidative browning) is considered as a major indicator in fresh meats deterioration (Mancini & Hunt, 2005).

2.3.4. Instrumental methods used for colour measurement of meat

Several methods for objective measurement of meat colour has been deployed. For example, using reflectance spectrometry measurement and Near Infrared Reflectance (NIR). The colour of meat can be evaluated by using colour standards, by reflectance spectrometry measurement, or by video analysis (VIA). The NIR spectroscopy is characterized by speed, ease of use, non-destructive, and less interference from moisture or colour of meat (Liu et al., 2003; Saláková, 2012). Three basic properties can be used in colour measurement which is (1) Brightness which describes the colours on a scale of dark-light, (2) Hue which describes the difference in colours redness from blue, green from yellow, and (3) Saturation which describes the neutral gray to a pure hue. Various systems for colour measurement has been introduced and applied by a foundation of CIE (Commission Internationale de l'Eclairage) in 1931, including (1) Munsell System which describes the colour by three attributes as hue (h^*) value or lightness (V) and chroma or colour purity (C^*) (AMSA, 2012), (2) CIEXYZ System, the CIE system in instead of the "real" primary colours of red, green and blue, it uses an X, Y, and Z space, (3) CIELAB system this system is one of the most commonly applied to measure the colour of an object in this system the colour coordinates (L^*, a*, b^*) used, the a* is part of a spectrum of wavelengths corresponding to colours from green to red ($-a^*$ to $+ a^*$), b^* from blue to yellow ($-b^*$ to $+b^*$) and L^* signifies the lightness of the meat (100 = white, 0 = black), Hue angle = $\tan^{-1}$ (b^*/a^*) the larger angles the more yellow and the more discoloured, and the saturation index = $(a^{*2} + b^{*2})½$, the larger values the more intense colour (Konica Minolta, 2006).

2.3.5. Myoglobin pigment and oxidation of colour in the meat

The most abundant pigment compounds found in meat are myoglobin (Mb) and haemoglobin (Hb) that physiological function is to carry and distribute oxygen to the different tissues. The chicken meat particularly the breast meat is primarily made of white muscle fibers, which is low in Mb while the thigh part is composed of red fibers, which is higher in Mb making them appear darker (Barbut, 2015). Myoglobin which is a haem-containing sarcoplasmic and water-soluble globular protein with a molecular weight of ~16.7 kDa is the main molecules pigments that responsible for the red colour of the meat. Mb represents 70 – 90 % of the total concentration of haem proteins it might present in any forms dictated by the redox state and nature

of the sixth ligand species of the haem iron, containing 8 a-helices (A to H) linked by short non-helical parts (Mancini & Hunt, 2005). The remaining colour of the meat comes from the Hb which is occurring and circulates mainly in the blood and it plays a minor role in meat colour due to its small quantity that exists in the tissues after slaughtering (Muchenje et al., 2009).

During the fresh meat processing, the aeration can facilitate oxidation of Mb. Mb normally can be available in the muscle in four different redox states depending on the state of the haem group (Figure 2) (Mancini & Hunt, 2005), namely oxymyoglobin (OxyMb), metmyoglobin (MetMb), deoxymyoglobin (DeoxyMb) and carboxymyoglobin (COMb). Oxymyoglobin (Fe^{2+}) is formed through the oxygenation which is the interaction of Mb with oxygen (O_2) it gives a meat desirable red colour. Mb and OxyMb have the capability to lose electrons (Brewer, 2004). Further oxidation is due to the interaction of present O_2 with the metal surfaces pigments and the O_2 is absorbed and binds to the iron. The Mb is then oxygenated to form OxyMb that produces MetMb with the production of brown colour in the product (Mancini & Hunt, 2005). The reduction of MetMb can produce OxyMb and DeoxyMb. The reduction of MetMb is significantly dependent upon scavenging enzymes of muscle's O_2, enzyme systems reduction, and the nicotinamide adenine dinucleotide (NADH) pool. Inconsistently, both NADH pool and enzyme activity could be reduced due to the time progresses at post-mortem (Mancini & Hunt, 2005). Additionally, at low-O_2 partial pressure circumstances, the OxyMb proceeds through the ferric redox state via consuming O_2 to form DeoxyMb. The meat pigments Mb (or DeoxyMb), OxyMb and MetMb in meat might change from one to other depending on the storage conditions. Oxidation of ferrous OxyMb (ferrous Fe^{2+}) to ferric brown MetMb (ferric Fe^{3+}) state is related to meat discolouration and results in loss of value (Smith et al., 2000). Also, carbon monoxide (CO) can react with DeoxyMb to red colour COMb while oxidation of COMb can form MetMb. It has been known that DeoxyMb is more easily converted to COMb than is OxyMb or MetMb (Mancini & Hunt, 2005). Meat colour is strongly influenced by the amount of available Mb, storage conditions, pH, available O_2, and meat temperature. An example is an increase in pH value caused decreases in the lightness of breast meat colour, also the shelf-life of meat is could be limited by the formation of layers Mb oxidation (Fletcher, 1999). Low pH can also stimulate the oxidation of Mb and OxyMb to MetMb (Kralik et al., 2017). Moreover, the total amount of Mb in meat depends on the bodyweight of the animal, Mb concentration in the muscle (red muscles are rich in Mb and white muscle is poor in Mb), and the degree of muscle development.

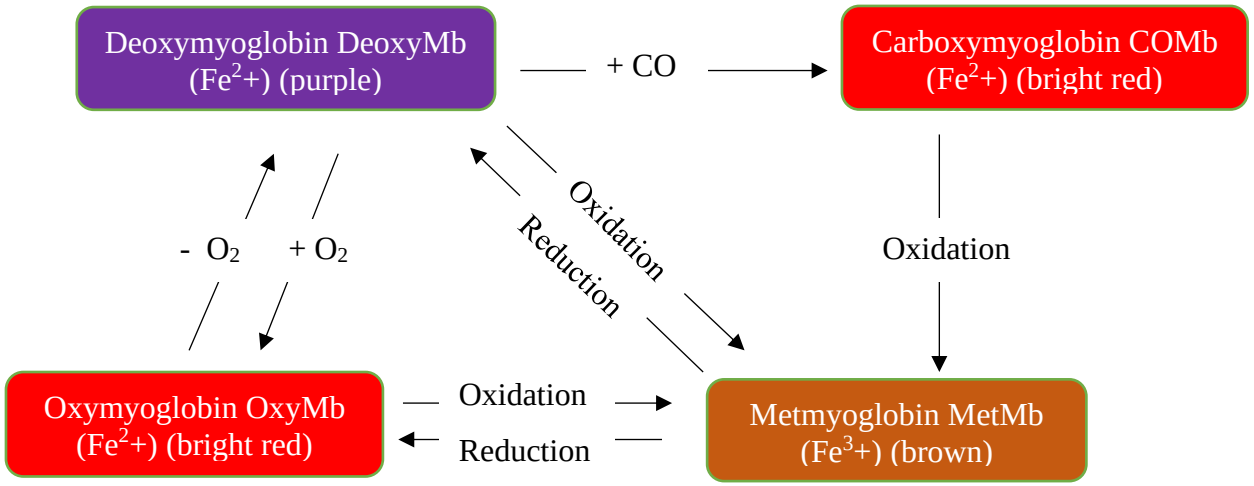


Figure 2: Myoglobin redox interconversions forms in fresh meats (from Mancini & Hunt, 2005).

2.3.6. Methods for assessing the meat pigments

Several methods have been implemented to estimate the amounts of each Mb redox form the meat surface (AMSA, 2012). Reflectance spectrophotometry and isosbestic wavelengths are most commonly applied in meat to assess the Mb values with quantification methodology. The *in vitro* determination MB redox state in meat samples can be achieved through using absorbance at 525 nm representing myoglobin, 503 nm for MetMb, 557 nm for DeoxyMb, and 582 nm for OxyMb concentration (Tang et al., 2004). For *in vitro* determination oxidation of COMb solutions A503/A581 can be used. Additionally, A543/A581 can be applied for distinguishing between 100 % COMb and 100 % OxyMb (Kerry & Ledward, 2009).

2.3.7. Texture characteristics and spreadability properties of meat

The texture is one of the main structural-conformational attributes of meat that could be explained by juiciness, cohesiveness, and tenderness (hardness, firmness or toughness) besides other quality attributes appearance, flavour. During the storage, the texture as important palatability trait of a food can changes, and any downgrades in these properties of food can affect the kinaesthetic and tactile senses perceived in the mouth (Arya et al., 2017). Different methods have been used for evaluating the meat texture which could be described in three types namely; instrumental methods (objective, physical or mechanical assessing), sensory evaluation methods by panelists and indirect methods (assessment of collagen content in meat, amount of dry matter, and so on). Sensory evaluation rates the deformation and the heterogeneity of the meat sample, whereas the instrumental method, is based on the measurement of the resistance of external physical force or energy in meat. These tests can perform shear, torsion, compression, tension, and

penetration of samples (Hansen et al., 2004). Some examples for the instrumental method used for assessing sensory meat tenderness are (1) Warner-Bratzler shear force (WBS) which is the most widely used estimator, the higher shear force means the tougher meat quality (Cavitt et al., 2004). (2) Texture profile analysis (TPA) is also a very common method to evaluate the texture of various food items. It has an advantage in measuring multiple variables at one time including springiness, adhesion, gumminess, tenderness (hardness), chewiness and cohesiveness (de Huidobro et al., 2005). Applying the TPA in food using spreadability tests has been used commonly for a product like jam, jelly, mayonnaise, and cheese spread. Which refers to the capability of being spread on a surface in a layer because of distributing or extending. Spreadability could be measured using the crosshead pushed the 90° cone probe of spreadability rig with 2 mm/sec speed into the sampling holder. However, this method was not been commonly used for minced meat samples.

Protein occupies about 20-23 % of poultry meat. Skeletal muscle consists of muscle fibers, connective (endomysium, perimysium, and epimysium) and fat tissues. Based on the solubility properties of protein three categories of native muscle proteins can mention myofibrillar (salt soluble), sarcoplasmic (water-soluble) and stromal proteins (Singh & Deshpande, 2018). The main myofibrillar protein is myosin (50-55 %) followed by actin which is about 22 %. Whereas sarcoplasmic protein mainly consists of creatine kinase, myoglobin, and other enzymes. The stromal protein composed of collagen and elastin (Figure 3) (Listrat et al., 2016).

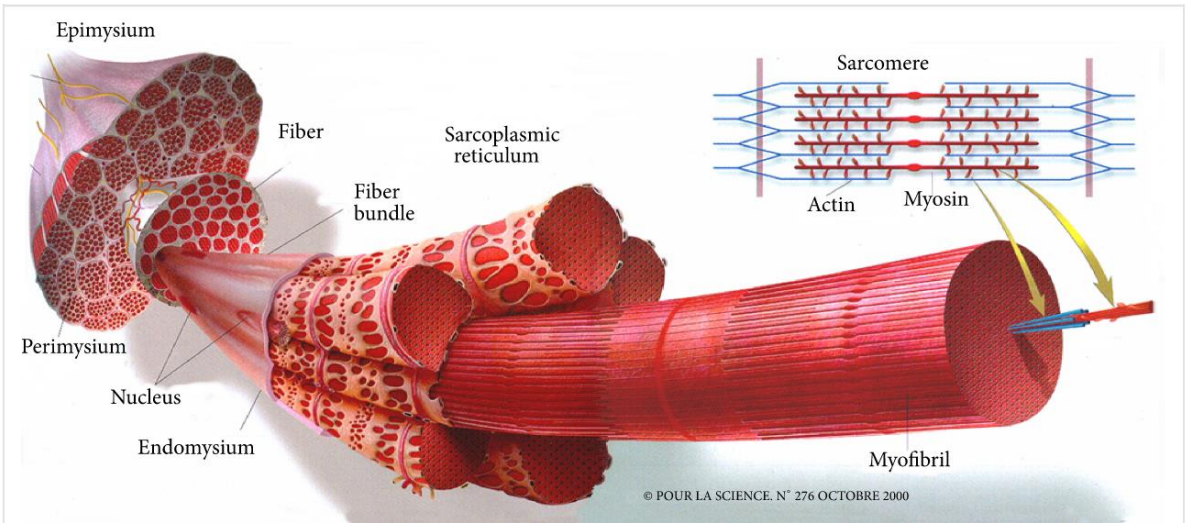


Figure 3: General organization of the muscle (adapted from Listrat et al., 2016)

2.4. Lipid Oxidation

Lipids are important component that contributes to several desirable characteristics in meat. Lipid consists of one of the following categories: phospholipids, free fatty acids, sterols, and mixture of mono-, di- and triglycerides. Triglycerides have been measured as the main lipid

responsible for the development of rancidity. It is produced by the esterification of a molecule of glycerol with three fatty acids, the rancidity reactions are supported by phospholipids that exist in the membranes and subcellular structures (Amaral et al., 2018). LO causes the development of rancidity in meat at the time of slaughter after releasing the phospholipids from the membrane and phospholipids strongly enhance LO during processing and storage (Djenane & Roncalés, 2018). LO often occurs when processed or comminuted meat is stored for a longer period in both refrigerated and frozen storage, it depends on the chemical composition of meat, storage temperature and access to light and O_2 (Dawson et al., 1988). High abundance and concentration of polyunsaturated fatty acids (PUFAs) and free iron increase sensitivity of meat and particularly poultry meat to LO (Kanner et al., 1988; Mercier et al., 2001). Whereas in beef LO occurs at a slower rate than pigment oxidation or microbial spoilage that is why LO might not be considered a limiting factor for shelf-life (Djenane & Roncalés, 2018).

2.4.1. Implications of lipid oxidation in the meat industry

LO is among the most vital quality parameters because it not only causes meat discolouration through protein oxidation but also is primarily a process that responsible for effecting the eating quality properties and causes serious challenges for product development specialists in meat and food production industry (Min & Ahn, 2005). Lipid oxidation may develop; (a) chemical spoilage, (b) degradation of pigments, (c) destruction effects on lipids, essential fatty acids, proteins, and fat-soluble vitamins and decrease of the energy content, (d) precipitate health hazards through the formation of carcinogenic substances (Malondialdehyde (MDA) has been criticized as a carcinogenic product of LO) and developing potentially toxic substances in meat and food products (i.e. aldehydes, ketones, and alkanes), (e) contribute to drip losses, (f) changes in the sensory properties (texture, off-odours formations, causing discolouration and off-flavours) of meat, and (g) reduced shelf life, loss of nutritional value and loss of functionality in meat products (Coronado et al., 2002; Fukumoto & Mazza, 2000; Morrissey et al., 1998). Ultimately, creating economic concerns for meat producers and effecting the consumer's preference for purchasing the meat products (Mercier et al., 2001; Reig & Toldra, 2010).

2.4.2. Mechanism of Lipid Oxidation

Lipid oxidation (LO) is a very complex phenomenon whereby the peroxidation of unsaturated fatty acids (UFAs) of membrane phospholipids oxidized by molecular oxygen through free radical mechanisms from fatty acylhydroperoxides, to form peroxides or hydroperoxides and conjugated dienes the primary products of the LO. Hydroperoxides are further susceptible to

several enzymatic reactions and further oxidation or decomposition to secondary oxidation products i.e. short-chain aldehydes, alkenes, alcohols, and ketones (propanal, hexanal, and malondialdehyde) (Gray, 1978), by which off-flavors and odours produced that negatively affects the overall quality and acceptability of meat and meat products. Lipids oxidation can occur in three main ways that include complex reactions: photo-oxidation, autoxidation and enzymatic-catalyzed oxidation. The most common way for LO in meat is autoxidation (Amaral et al., 2018). Photo-oxidation results from the direct exposure of meat and meat products to light this makes it much faster than autoxidation (Domínguez et al., 2019). In LO by an enzyme-catalysed method, the enzyme is involved in producing the peroxides and hydroperoxides with conjugated double bonds and the main enzyme involved in the initiation of oxidation is lipoxygenase (Domínguez et al., 2019). The mechanism of LO by autoxidation is complex and consists of three main steps of radical chain reactions: initiation, propagation, and termination (Kanner, 1994). The autoxidation radical reactions depend on catalytic action (light, temperature, pH, metal ions or metalloprotein catalysts, and free radicals) (Shahidi & Ambigaipalan, 2015). These steps are:

1- The initiation phase process began by the abstraction of a hydrogen atom ($H^{\bullet}$) from methylene group in lipid molecules of UFAs (LH) which can initiate lipid peroxidation (Min et al., 2008). Hydrogen peroxide can react with MetMb to generate ferrylmyoglobin, and to form lipid peroxy radical's alkyl ($L^{\bullet}$) (equation A) (Velioglu et al., 1998). The abstraction of ($H^{\bullet}$) which has only one electron from the lipid chain leaves unpaired electron on the carbon of the chain ($L^{\bullet}$) (Figure 4). This carbon radical tends to be stabilized by a molecular rearrangement to form a conjugated diene (Min & Ahn, 2005).
2- Under conditions like the presence of O2, the most likely fate of conjugated dienes is to react with molecular O_2 to form a LOO radical (equation B and C). In this propagation step, $LOO^{\bullet}$ forms and can abstract $H^{\bullet}$ from another susceptible lipid molecule in neighbouring or surrounding FAs to form lipid hydroperoxide (LOOH). This propagation continues and may occur up to 100 times before one of the radicals is been removed by reaction with another radical or with an antioxidant, whose resulting radical (A) (Huss, 1995). LOOH may undergo various reactions, depending on cell or tissue environments. For example, further combination reactions, intramolecular rearrangement, and further reactions with additional O_2 molecule resulting in the formation of numerous secondary derivatives such as cyclic peroxides, prostaglandin-like bicycloendoperoxides (Gardner, 1989).
3- The last step of LO is termination process in which the $LOO^{\bullet}$s reacts with each other and/or self-destruct to form non-radical products (equation D) (Min & Ahn, 2005). The LO ends with the formation of secondary products volatile and non-volatile compound with different functional

groups such as carbonyls, aldehydes, ketones, epoxides hydrocarbons, esters, alcohols, brans, furans, and lactones. The most abundant type of aldehydes observed in meat products are 4-hydroxy-2-trans-nonena, hexanal, propanal, and malondialdehyde (MDA) (Estévez, 2015). These secondary products are largely responsible for rancid flavours and sensory defects, deterioration of protein stability and functionality in meat (Amaral et al., 2018).

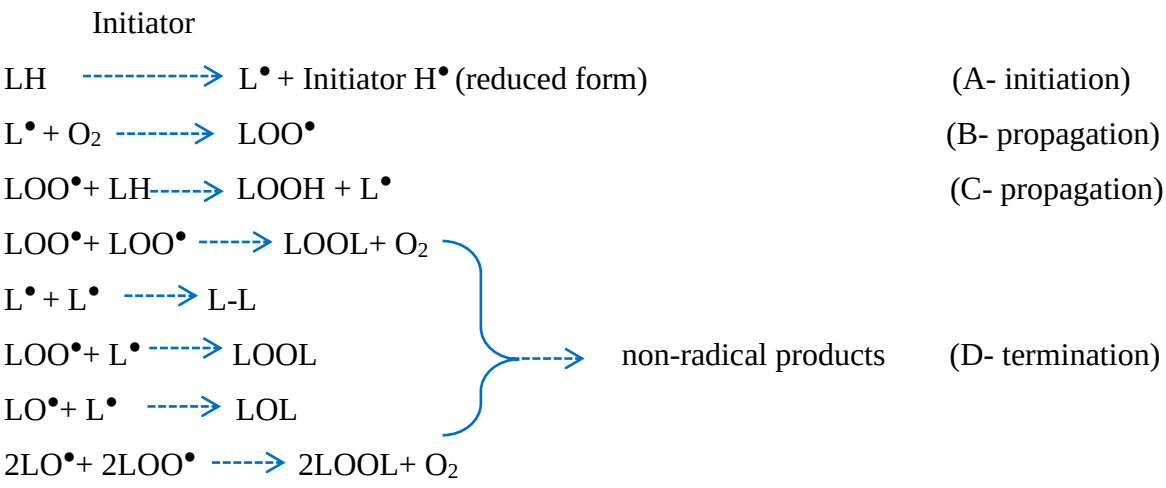


Figure 4: Mechanisms of LO (Adapted from Erickson, 2008).

*Where: LH is a fatty acid, H is a hydrogen atom, L• is an alkyl radical, LOO• is a peroxyl radical, LOOH is lipid hydroperoxide, LO• is alkoxy radical and LOOL is non-radical.

2.4.3. Factors affecting lipid oxidation

The rate of LO in meat depends on the balance between endogenous/ intrinsic factors (meat composition) and exogenous/ extrinsic (processing and storage condition) factors of the meat (Min et al., 2008). Endogenous factors include total lipid contents, composition of FAs (degree of PUFAs), concentration and types of metal ions present, haem-proteins, reducing compounds (e.g. ascorbic acid), natural antioxidants compounds, vitamins, and pro-oxidant enzymes (catalase, superoxide dismutase, peptides, peroxidases and dioxygenases) (Calkins & Hodgen, 2007; Djenane & Roncalés, 2018; Domínguez et al., 2019; Min et al., 2008). Besides, it has been reported that the extent of LO of the muscle depends on iron catalysts such as Mb and free iron. *Sartorius* muscle that exists in turkey's leg found to have a higher rate of LO than *Pectoralis* muscle, which is found in turkey's breast. This because the leg muscle has more Mb receiving more blood for movement. Moreover, exogenous factors affect the extent of oxidation in meat including; exposure to O_2 and light, additives (salt, nitrate, and BACs, spices), temperature abuse during handling and distribution, and prolonged storage, as well as preservative and processing techniques (such as

chilling, freezing, cutting, deboning, grinding, cooking, irradiation, high-pressure processing and packaging), can accelerate the development of LO (Falowo et al., 2014; Min et al., 2008).

2.4.4. Methods used for the determination of lipid oxidation

Several methods have been implemented for monitoring the LO in meat and other food, based on the purpose of measurements, the absorption of oxygen, the loss of initial substrates, the formation of free radicals, and the formation of primary and secondary oxidation products (Dobarganes & Velasco, 2002). These methods include: (a) assessing changes in the substrates, this method is not commonly used in assessing the LO, (b) measuring the peroxide value (PV) also called hydroperoxides, this is the most common method used for measuring LO in meat, which consist of assessing the amount of PV as the primary products of oxidation in meat and meat products. The measurement of PV could be performed through iodometric Titration and ferric-xylenol Orange (FOX), (c) conjugated dienes and conjugated triene hydroperoxides, measuring the conjugated compounds used as an indicator for LO in meat that asses the increased formation of hydroperoxides (Shahidi & Wanasundara, 2008), (d) measurement of cholesterol oxidation products, this method involves lipid extraction, saponification, purification, and derivatization. The common apparatus used for performing this analyses are gas chromatographic (GC) and high-performance liquid chromatography (HPLC) (Sanches-Silva et al., 2014), (e) Thiobarbituric acid-reactive substances (TBARS) this method used to measure the values of MDA (1,3-propanedial) as the most important aldehydes produced during the process of secondary LO of PUFA. The apparatus GC and HPLC are used to quantify MDA (Shahidi, 1994), (f) Another method for monitoring the secondary product of LO is measuring of carbonyls group (aldehydes and ketones) in meat (Shahidi, 1994), and (g) volatile compounds; the measurement of propanal, pentanal especially hexanal considered to be the greatest indicator of LO in meat and meat products because they are much more stable than unsaturated one. Hexanal is the most abundant component of aldehydes in meat compared to other components propenal, 4-heptenal, 2,4-heptadienal, 2-octenal and 2-nonena. These compounds are commonly measured by GC and liquid chromatographic-mass spectrophotometer (LC–MS) (Domínguez et al., 2019).

2.5. Microbial deterioration in meat and meat products

Meat and meat products provide an excellent growth environment for a variety of microflora (bacteria, moulds, and yeasts) some of which are pathogens (Jay et al., 2005). Due to the incidence of chemical and enzymatic activities as well as high-value nutrient composition, high water content and moderate pH, these make the meat one of the most perishable compared to a

variety of foodstuffs (Dave & Ghaly, 2011). Microbial spoilage of meat during the supply chain is one of the major concerns causing quality defects and has the potentiality to cause food-borne illness, food insecurity and public health issues, degradation of proteins and lipids and develop unpleasant quality characteristics. Despite the application of recent and advance techniques, chemical preservatives and refrigeration chains, it has been projected that microbial and chemical spoilage with other factors causes the massive wastage about 1.3 billion tons/year (25 %) of all food globally produced during post-harvest or post-slaughter (FAO, 2011; Iulietto et al., 2015). Consequently, creating a serious concern to consumers, governments and food industries (Sant'Ana et al., 2012). It has been estimated by the World Health Organization (WHO) that each year about 600 million cases of food-borne illnesses and the deaths related cases about 420,000 globally (WHO, 2015).

2.5.1. Factors affecting microbial deterioration in meat

A variety of intrinsic and extrinsic factors can influence the rate of spoilage of meat and meat products and fuel the growth of microorganisms. Some of these technological factors/condition's perform a primordial role in the shaping of microbial consortia during the fermentation of meat (Charmpi et al., 2020). Intrinsic factors, such as meat composition pH (acidity of the meat), presence of oxygen, oxidation-reduction potential (E*h*), water activity (a_w), fat and protein content of meat, antimicrobial hurdles (salt and preservatives). Extrinsic factors; hygiene, storage conditions (freezing, irradiation, dehydration, temperature, time and relative humidity), packaging conditions, and the gaseous composition (Iulietto et al., 2015; Nychas et al., 2008). These factors can enhance spoilage and wastage of meat due to accelerating the growth of different bacteria such as psychrophile, psychrotrophic, mesophile and thermophile**,** pathogenic microorganisms (*L. monocytogenes, E. coli* O157:H7, *Salmonella spp, St. aureus, Bacillus cereus, Campylobacter spp, Clostridium perfringens, Aspergillusniger,* and *Saccharomycescerevisiae*), other bacterial species such as; (*Pseudomonas*, *Proteus* spp, *Lactobacillus* spp, *Enterobacter*), yeast (Candida and Torulopsis), mould (Rhizopus, Fusarium and Aspergillus) (Fratianni et al., 2010; Lucera et al., 2012; Sant'Ana et al., 2012). Russell et al. (1998) reported that pH about 5.5 to 7.0 is preferred for the growth by spoilage bacteria. It has been reported that *Salmonella* serotype Typhimurium, *E. coli* O157:H7, *L. monocytogenes* are among the most emerging pathogens that have a dominant influence in causing poisoning and intoxications in meat and food products rather than effecting the sensory properties of meat (colour, odour, taste, texture) (Mor-Mur & Yuste, 2010). Therefore, the important concern ensuring microbial safety to produce the highest quality meat and meat products possible (Fratianni et al., 2010; Lucera et al., 2012). Several technologies

and treatments have been developed and applied in order to control spoilage and pathogenic microorganisms in meat and food such as sonication, microwave, irradiation, ohmic heating, ozonation, pulsed electric fields, and cold plasma (Bahrami et al., 2020), organic acids (Mani-López et al., 2012), BACs with bacteriophage (Moon et al., 2020), HHP (Chuang et al., 2020), and MAP (Chouliara et al., 2007).

2.5.2. Aerobic mesophilic count

The aerobic mesophilic count (AMC) is used as an indicator of bacterial populations it is known with other names including aerobic plate count, aerobic colony count, standard plate count, or total plate count (TPC). AMCs do not associate directly with the presence of pathogens or toxins. AMC does not measure the entire bacterial population; it is a generic test used for detecting the organisms that grow aerobically at mesophilic temperatures (25 to 40 °C). The application for the AMCs has been widely implemented in different media including soil and water using specific solid agar media at temperatures in the range of 35 – 44 °C incubated for 24 h. It is used also in the fruit and vegetable industry in monitoring and tracing microbiological problems (Brackett et al., 1994). The AMCs provides information about the total microbial load in food, fresh meat, and poultry products, but it has some limitations such as the AMCs only reveals the number but not kinds of cells are present, and AMCs enumerate only relatively rapidly growing aerobic organisms such as it might include viable but non-culturable organisms (Brackett et al., 1994; Rouger et al., 2017). AMC also widely used to determine the overall quality and safety of the product shelf life, during handling, transportation, processing, and storage, however regarding the plating medium, temperature, and length incubation no consensus has been established. The most widely applied temperature-time in studies was observed to be 25.8 °C to 35.8°C /48 h (Jay et al., 2005). Generally, the growth of AMC is fast within 5 days at 7 °C. In contrast, the psychrotrophic bacteria grew slowly or did not grow at all and within 3 days at 7 °C (Ercolini et al., 2009).

In chicken meat as one of the most perishable foods, the availability of AMCs is an indicator of the hygienic level and usually in-ground chicken meat the AMCs is always high, and consequently increases the risk of microbiological spoilage disintegration. Álvarez-Astorga et al. (2002) stated that the AMCs in processed various cuts products of poultry (hamburgers, sausages) were approximately 7 log CFU/g and higher compared to fresh cuts (thighs, wings) with approximately 5.7 log CFU/g. This could be due to an increase in the surface area of meat in contact with surfaces and air to increase the possibility of contamination. In a comparative study on the microbiological quality of poultry meat, the result showed that chicken meat had AMCs about 4.5 - 6.6 log CFU/g and less contaminated than turkey meat with AMCs about 5.4 - 7.4 log

CFU/g (Cohen et al., 2007). Kim et al. (2017) also indicated that post-chilling with peracetic acid (a mixture of acetic acid and hydrogen peroxide) in chicken meat resulted in a significant reduction of AMCs about 4.08 log CFU/chicken. While Zhang et al. (2011) reported post-chill results with an AMCs mean log 1.79 CFU/mL. Additionally, 7 logs CFU/g has been used in some studies to define the spoilage for the criterion of microbiological acceptability of meat (Höll et al., 2016; Rouger et al., 2017).

2.5.3. *Escherichia coli* (enterohaemorrhagic *E coli* or EHEC)

Escherichia coli (O157:H7 BO1909) is considered as a common portion of the normal facultative anaerobic microflora that exists in warm-blooded animal and human intestinal tract. Based on the distinct serotypes (O:H), pathogenicity mechanisms, virulence characteristics and clinical syndromes the isolates of diarrheagenic *E. coli* are grouped into various specific pathotypes which are; (a) enterohemorrhagic *E. coli* (EHEC), (b) enterotoxigenic *E. coli* (ETEC), (c) enteropathogenic *E. coli* (EPEC), (d) diffuse-adhering *E. coli* (DAEC), (e) enteroinvasive *E. coli* (EIEC) and (f) enteroaggregative *E. coli* (EAEC) (Meng et al., 2007). EHEC is considered as an emerged important zoonotic strain of *Esherichia coli* that triggers the foodborne illness. EHEC (*E. coli* O157:H7) organism was first identified as human pathogens with significant public health threats in 1982 (Mead et al., 1999), it was recognised as predominant cause responsible for two outbreaks of haemorrhagic colitis. Ever since various serotypes of *E. coli* such as O26, O111 and sorbitol fermenting O157:NM has been determined to be able to cause haemorrhagic colitis, several sporadic cases and humans outbreaks of foodborne disease including symptom-free carriage to non-bloody diarrhea, haemorrhagic colitis, haemolytic uraemic syndrome, and even death (Havelaar et al., 2015). About 73 outbreaks were reported in the EU in 2013 caused by Shiga toxin *E. coli* (STEC) and mainly sourced from meat and its products (EFSA, 2015). It has been reported that organisms of *E. coli* produce STEC and associated with the identification of over 600 serotypes of STEC with approximately 50 H types and 160 O serogroups (Meng et al., 2007). The name of *E coli* O157:H7 refers to the expression of the 157th somatic O and the 7th flagellar antigen H. *E coli* O157 is genetically related to an enteropathogenic strain *E coli* O55:H7, that generally produces diarrhea among infants (Mead et al., 1999). The production of Shiga toxin 1 and 2 by *E coli* O157 is considered one of its important virulence attributes, this Shiga toxin 1 is identical to the Shiga toxin produced by *Shigella dysenteriae* type 1 (Mead et al., 1999). The common characteristics of *E. coli* O157:H7 and Shiga toxin that cannot be seen in most of the other strains of *E. coli* are the inability to grow well at a temperature such as at 44.5 ℃ or higher, inability to ferment sorbitol in 24 h, possession of a pathogenicity island (Brackett et al., 1994).

Additionally, the minimum pH for the growth of *E. coli* O157:H7 is 4.0 to 4.5, however other growth factors can interact with this pH (Mead et al., 1999). Preventing infections by *E coli* O157 could be achieved through avoiding eating and ingestion of minced meat that is undercooked and unpasteurised raw milk products and juices. Proper heating of foods of animal origin, for example reaching an internal temperature of at least 68.3 °C for several seconds is an important critical control point to ensure the inactivation of *E. coli* O157:H7. Additionally, an irradiation dose of 1.5 kGy should be sufficient to eliminate *E. coli* O157:H7 at the cell numbers likely to occur in ground beef (Meng et al., 2007). In addition, the use of EO and BACs can reduce the growth of *E. coli*. Busatta et al. (2008) reported that the inclusion of marjoram EO (11.5 mg/g) to fresh sausages case significant reduction in the populations of *E. coli* during 35 day of storage. Yegin et al. (2016) reported that BACs (geraniol) loaded polymeric nanoparticles can inhibit the *in vitro* growth of *Salmonella enterica* Typhimurium, and *E. coli* (O157:H7).

2.5.4. *Salmonella* Typhimurium

Salmonella spp. (B1310) are anaerobic Gram-negative (G-veB) rod-shaped facultative bacteria belonging to the family *Enterobacteriaceae* (Figure 5). *Salmonella* spp. consist of microorganisms that are resilient and readily adapt to severe environmental circumstances (D'Aoust & Maurer, 2007). According to the variation in biochemical characteristics, *Salmonella* can be categorised into two major species*: S. enterica* and *Salmonella bongori*. *S. enterica* is divided into 6 subspecies while *S. bongori* comprises 22 serotypes (Lamas et al., 2018). The subspecies *S. enterica* is responsible for more than 99 % of human salmonellosis, and it consists of 1,531 serotypes among which are *S.* Typhimurium and *Salmonella* Enteritidis (Lamas et al., 2018). Some strains of *Salmonella* such as Typhimurium capable of growth at temperatures 54 °C or higher, and others demonstrate psychrotrophic properties in their ability to grow in foods stored at 2 °C to 4 °C and has the ability to proliferate at pH values ranging from 4.5 to 9.5 (with an optimum growth pH of 6.5 to 7.5) (Droffner & Yamamoto, 1991). In both developing and high-income countries, *S.* Typhimurium is associated with foodborne outbreaks as the most dominant serovar globally (Mohammed, 2017). *S.* Typhimurium has been associated mainly with the consumption of undercooked meat or ground meat (poultry and beef), dairy products, and especially raw eggs. Salmonellosis has been known as one of the most common foodborne diseases globally, accounting for around 93.8 million foodborne illnesses and about 155,000 cases of deaths per year worldwide (Eng et al., 2015). It is believed that the increased salmonellosis contributed by the increase in the consumption of poultry meat and table eggs (Foley et al., 2011). In the EU, *Salmonella* was found at 4.1 % throughout the prevalence in 51,093 fresh broiler meat units (EFSA

& ECDC, 2014). Human infection by *Salmonella* can exhibit several clinical conditions such as enteric fever (a serious human disease-associated typhoid and paratyphoid), uncomplicated enterocolitis, and systemic infections by nontyphoid microorganisms. Whereas human infections with nontyphoid *Salmonella* commonly result in enterocolitis (D'Aoust & Maurer, 2007; D'Aoust, 1991; Dougan, 1994). Various method has been applied to control *Salmonella* strains in meat such as BACs alone or combination with MAP (Shin et al., 2010; Ward et al., 1998), the application of different lytic bacteriophages and the combination of bacteriophage with other antimicrobials (Moon et al., 2020), organic acids to control Salmonella in poultry products (Mani-López et al., 2012). However, vacuum storage at 4 and 10 °C for 28 days did not result in dramatic reductions in the mean numbers of *C*. jejuni and *S.* Typhimurium in 10 or 20 % moisture-Enhanced Pork (Wen & Dickson, 2012). Moon et al. (2020) observed that the dipping treatment of inoculated chicken meat in both bacteriophage (1.1×10^8 PFU/ml) and 1.6 % (w/v) thymol or CARV for 3 min resulted in reductions of 1.9 - 2.0 log CFU/g of a cocktail of *Salmonella* strains (*S.* Typhimurium, *Salmonella* Enteritidis, and *Salmonella* Dublin SP.).

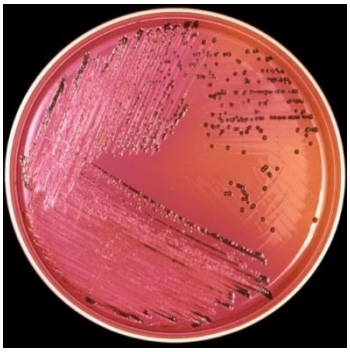


Figure 5: *Salmonella* Typhimurium on XLD agar.

2.5.5. Staphylococcus aureus

Staphylococcus aureus (ATCC 6538) is an important human pathogen that extremely versatile and responsible for a variety of infections and food poisoning outbreaks (Boucher et al., 2010). Most strains of staphylococcus about 90 % were described with potentially enterotoxigenic and pathogenicity that causes staphylococcal food poisoning (SFP) (Rodríguez-Lázaro et al., 2017). The genus *Staphylococcus* is consisting of 36 species and 21 subspecies (Mellmann et al., 2006). Nowadays 23 various serological types of staphylococcal enterotoxins (SEs) that possess emetic activity have been detected and the 5 major types are; SEA, SEB, SEC, SED and SEE, within these SEs the SEA is considered to be the most common in staphylococcus-related food poisoning (Ono et al., 2015; Pinchuk et al., 2010). *St. aureus* is Gram-positive (G+veB), non-motile cells, non-spore-forming, cocci-shaped (diameters ranging from 0.5-1.5 μm) bacterium. *St. aureus* (aureus means golden or yellow which is the colour of colonies) this organism's opportunistic foodborne pathogen has the capability to grow facultatively (aerobically or anaerobically). It can grow at temperatures ranged between 7 - 48.5 °C, pH between 4.2 to 9.3, and can tolerate salt concentrations up to 15 % (Kadariya et al., 2014). For *St. aureus* strains catalase positivity and coagulase positivity as biochemical properties can be used to differentiate

potentially pathogenic and non-pathogenic. *St. aureus* responsible for SFP outbreaks and variety of infections including allergic (staphylococcal scalded-skin syndrome), autoimmune reactions, toxic shock syndrome, osteomyelitis, endocarditis, pneumonia, bacteremia, abdominal cramping, vomiting, diarrhea, nausea, and staphylococcal scarlet fever (Rodríguez-Lázaro et al., 2017).

St. aureus has been observed in air, dust, soil, water, insects, and plants, it has been isolated from surfaces of live poultry, and raw retail meat is also an important reservoir (Hennekinne et al., 2012). It can be found in milk, the consumption of raw milk or recontaminated milk (e.g. approximately 0.5 ng/mL concentration of contaminated chocolate milk with SEs) (Murray, 2005), can increase the chances of infections with *St. aureus* enterotoxins and SFP (EFSA, 2017). Additionally, it has been reported that approximately 0.1 μg of SEs able to cause SFP in humans, also SFP is one of the most prevalent causes of gastroenteritis globally. SFP differs from other foodborne illnesses due to its incidence shortly after the ingestion of food contaminated with enterotoxin within 30 min to 8 hrs (Kadariya et al., 2014). The methods for controlling these infections are often difficult due to the emergence of strains resistant to multidrug. However, good application and adherence to the microbiological guidelines such as Good Hygienic Practices (GHPs), Hazard Analysis and Critical Control Points (HACCP) and Good Manufacturing Practice (GMPs) established by WHO and FDA Food and Drug Administration can eliminate the risk of food contamination to preventing *St. aureus* (Kadariya et al., 2014). Additionally, several reports investigated the inhibition or reduction in the growth of *St. aureus* through the application of EO and BACs *in vitro* and meat model (Guimarães et al., 2019; Lambert et al., 2001; Luz et al., 2013; Zahi et al., 2017).

2.5.6. Pseudomonas lundensis

The *Pseudomonas* genus is one of the most significant distinct biologically groups of identified bacteria (Molina et al., 2013). *P. lundensis* (CCP5) belong to the family *Pseudomonadaceae,* which has morphological characters like; G-veB reaction, rod-shaped cells (0.5 to 3.0 μm), presence of motile with polar flagella they have pili or fimbriae, oxidase-positive, catalase-positive bacteria, absence of spores, obligate respiratory metabolism and they are considered opportunistic pathogens for humans and animals (Iglewski, 1996; Meliani & Bensoltane, 2015) (Figure 6), whereas the majority of *Pseudomonas* species are non-pathogenic (Molina et al., 2013). *Pseudomonas* spp. can grow at a temperature range between 4 – 42 °C (optimal temperature above 20 °C) (Meng et al., 2017). *Pseudomonas* genus consists of more than 140 species and over 25 species are humans related. Many of these species cause opportunistic infections in humans such as *P. aeruginosa*, *P. fluorescens*, *P. putida*, *P. cepacian*, and *P. stutzeri*.

Only 2 species, *P. mallei*, and *P. pseudomallei* are known to cause specific human diseases which are glanders and melioidosis (Iglewski, 1996).

Pseudomonas is found in ubiquitous natural habitats including soil, vegetation, freshwater, and marine environments, animals, plants, it can be isolated in healthy persons from the throat, stool and skin and species like *P. fragi, P. fluorescens, P. putida, P. gessardii, P. lundensis* are often isolated from spoiled foods (Ercolini et al., 2010; Molina et al., 2013; Yagoub, 2009). It has been determined that during aerobic storage and at low temperature *Pseudomonas* can affect fruits and vegetables. *Pseudomonas* spp plays a significant role in spoilage of milk, and dairy products mainly at post-pasteurization (Nychas et al., 2008). These occur after the processed milk produces several thermo-tolerant lipolytic and proteolytic enzymes by *Pseudomonas* spp. Eventually, reduce the quality and shelf life of the product. Three species of *Pseudomonas* (*Pseudomonas* fragi, *Pseudomonas* fluorescens, and *Pseudomonas lundensis*) are mainly responsible for spoilage for a variety of foodstuffs and colonize fresh meat and meat products (beef and chicken) (Ercolini et al., 2009, 2010; Mellor et al., 2011). *Pseudomonas* spp. from diverse sources of contamination including carcass microbiota at slaughter can produce volatile compounds (aldehydes, ketones, and esters) and alcohols (e.g. butanol, 1-heptanol, 1-hexanol) during refrigerated storage of meat causing off-flavour in a product that associated with economic costs and health concerns (Yagoub, 2009). It has been noticed that during processing the scalding of poultry may destroy *Pseudomonas*, but it may increase the sensitivity of the carcass product to recontaminate at followed steps of processing (Mead, 2005). Additionally, it has been reported that at the aerobic condition the meat product should have an initial load of *Pseudomonas* spp. fewer than 100 CFU/g to achieve an ideal shelf life and sensory demand (Mead, 2005). It has been reported that the inhibition or reduction in the growth of *Pseudomonas* spp. can be achieved through the application of EO and BACs *in vitro* and meat model (Karam et al., 2019; Lambert et al., 2001; Mastromatteo et al., 2009, 2009; Sangkasanya et al., 2018).

Figure 6: *Pseudomonas* on cetrimide agar.

2.5.7. Listeria monocytogenes

Listeria monocytogenes (CCM 9699) is considered one of the important foodborne pathogens that has been emerged causes for the foodborne disease called Listeriosis. The genus *Listeria* consists of 17 species, some examples are *L. monocytogene, L. ivanovii, L. grayi, L. innocua, L. seeligeri* and *L. welshimeri* (Orsi & Wiedmann, 2016). *L. ivanovii* has been identified

in two subspecies *ivanovii* and subspecies *londoniensis* and within the genus *Listeria* only two species described as pathogenic, *L. monocytogenes* (human pathogen) and *L. ivanovii* (animal pathogen) (Orsi & Wiedmann, 2016). Additionally, *L. monocytogenes* consists of about 13 serotypes that can cause diseases such as serotypes: 1/2a, 1/2b, and 4b (Salova et al., 2005) (Figure 7). *L. monocytogenes* are G+veB rod-shaped, ubiquitous, intracellular, non-spore-forming, flagellated, and facultatively anaerobic bacterium (it has a width of 0.5 μm and length of 1-2 μm). *L. monocytogenes* considered as representative species of the genera which is able to initiate growth aerobically and facultatively anaerobically in the temperature between 0 to 45 °C and while the growth of bacterium is slow or moderately inactive at low temperatures below 0 °C and the growth could be inactivated by exposure to temperatures above 50 °C. The optimal a_w for the growth of *L. monocytogenes* higher than 0.97 and the minimum a_w for growth of most strains is 0.93. This bacterium can initiate growth at pH values as low as 4.3 and can grow at a high salt concentration up to 16 % (Swaminathan et al., 2007).

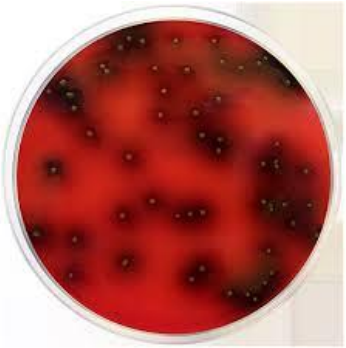


Figure 7: *L. monocytogenes* on Palcam base.

L. monocytogenes causative microorganism responsible for sporadic cases and several emerged outbreaks of foodborne diseases such as listeriosis that observed in Canada in 1981. Listeriosis has high severity rate of infection can be non-invasive or invasive that can spread to the nervous system (symptoms such as convulsions, headache, confusion, loss of balance) can occur, cause meningitis, septicemia, fever and gastrointestinal symptoms (Orsi & Wiedmann, 2016; Swaminathan et al., 2007). *L. monocytogenes* organism is widely distributed in the plant, soil, and surface of the water and can multiply in suitable environmental conditions especially during the preservation of food by refrigeration such food include; unfermented cheeses, unpasteurized milk, vegetables, juices, unheated frankfurters, seafood (shellfish) and several delicatessens, ready to eat (RTE) meats and poultry products (Roberts et al., 2020). The occurrence of *L. monocytogenes* in fresh broiler meat can varies from 0 % to 64 % that can be caused during manufacture, ageing, transportation, and storage (Loncarevic et al., 1994). That is why *L. monocytogenes* causes a considerable economic influence on the food production and food preservation industry and society in general. Since the discovery of *L. monocytogenes* many control measures have been implemented, while an increase in listeriosis cases can be noticed over time for instance compared with 2012 EU reported an 8.6 % rise in listeriosis in 2013 (Heredia & García, 2018), and an increase of 9.3 % between 2016 compared to 2015 was recorded by EFSA,

(2017). The avoidance of the consumption of uncooked animal products, appropriate methods for cooking of the food products, and the use of sublethal multiple hurdles in food processing and preservation could be implemented to reduce the risk of *L. monocytogenes.* Several studies have been conducted and reported the inhibition or reduction in the growth of *L. monocytogenes* by the use of EO and BACs *in vitro* and meat model (Ahn et al., 2001; Churklam et al., 2020; Hao et al., 1998; Hugas et al., 2002; Kim, et al., 1995; Li & Gänzle, 2016; Lin et al., 2000; López et al., 2007; Shin et al., 2010) (Table 2-Appendix).

2.5.8. Bacillus cereus

The *Bacillus cereus* group consists of 6 various closely related species including *B. cereus*, *B. weihenstephanensi*, *B. mycoides*, *B. pseudomycoides*, *B. thuringiensis* and *Bacillus anthracis* (these species possesses large virulence plasmids compared to others). *B. cereus* is an anaerobic, G+veB facultative bacterium. Members of *B. cereus* group are mostly found in vegetables, soil, plant origin (grains, raw rice, and pasta), eggs, dairy, and meat products (Stenfors Arnesen et al., 2008). *B. cereus is* known as a foodborne endospore-forming pathogen that might be a source of food poisoning and making great concern in the food industry. These food poisonings could be: (a) emetic type produced by growing cells in the food which associated with farinaceous foods, especially fried or cooked rice, and pasta, or (b) the diarrheal type is caused by enterotoxins which linked to proteinaceous food such as meat and also outbreak linked with vanilla sauce (Schoeni & Wong, 2005). *B. cereus* although it is not a competitive microorganism it can grow well during heat treatment that causes spore germination after cooking and cooling at temperatures less than 48 °C. Moreover, despite foodborne illness, due to the intake of large amounts of the emetic toxin death cases have also been reported (Anderson Borge et al., 2001). *Bacillus* spores can be seen in a range of food products including rice, pasta, eggs, and milk (Parihar, 2014). The spores possess high resistance and capability to survive in extreme environmental conditions like starvation, elevated temperatures, ionizing radiations, desiccation, hydrolytic enzymes, and toxic chemicals (Nicholson et al., 2000). They have the capability to activate germination and change to the vegetative state when nutrients are available and eventually cause foodborne illnesses (Setlow, 2014)**.** Several methods used in controlling and eliminating the *Bacillus* spores in food including heat treatments, UV irradiation (Koutchma, 2008), a non-thermal method like pulsed electric field (PEF) (Soni et al., 2016), ultrasound (Raso et al., 1998), high hydrostatic pressure (Zhang & Mittal, 2008), PEF and antibacterial/natural extracts (polyphenol and EOs) (Pina-Pérez et al., 2009).

2.6. High-pressure processing

High hydrostatic processing (HHP) is an emerged non-thermal preservation/processing technique. It is being used to prolong the shelf life of processed meat and meat products with a minimal impact on nutritional, functional, and sensorial quality (Marcos et al., 2013). The HHP has been widely applied for the pasteurization of food products (Huang et al., 2020), however, the average unit price of HHP products is higher compared to non- HHP products. The treatments of product using HHP depend on placing it (liquid or solid product) in a pressure vessel that is filled with the pressure-transferring medium (PTM) compressed by a pump (generally water used for this medium in food applications). During the application of this procedure, the hydrostatic pressure is transmitted uniformly and immediately to the sample through the PTM and this process is based on the Pascal or isostatic principle. One of the characteristics of applying this technology is that the effects of pressure are not dependent on the size and geometry of the products as it is the case using heat treatments. Nevertheless, the classical limitation of heat transfer must be taken into consideration. Using the technology, the adiabatic heat of compression is reversible and estimated to about 3 °C/100 MPa for most of the foods and can reach 8 – 9 °C/100 MPa for high-fat products (Figure 8) (Picart-Palmade et al., 2019; Toepfl et al., 2006).

HHP is a method that subjects food to intense pressure loads of up to 1000 MPa using non-compressible pressure-transmitting fluid. Relatively moderate to intense pressure loads are applied aiming at eliminating pathogenic and spoilage microorganisms and inactivating deteriorative enzymes, preventing the main food degradation process mechanisms from occurring (Huang et al., 2015; Rendueles et al., 2011). Besides, it has been documented the cytoplasmic membrane of bacterial considered as the main site to HHP action. However, the exact mechanisms of microbial inactivation and cellular damage by HHP may be complex, it consists of unfolding the structure of the protein, causes loss of cell membrane integrity through changing its fluidity and causing denaturation, loss of intracellular pH, induced denaturation of membrane-bound enzymes, and ultimately leads to cell disruption (Georget et al., 2015; Teixeira et al., 2018). The cell morphology analysis of microorganisms depicted that HHP changes the external cellular structures and cause the release of intercellular contents especially at >400 MPa (Huang et al., 2015; Liu et al., 2017). During favourable environment storage, functional recovering can occur in several sub-lethally injuries of microorganisms (Ait-Ouazzou et al., 2013), and the environment with temperatures higher than 40 °C and with HHP more than 600 MPa, the rate of sub-lethal damages decreases to more lethal ones (Ates et al., 2017). Application of HHP in meat can control microorganisms associated risks in marinated beef loins (Hugas et al., 2002), however, the pressure of 600 MPa

does not effectively eliminate the growth of spores and the resistant strains of *E. coli* or *L. monocytogenes* in the products (Jofré et al., 2009; Liu et al., 2012; Marcos et al., 2013).

HHP can impact the structural, physicochemical, morphological, and textural characteristics of the meat, and can cause partial discolouration of fresh red meat (Kim et al., 2007). Additionally, HHP alone might not achieve and satisfy the consistent reduction of pathogenic bacteria to meet the requirement of FDA and HACCP to reach up to 5-log reduction and achieve the enhancement of the health benefits to consumers particularly in RTE products (Bahrami et al., 2020). Therefore, the application of an additional hurdle with HHP could achieve synergistic or additive effects to improve the preservative effects of HHP in maintaining the quality of food, assuring sufficient reduction in microbial growth and extend the shelf-life (Bahrami et al., 2020). Jofre et al. (2008) studied the efficiency of combining HHP with bio-preservatives through applying the interleavers containing preservative nisin in the packaging material on cooked ham at 6 °C during 3 months of storage. They found that only the combined use of interleavers and HHP treatment of 400 MPa achieved complete elimination of *Salmonella* spp. in cooked ham. While the combination of 600 MPa with nisin completely inhibits the *L. monocytogenes* and *St. aureus* growth. Teixeira et al. (2018) treated RTE ham with HHP 500 MPa with or without rosemary extract or nisin at 5 °C for 1 or 3 min stored for 4 weeks. They found that HHP or nisin alone does not eliminate *Listeria* or other microbiota, while the combination of nisin with HHP treatment reduced counts of *Listeria* and meat microbiota by >5 logs CFU/g. The combination of direct application of BACs with HHP could have a promising efficacy than HHP or BACs alone to improve the meat quality.

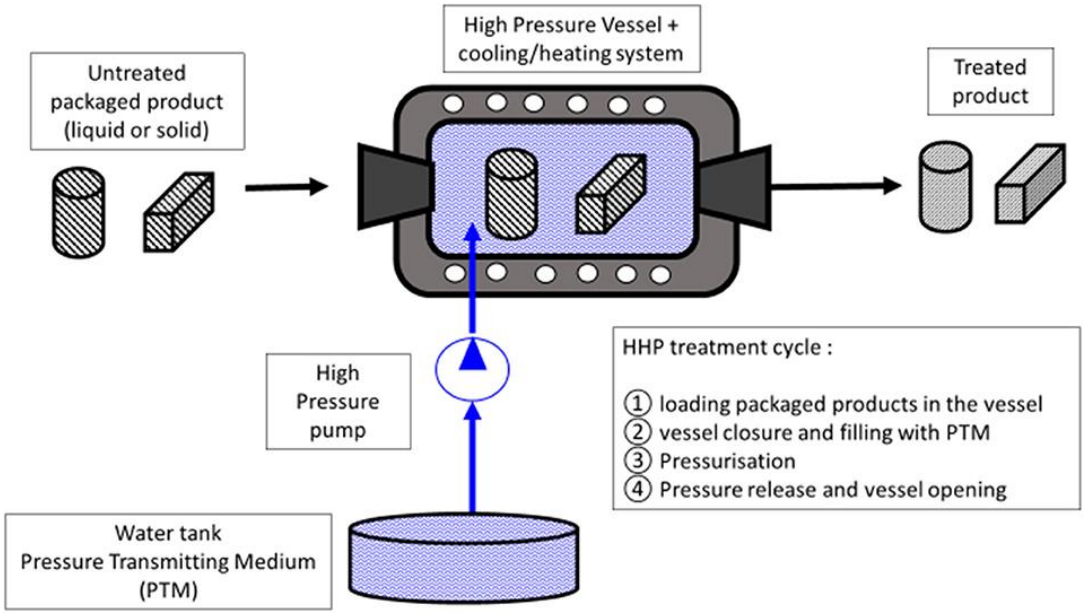


Figure 8: Schematic layout for a High Hydrostatic Pressure (HHP) treatment pilot (adapted from Picart-Palmade et al., 2019).

2.7. Essential oil (EOs) and their bioactive compounds (BACs): varieties and sources

Essential oil (EOs) is the term used to describe natural, complex, volatile compounds or combinations of secondary metabolites from plant liquids which consist of terpenes and phenylpropenes. EOs characterized by being oily, generally lower density than water, aromatic and volatile liquids with a strong odour, rarely coloured. EOs and BACs can be extracted from plant organs buds, bark, seeds, leaves, fruits, twigs, wood, roots, herbs and flowers (e.g. basil, thyme, oregano, cinnamon, clove, and rosemary, tea, sage, mint, ginger, marjoram, and caraway), fruits (e.g. grapes, pomegranate, and date), vegetables, (e.g. broccoli, potato, drumstick, pumpkin, curry, nettle and bulbs of garlic and onion) and naturally occurring polymers (chitosan) (Burt, 2004; Hyldgaard et al., 2012; Naveena et al., 2006; Rojas & Brewer, 2007; Shah et al., 2014; Holley & Patel, 2005; Othman et al., 2019; Tiwari et al., 2009).

EOs are often stored in plant secretory cells, cavities, canals, epidermic cells of glandular trichomes (Burt, 2004). These plants are mostly grown in Mediterranean and Middle Eastern regions and widely used for centuries in food preservation, cooking, medicine, and locally anaesthetic remedies, perfumery, and cosmetics worldwide (Burt, 2004; Dorman & Deans, 2000; Erkan et al., 2011). Besides, they are recognized to have a wide spectrum of antiseptic against various biological targets such as virucidal (Wu et al., 2010), antimicrobial properties and able to retard or inhibit the growth of yeast, and moulds and play a crucial role in plant defence (Bakkali et al., 2008; Chorianopoulos et al., 2008; Oussalah et al., 2007; Tajkarimi et al., 2010), antioxidant (Brenes & Roura, 2010), fungicidal (Silva et al., 2011), antiparasitic (George et al., 2009), pharmaceutical and medicinal properties (anticancer agents) (Suhail et al., 2011), insecticidal (Baser, 2008), cosmetics (perfumery, fragrance and skin products) (Naveed et al., 2013), and to enhance the growth for animals (Brenes & Roura, 2010) (Figure 9). Besides they have been used in embalmment, food preservation, and as antimicrobial, sensory preservatives (flavour), analgesic, sedative, spasmolytic, and anti-inflammatory activity (Bakkali et al., 2008; Burt, 2004). Additionally, due to their containing volatile compounds such as ketones, aldehydes, and aromatic compounds, the EOs have been applied in aromatherapy especially to decrease the level of mental and physical stresses. The chemical composition and BACs of EOs are affected by some factors for example plant part, species and subspecies, extraction method, geographical location, and season of harvest (Rasooli, 2007).

The various method has been implemented to obtain EOs including; mechanical process such as extraction, hydro-distillation fermentation, enfleurage, expression, and dry distillation, without heating of a plant or its parts (e.g. citrus fruits), solvent extraction microwave-assisted EO extraction, however, the most commonly used method is steam distillation (Rubiolo et al., 2010).

Furthermore, the chemical composition of EOs is preferably analyzed using apparatus such as GC. However, obtaining good chemical profile analyses may need to apply other analytical tools together with GC to deliver enough data. Consequently, other analytical tools such as mass spectrometry (MS), nuclear magnetic resonance (NMR), infrared spectroscopy (IR), high-pressure liquid chromatography (HPLC) multidimensional HPLC, HPLC-MS, and HPLC-GC have been used to analyse the chemical compounds of EOs (Fokou et al., 2020).

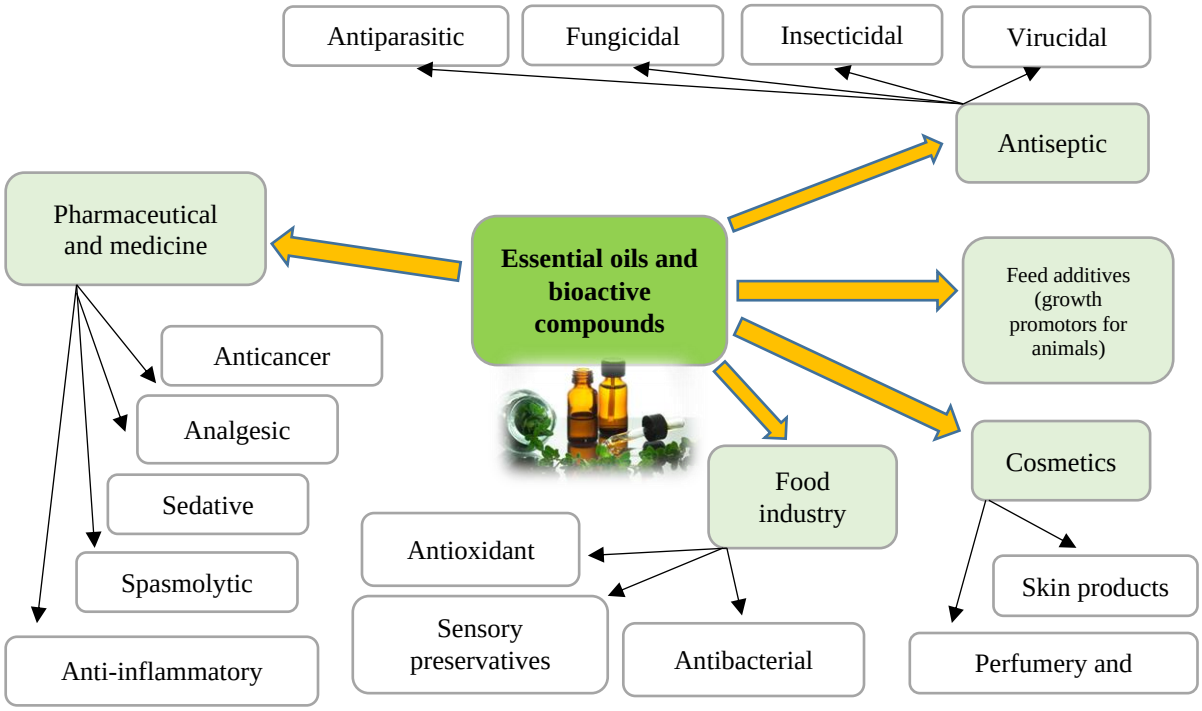


Figure 9: Various activities and uses of essential oils and bioactive compounds.

2.7.1. Essential oils chemical components, classification, and their structures

EOs have a complex composition, which consists of more than 300 different components with various concentrations. These compounds have less than 1000 Da molecular weights and they have large differences in antimicrobial and antioxidative activities (Bhavaniramya et al., 2019; Hyldgaard et al., 2012). The percentage of the components of EOs varies amongst species and the part of the plant (Solórzano-Santos & Miranda-Novales, 2012). They are mostly dominated by two or three key components at fairly high percentages (20 to 70 %) compared to other components present in trace concentrations (Bakkali et al., 2008). The percentage composition of BACs in some plant of potential application in meat and meat products are summarized in Table 1-Appendix. The main groups of BACs identified in EOs are found to be of four groups, with distinctive biosynthetic origin based on the chemical structure (Pichersky et al., 2006; Tajkarimi et al., 2010). These groups include (1) terpenes, (2) terpenoids, (3) phenylpropenes, and (4)

miscellaneous group. This later group possess different structures not observed in the first three groups.

Terpenes can be acyclic, monocyclic, bicyclic, or tricyclic (Abed, 2007). These BACs synthesized in the cytoplasm of plant cells and create a large class and serve multiple roles in plant structure and function (Falara et al., 2011). Many thousands of these compounds are recognized in the plant kingdom, each species of plant capable to synthesize a small fraction of this repertoire only (Chen et al., 2011). The main terpenes are the monoterpenes (C10) and sesquiterpenes (C15), however, hemiterpenes (C5), diterpenes (C20), triterpenes (C30) and tetraterpenes (C40) also exist (Flamini et al., 2007). Some examples of these compounds with their structures have been shown in Figure 10-Appendix. The monoterpenes are the most representative molecules constituting 90 % of the EOs with a great variety of structures and mostly formed from combining two isoprene units (Flamini et al., 2007). Besides, terpenes are hydrocarbons formed by the combinations of several 5-carbon-base (C5) units are named as isoprene that serves specific physiological and ecological roles (Caballero et al., 2003; Pichersky et al., 2006). Some examples of these BACs are; monoterpene hydrocarbons (*p*-Cymene, limonene, α-Pinene, and α-Terpinene), monoterpene alcohols (geraniol, linalool, and nerol), oxygenated monoterpenes (camphor, CARV, eugenol, and thymol), sesquiterpene hydrocarbons (β-caryophyllene, germacrene D, and humulene), sesquiterpene alcohol (patchoulol), oxygenated sesquiterpenes (spathulenol, caryophyllene oxide), diterpenes (cembrene C, kaurene, and camphorene).

Terpenoids are considered the terpenes that resulted from biochemical modifications supported by enzymes that increase O_2 molecules and eradicate or move methyl groups (Hyldgaard et al., 2012). Terpenoids oxygenated derivatives or not can also be subdivided into alcohols, esters, aldehydes, ketones, ethers, phenols, and epoxides. Such examples of these subcategories of terpenoids are; oxygen-containing hydrocarbons or alcoholic compounds; aldehydes (citral, cuminal, benzaldehyde, cinnamaldehyde, citronellal, phellandral, carvone, camphor), acids (geranic acid, benzoic acid, cinnamic, myristic acids), ketonic bodies (thymol, eugenol), lactones (bergapten), esters (bornyl acetate, linalyl acetate, ethyl acetate), and phenols (CARV, thymol, linalool, safrole, eugenol, catechol, ascaridole, and anethole) (Calsamiglia et al., 2007; Dorman & Deans, 2000; Swamy et al., 2016). The functionality of terpenoids is largely associated with possessing a hydroxyl group of its phenolic properties and the existence of delocalized electrons can enhance their antimicrobial activity (Rhayour et al., 2003).

Phenylpropanoid are compounds with a three carbons chain bound to an aromatic ring of six carbons (Calsamiglia et al., 2007). These aromatics are found in variable mixtures of main terpenoids. Some examples of phenylpropanoids are cinnamyl alcohol, chavicol, estragole, methyl

eugenols, and methyl cinnamate (Bakkali et al., 2008; Hyldgaard et al., 2012; Jayasena & Jo, 2013). Miscellaneous are sulfur or nitrogen-containing compounds such as allyl- and propenylphenols (phenylpropanoids), for example, allyl-isothiocyanate (mustard oil), and llicin (garlic) (Solórzano-Santos & Miranda-Novales, 2012). Bampidis et al. (2005) reported that the major compounds of oregano EO were CARV, thymol, γ-Terpinene, and *p*-Cymene. Range of 6-60 % CARV and 19-64 % thymol in oregano were obtained (Burt, 2004; Fasseas et al., 2008; Karabagias et al., 2011) (Table 1-Appendix). The *in vitro* tests indicate that terpenes are inefficient as antimicrobials when applied as single compounds (Hyldgaard et al., 2012). The use of natural BACs in meat and meat products is multifunctional. It plays an antimicrobial, antioxidant, and preservative role in meat during processing and storage. The use of EOs and BACs has been extensively studied in different *in vitro* and food matrix as they are considered as GRAS. However, the application of EO and BACs commercially in food products is controlled by regulatory laws of a specific country or international standards (Karre et al., 2013).

2.7.2. Mechanisms of antioxidant activity of BACs

Various valuable BACs have been identified in different plants and proposed to possess antioxidant activity, but only a few of these BACs can be used to improve and preserve the quality of meat and meat products. The use of BACs as antioxidants from natural origins like medicinal plants can promote consumers' health and wellness and has been more demanded in the meat industry compared to synthetic preservatives. It has been reported that despite the prevalence of antioxidants such as vitamin E and C the phenolic acids and flavonoids BACs have the most responsibility as antioxidant activity in the plant, fruits, and vegetables (Lee et al., 2017). The addition of natural BACs as antioxidants to meat, it can donate an electron to a rampaging free radical and neutralize it, they possess the capability to stabilize the level of cholesterol, prevents the formation of cholesterol oxidized products, besides they can reduce the formation and absorption of MDA and heterocyclic amine in cooked meat, this amine is considered to be mutagenic and may lead to changes in DNA that increase the risk of cancer (Falowo et al., 2014; Lobo et al., 2010). The application of BACs as nutraceutical ingredients and potent antioxidants in meat and food system depends upon properties of BACs such as effectiveness at low concentrations, good solubility to incorporate in the product, less or no odour, taste or colour of their own that can change the original sensory properties of meat, remain stable and capable to survive during processing, inexpensiveness and nontoxic (Kiokias et al., 2008).

The mechanisms in which BACs and antioxidants react to inhibit the oxidation process depends upon their skeleton structure and pattern of functional groups on this skeleton (Wojdyło

et al., 2007) and it include several pathways; (a) prevention of formation of lipid and protein radicals and chain inhibition through radical scavenging activity which proceeds through the transfer of H atom or via donation of electrons. The scavenging action is carried out either for reactive species such as reactive oxygen species (ROS) including OH and O_2, or it could be toward lipid peroxidizing radicals such as L*, $LO^{\cdot}$, and LOO. This cause terminating the oxidation cycle that takes place at the propagation step (Allen & Cornforth, 2010; Dangles & Dufour, 2006) (Figure 11), (b) breaking chain reaction catalysts (radicals) by decreasing localized O_2, removing or limiting free radical (ROS) and scavenge chelate prooxidants (prevention of metal transition) that utilize metal chelation (example for chelators; iron and copper) through inhibition of the oxidation of low-density lipoproteins (LDL) in order to produce catalyzed reactive species in the form of inactive or insoluble (Allen & Cornforth, 2010; Dorman et al., 2003), and (c) interaction between antioxidants (such as collaborative or supportive actions), localization, and movement of the antioxidant at the microenvironment (Apak et al., 2007). Generally, it has been reported that the presence of multiple free hydroxyl (OH) groups enhance the antioxidant potential of polyphenol, phenolics, flavonol BACs and vitamins (e.g. E and C) and has potent radical scavengers (Kumar & Pandey, 2013). However, the reaction becomes auto-propagative in the lack of antioxidants, by which non-radical products could be produced. Phenolic BACs which are nutraceutical ingredients like flavonoids in plants, they are characterized to show anti-inflammatory, antiviral, anti-allergenic activity (Sakakibara et al., 2003), inhibiting the *in vitro* oxidation of LDL (Riemersma et al., 2001), decrease in risks of neurodegenerative diseases, for example, cardiovascular disease (Paran et al., 2009), anticarcinogenic activities (gastrointestinal and colon cancers), hepatoprotective and leukemia (Santos-Sánchez et al., 2019).

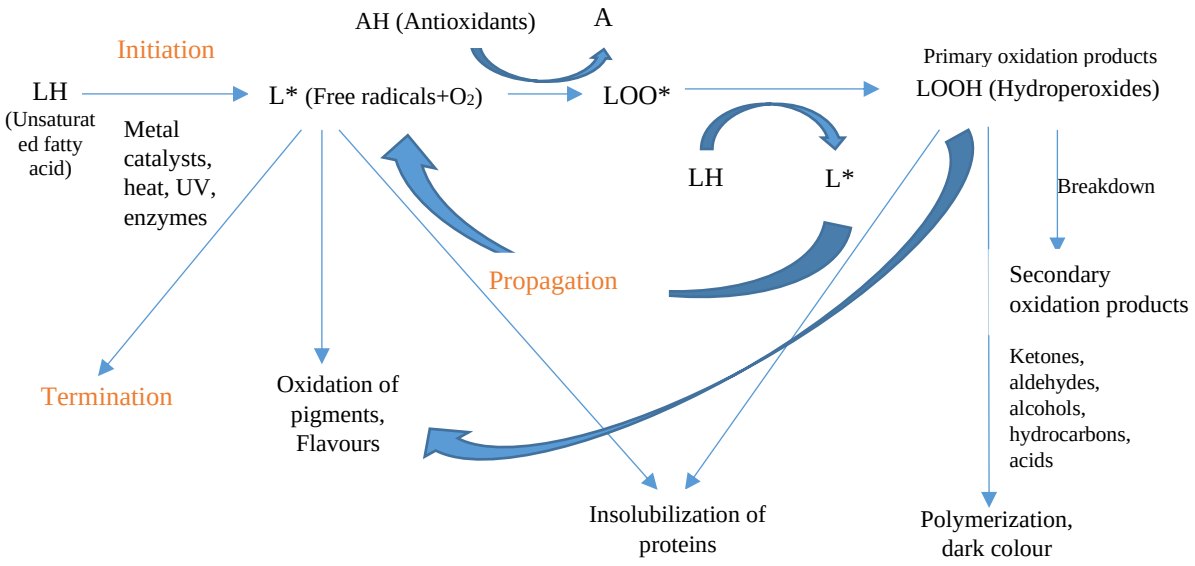


Figure 11: Different steps of oxidation in meat - lipids containing PUFAs (LH) and their consequences (modified from Guyon et al., 2016).

2.7.2.1. Methods used for the assessment of antioxidant activity of bioactive compounds

The antioxidant capacity of BACs can be evaluated by different *in vitro* models. The most common tests used to evaluate the antioxidant activity of the plant extracts, EO and their BACs are; (a) free radical scavenging activity methods; 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (Bondet et al., 1997) and Trolox equivalent antioxidant capacity (TEAC), this TEAC is based on the relative ability of antioxidants to scavenge the radical 2, 2-azinobis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS). These methods have procedures that are reproducible, simple, sensitive, and rapid (Lü et al., 2010; Rice-Evans, 1995). In these methods, the reaction required antioxidants that can donate an electron or an active hydrogen atom as the case with hydroxyl group that is occurring in some BACs and characterised with potent radical scavengers, (b) superoxide scavenging activity of anion radicals and other reactive oxygen species (ROS) it is also named as oxygen radical absorption capacity (ORAC) assay, this method used to determine the antioxidant activities by direct O_2 scavenging through using Nitro blue tetrazolium and other tetrazolium salts like other enzymes that used in the determination of O_2 determination body such as NADH oxidase, monooxygenases, and cyclooxygenases. ORAC has been applied to evaluate the antioxidant capacity of water-soluble phytochemicals (Ak & Gülçin, 2008; Cao et al., 1993), and (c) thiobarbituric acid reactive substance (TBARS) or hydroxyl radical scavenging assay, in this method the scavenging ability of antioxidants can be determined via Gutteridge method. Hydroxyl radical (OH) is extremely reactive and can attack DNA, lipids, and proteins. The Gutteridge method is based on the fact that the OH can degrade deoxyribose and forms a reactive species MDA, which forms an adduct with thiobarbituric acid (TBA). This essay is based on the stable product that could be detected which is formed between aldehydes (MDA) and TBA in the aqueous phase which assayed spectrophotometrically at absorption at 532 nm (Buege & Aust, 1978; Haces et al., 2008; Lü et al., 2010). Other methods used are total antioxidant activity by phosphomolybdate assay, hydrogen peroxide (H_2O_2) scavenging activity assays are used to measure the antioxidant activity of BACs (Jan et al., 2013; Shah et al., 2014). It has been studied that the extraction method and the solvent used can affect the antioxidant activity of a BACs and composition of EOs (Jan et al., 2013).

2.7.3. Mechanism of antimicrobial activity of BACs

The antimicrobial activity of plant EOs is believed to have a strong relationship with their great number of complex constituents and BACs metabolites. The mechanism of antimicrobial action of these BACs depends upon their chemical structure and variability of chemical groups. This may result in various action modes and hard to identify from the molecular point of view

(Burt, 2004; Carson et al., 2002). In general, several mechanisms have been suggested to mediate modes of antimicrobial action of BACs against different spoilage bacteria in fresh meat: (1) It has been suggested that the cytoplasmic membrane is an important cellular target to BACs (Burt, 2004). BACs disrupt cell membranes and cause necrosis of prokaryotic and eukaryotic microbes. Thus, the lipophilic moieties that exist in most BACs and EOs can freely pass through the cell wall enhances membrane permeability and cause cell wall disruption and lysis. The accumulation of these lipophilic moieties in the cytoplasmic membrane of the bacterial cell causes noticeable changes in the stability, composition of fatty acid, fluidity and hydrophobicity and/or lipophilicity of the membrane and leads to a subsequent loss of cellular components (Sikkema et al., 1995; Ultee et al., 2002) (Figure 12), (2) The accumulation of these lipophilic constituents in the cytoplasmic cell membrane can cause acidification inside the cell leading to the hydrolysis of adenosine triphosphate (ATP), decreasing in the synthesis of ATP and reduction of the intracellular ATP pool. A further effect of BACs in the side cytoplasmic membrane can cause ion loss, leakage of lipopolysaccharides, induce production of ROS, change on fatty acids and phospholipids, disrupting proton motive force and membrane potential that leads to inhibition of cellular energy production and compulsory into a bioenergetic compromise, and eventually lose viability (Bakkali et al., 2008; Hansen et al., 2004; Li et al., 2014). BACs can cause loss of potassium that leads to cell death because of potassium is obligatory for maintaining osmotic balance, cellular enzymes activation, pH, turgor pressure and metabolism of glucose in prokaryotic and eukaryotic cells (Preedy, 2015), and (3) BACs also cause coagulation of the cell content and disintegration of the plasma membrane affecting nutrient absorption in the cell, deactivate activity of enzymes, damage lipids, prevents the metabolism of energy, destruction of genetic materials loss of RNA and proteins, inhibit microbial DNA replication and ultimately results in the death of organisms (Bakkali et al., 2008; Burt, 2004; Hernández-Ochoa et al., 2014; Lambert et al., 2001).

Dorman and Deans, (2000) suggested that hydrophobicity and/or lipophilicity of BACs is mainly due to the presence of hydrophilic functional groups, such as hydroxyl groups in the structure of phenolic BACs and makes the BACs active against G-veB and G+veB bacteria. However, in some studies, it has been reported that G-veB is more resistant to antimicrobial BACs and EOs than G+veB (Amensour et al., 2010; Nychas, 1995; Solórzano-Santos & Miranda-Novales, 2012). This is because of lacking outer membrane makes G+veB more susceptible to the BACs and EOs. While the resistance of G-veB is likely due to the fact of having a wall associated with an outer complex membrane and hydrophilic cell wall, which blocks the passage of hydrophobic BACs (Inouye et al., 2001). It has been reported that EOs that are rich in BACs (e.g.

thymol and CARV) (phenolics) showed higher activity in damaging the membrane of bacteria than those contain less amount of these phenolics (Preedy, 2015).

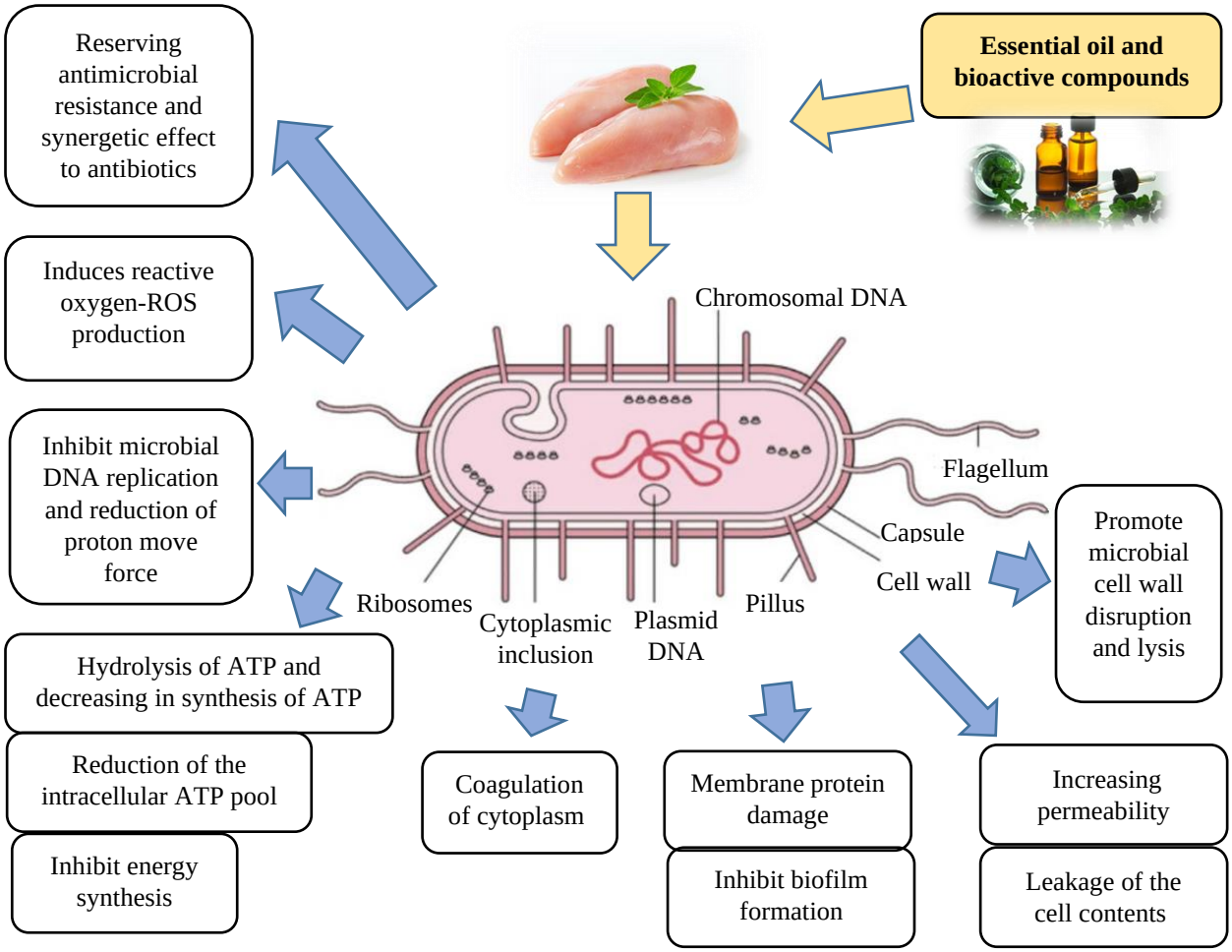


Figure 12: Possible mechanisms of antibacterial action of BACs against the bacterial cell.

2.7.3.1. Methods used for assessing the antimicrobial properties of EOs and BACs

The antimicrobial activity of BACs has been evaluated using several *in vitro* and *in vivo* methods. Some of the *in vitro* method has been implemented are: (a) the micro-atmosphere method in the vapour phase. In this method the microorganism is inoculated on the surface of agar on Petri dish which is inversely incubated at optimum temperature, a few drops of BACs can be placed on a blotting paper or a small cup placed at the bottom and the centre of the cover (Cardiet et al., 2011), (b) diffusion methods using solid media (disk diffusion method and well diffusion method). Well, and disk diffusion methods are most commonly applied for screening of large amounts of BACs, EOs and/or microbial isolates due to the great simplicity and cost-effectiveness. It can be stated that the only variation between the two is that a sterile well of 6 mm diameter in the inoculated agar filled with BACs using a micropipette is used instead of impregnated and blotting

paper in disk method. However, diffusion methods have limitations due to various factors such as the deposited volume of BACs, culture thickness, inoculum density, microbial strains, and solvent nature for solubilization od BACs. These methods were applied in the current study, and the antimicrobial activity is determined by measuring zone inhibition of inhibition in mm or cm (Dorman & Deans, 2000; Dussault et al., 2014; Fernández-López et al., 2005), (c) dilution method (broth and agar) the most appropriate method that used to quantitatively define the minimum inhibitory concentration and minimum bactericidal concentration (MIC and MBC) of BACs against the growth of the tested bacteria either through macro-dilution or microdilution. MIC is the lowest concentration of BACs that completely inhibits the growth of the organism up to 24 or 48 h incubation whereas the MBC is the lowest concentration of BACs required to kill 99.9 % of the final inoculum within approximately 24 h of incubation (Delaquis & Sholberg, 1997). In this method, a series decreasing in the concentrations of antimicrobial agents performed using standardized bacterial suspension (~10^8 bacteria/ml) (Li et al., 2014; Turgis et al., 2012), (d) 'Omic' techniques have been developed to follow the antimicrobial activities of BACs and EOs and against a single pathogen, in particular, this method related to genomics, transcriptomics and proteomics terms that used in studying the antimicrobial effect of additives (Li et al., 2014), (e) the checkerboard method or antimicrobial gradient method (Etest) has been applied to assess the efficacy of possible interaction of combined BACs or between two drugs. This method determines the MIC value by combining the principle of dilution assay with that of diffusion assay. In this method, Fractional Inhibitory Concentration (FIC) index used to determine the interaction effect against pathogens and this effect could be additive, synergistic, or exhibiting no interaction or antagonist (Gutierrez et al., 2009; White et al., 1996), and (f) time-kill test, this method used to evaluate the bactericidal or fungicidal effect. Similar to the previous method this assay applied as a technique to show dynamic interaction between BACs and the microbial strain. This method shows the antimicrobial effect in terms of time-dependent or a concentration-dependent (Balouiri et al., 2016).

Furthermore, the assessments and efficacy of antimicrobial activities of BACs can be influenced by several factors: structural nature of BACs, water insolubility, the solvent used (absolute ethanol, Tween 80, dimethylsulfoxide-DMSO), amount of BACs applied, species of targeted microorganism, pH value, food composition and nature (presence of proteins, lipids, salts, and phenolic substances), the temperature of the environment, initial populations of bacterial cells and culture conditions (Aleksic & Knezevic, 2014; Sagdic, 2003).

2.7.4. The activity of EO and their BACs in maintaining sensory properties of meat

Nowadays the use of natural additives in food and meat preservation including EOs and BACs gained widespread popularity than synthetic chemical preservatives (Gutierrez et al., 2009). The decrease in the demand in preserving the meat with synthetic additives is due to the negative health perceptions of consumers towards synthetic chemicals related to detrimental residual effects and toxic properties that might promote the development of cancer, for example, carcinogenic and anticarcinogenic effects have been reported with synthetic antimicrobial and antioxidants; BHA and BHT (Botterweck et al., 2000; Yadav & Kamble, 2009). The pursuit of natural additives BACs and EOs as preservatives of meat not only focus on antibacterial and antioxidant effects but also the prevention of organoleptic characteristics of meat (flavour, odour, taste, appearance, texture). Various benefits of these constituents of EOs have been reported; the improvement of sensory properties including aroma, taste, texture, showing anticancer, hypoallergenicity and antioxidant benefits related to consumer's health (Bhat et al., 201; Bleasel et al., 2002). The direct application of BACs in meat preservation should be kept as low concentration as possible to avoid any harmful effect to the original sensory properties of meat because of most BACs and EOs possess an intense and strong aroma and flavours even at low concentrations and could overwhelm consumers and might cause negative organoleptic changes (Sharma et al., 2017). In addition to that because of the large scale, exert of BACs is a costly procedure (Skandamis & Nychas, 2001).

Although the potential of BACs has been observed in meat preservation during *in vitro* studies, it is challenging to extrapolate the findings from *in vitro* assays in meat models. Additionally, it has been reported that due to the potential interaction of BACs with meat constituents and the effect of factors such as a_w and pH, meat models require a greater concentration of BACs to achieve the same impact as it obtains by *in vitro* screening (Burt, 2004). Gutierrez et al. (2009) stated that individual BAC and EO not potent enough to inactivate both spoilage and pathogenic organisms in meat due to the high binding capacity of BACs to proteins and fats in meat followed by a decrease in the physical stability BACs.

Several procedures have been implemented to enhance the preservative effect of BACs in meat. It is critical to understand the effect of BACs such as AITC, thymol, and CARV individually or in combination to optimize the order of their combination to better exploit their synergistic effects in meat preservation (Lucera et al., 2012). The incorporation of BACs into the packaging materials as active packaging can support the diffuse or partition process into the meat might increase the stability of BACs (Skandamis & Nychas, 2001). Other possible solutions are; using sachets that provide a slow release of BACs and EOs be incorporated into packaged meat (Sánchez-González et al., 2011). More preservation technologies have been applied such as low

temperature (Skandamis & Nychas, 2001), applying low dose irradiation (Farkas, 1990), high hydrostatic pressure (Li & Gänzle, 2016), MAP (Chouliara et al., 2007; Lucera et al., 2009; Marino et al., 1999), and application of edible films and coatings such as chitosan on meat surface could increase the stability of the meat sensory attributes (texture, colour) (Dias et al., 2013; Guo et al., 2013; Sánchez-Ortega et al., 2014; Siripatrawan & Noipha, 2012).

2.8. Application of BACs in meat and meat products

The EOs are largely used in food preservation and have been extensively studied alone or in combination with other EOs and/or preservation methods to improve the quality attributes and extend the shelf life of meat and meat products. For example, oregano (*Origanum vulgare* L.), rosemary (*Rosmarinus officinalis* L.), and sage (*Salvia officinalis* L.), thyme, basil, balm, turmeric, lemon leaf, coriander, ginger, garlic, clove, savoury and fennel have been reported to exhibit a better potential in extending the shelf-life of fresh meat and meat products and to reduce lipid/protein oxidation, reduce discolouration, and limit the microbial growth in fresh meat, ground meat, seafood and their packaging/edible films (Djenane & Roncalés, 2018; dos Santos Rodrigues et al., 2017; Mohamed & Mansour, 2012; Shan et al., 2005; Sharma et al., 2017; Velasco & Williams, 2011; Wojdyło et al., 2007). However, higher than 1.0 % of some of these EOs (e.g. oregano), may produce low sensory quality foodstuffs due to the production of very strong unfavourable flavour (Camo et al., 2008; Zinoviadou et al., 2009).

The use of BACs is also getting more attraction by scientists and food producers. Some of widely evaluated BACs that have shown to possess preservative activity are volatile terpenes, terpenoids and phenylpropanoids: CARV, 1, 8-cineole, eugenols, carvone *p-Cymene*, γ-Terpinene, thymol, cinnamaldehyde, geraniol, menthol, chavicol, citral, estragole, geraniol, perillaldehyde, terpineol, eugenyl acetate, geranyl acetate, citronellol, menthol, and vanillin has been applied in various application in food and food products (Bakkali et al., 2008; Hyldgaard et al., 2012; Jayasena & Jo, 2013; Oyedemi et al., 2010; Tiwari et al., 2009). Many of these BACs did not show any major health concerns. They are included in the A-list published by the Council of Europe and classified as GRAS by the U.S. Food and Drug Administration (FDA), and also have been registered by the European Commission to be used as flavouring and as food additives (Bhavaniramya et al., 2019; López et al., 2007). On the other hand, some factors may impact or interfere with the preservative effect and reduce the stability of BACs in meat. Properties such as low solubility in water, volatility, and strong aroma may produce negative organoleptic effects, hence possibly will limit the use of some EOs and BACs (Bhavaniramya et al., 2019; Dias et al., 2013), reaction with constituents of meat (such as thiols, AAs, and the sulphydryl groups) of

proteins (Nadarajah et al., 2005), the solubility of some BACs example AITC in the fatty acids of the meat, the adsorption of the antimicrobial in packaging materials (Nadarajah et al., 2005), the fat in foods can provide a protective layer around the contaminating bacteria or absorb the lipophilic part of BACs (Chacon et al., 2006). Same as EOs the BACs can be applied in meat alone or in combination with other BACs and/or preservation techniques. However, careful selection of type and concentration of BACs needs to be considered (Burt, 2004). Additionally, there is a claim that individual BACs in inadequate quantities are not potent enough to show antimicrobial/antioxidant and desirable sensorial effects in food preservation. Therefore, synergism and antagonistic effects between several BACs have been suggested as a solution to overwhelming this claim (Andrés et al., 2017). Many experimental applications of natural BACS as antioxidant/antimicrobial agents in meat and meat products are presented in Table 2-Appendix.

2.8.1. Allyl isothiocyanate

Allyl-isothiocyanate (AITC) is a colourless, volatile, and aliphatic organosulfur compound. It is found in the seeds, stem, leaves, and roots of cruciferous plants (Lin et al., 2000) (Ohta et al., 1995; Clark, 1992; Okano et al., 1990). Studies have shown that other plants such as; horseradish, cabbage, wasabi, brussels sprouts, broccoli, cauliflower, kohlrabi, turnip, rutabaga, watercress, and papaya also contain AITC (Table 1-Appendix) (Delaquis & Mazza, 1995; Kyung & Fleming, 1997; Ono et al., 1998). It constitutes almost 90 % of the composition of horseradish root (Ward et al., 1998). The use of AITC for food preservation is previously approved in Japan (Dufour et al., 2015) and considered as GRAS by the Food and Drug Administration (FDA) of the United States (Dias et al., 2013). However, in Europe, the use of AITC as a food additive, flavouring, anti-spoilage agent in food is under revision (EFSA, 2010).

Additionally, AITC possesses antioxidant and antimicrobial characteristics that inhibit a range of pathogens at low concentrations in food. AITC has been applied in both vapour and liquid forms. However, it has been investigated to show more effective antimicrobial activity when used in the vapour rather than the liquid form against various food spoilage microorganisms and food pathogens (Delaquis & Mazza, 1995; Isshiki et al., 1992; Kim et al., 2002; Shin et al., 2010), and a broad spectrum of fungi (Delaquis & Sholberg, 1997; Nielsen & Rios, 2000). Chacon et al. (2006) highlighted that yellow or white mustard is highly inhibitory towards *E. coli* O157: H7 in fermented dry sausages, mainly because it comprises of constituents including AITC. Pang et al., (2013) reported that AITC with modified atmosphere reduced the growth of *P. aeruginosa* in catfish fillets in vapour-phase (Pang et al., 2013). Additionally, the antimicrobial activity of AITC has been reported against yeast and moulds in cottage cheese (Gonçalves et al., 2009), *Penicillium*

expansum and *Botrytis cinerea* on apples (Wu et al., 2011), *Listeria innocua* on frozen RTE shrimp (Guo et al., 2013), *Salmonella enterica* in liquid egg albumen (Jin & Gurtler, 2012), and spoilage organisms on blueberries (Wang et al., 2010). Luciano and Holley, (2009) observed that the metabolism of *E. coli* O157:H7 was affected by AITC and found that AITC was able to inhibit the activity of enzymes thioredoxin reductase and acetate kinase. However, AITC has some properties; poor aqueous solubility, pungent smell and flavour associated with isothiocyanate, instability at high temperature, and susceptibility to degradation by nucleophilic molecules may sometimes limit its application (Kim et al., 2002; Li et al., 2015b). Thus, flavour and odour changes may limit the use of a higher concentration of AITC in food preservation.

The mode of action behind the antimicrobial activity of AITC in causing cell membrane damage, leakage of cellular metabolites at all growth stages believed to be due to its chemical group (Luciano & Holley, 2009; Nadarajah et al., 2005). The AITC might possess a similar mode of action in both prokaryotes and eukaryotes (Hyldgaard et al., 2012). Whereas the G-veB could be more sensitive to be inhibited by AITC than G+veB (Isshiki et al., 1992; Lin et al., 2000). Lin et al. (2000) tested liquid AITC *in vitro* against G+veB (*L. monocytogenes*) and G-veB bacteria (*Salmonella* Montevideo and *E. coli* O157:H7) their result indicates that *Salmonella* Montevideo and *E. coli*, were more sensitive to AITC than *L. monocytogenes*. They observed that AITC caused cell membrane damages to *E. coli* and *Salmonella* Montevideo, it did not show cell lysis but lead to the leakage of cellular metabolites (Lin et al., 2000). AITC can cause cell wall damages (Ahn et al., 2001), inhibit enzymes (e.g. sulfhydryl) and alter proteins through an oxidative process and cause cleavage of disulfide cysteine bonds, attacks free amino groups (proteins), glutathione, arginine residues, and react with oligopeptides, sulfites, and water (Delaquis & Mazza, 1995; Kawakishi & Kaneko, 1987). The central electron-deficient carbon atom of isothiocyanate is highly electrophile and prone to reactions with oxygen-, amines, AAs, sulphur-, alcohols, or nitrogen-centred nucleophiles and produce carbamates, thiocarbamates, or thiourea derivatives, respectively (Cejpek et al., 2000; Zhang & Talalay, 1994).

2.8.2. Carvacrol

Carvacrol (CARV) is a major phenolic monoterpenoid constituent found in oregano (*Origanum vulgare*) and constitutes about 6-80 % (Burt, 2004; dos Santos Rodrigues et al., 2017; Gutierrez et al., 2008; Karabagias et al., 2011) (Table 1-Appendix). CARV ($C_{10}H_{14}O$) represented by the synonyms: isopropyl-o-cresol, pcymen-2-ol, 2-hydroxy-*p*-Cymene, 5-isopropyl-2-methylphenol and iso-thymol (Sikkema et al., 1995). CARV is highly lipophilic and insoluble in water, the cytotoxic effect of CARV can make it an effective antiseptic and

antimicrobial agent (Yadav & Kamble, 2009). CARV is one of the most extensively studied BACs together with its closely related isomer thymol (Sikkema et al., 1995). It has been reported that γ-Terpinene play the main role in the aromatization process for biosynthesizing thymol and CARV, by which γ-Terpinene produces *p*-Cymene the latter is the fundamental precursor for oxygenated compounds which is converted into thymol and CARV by hydroxylation (Figure 13) (Nhu-Trang et al., 2006).

The mode of action of CARV is mostly identified in the cell membrane as the main cite of action and depends on its chemical composition. CARV has a hydroxyl group that supports interaction with the cell membrane proteins and periplasmic enzymes and acts as a transmembrane carrier (Hyldgaard et al., 2012). Additionally, it has shown antioxidant effects which are related to the hydroxyl groups linked to the aromatic ring, which are capable of donating hydrogen atoms with electrons and stabilizing free radicals (Hernández-Ochoa et al., 2014; Zengin & Baysal, 2014). CARV causes a reduction in LO and causes colour stability of poultry meat (Lucera et al., 2009). Ultee et al. (1999) noticed that CARV (2 mM) caused a significant reduction in intracellular ATP pool that associated with a change of the membrane potential, while the extracellular ATP pool has no relative increase. They also found that CARV enhances the fluidity and permeability of membranes (e.g. *B. cereus*) for monovalent cations (carrying H+ in and releasing K+ back out of the cell cytoplasm). The disintegration the outer membrane of example G-veB is leading change the release of lipopolysaccharides, composition of fatty acid of the membrane leads to the destruction of essential processes in the cell, leakage of cellular material and finally to cell death (Ben Arfa et al., 2006; Helander et al., 1998; Ultee et al., 2002). Kim et al. (1995) observed that CARV showed strong bactericidal activity against G-veB (*E. coli*, *E. coli* 0157:H7, *S.* Typhimurium, and *Vibrio vulnificus*) and G+veB (*L. monocytogenes*). Luz et al. (2014) found that the exposure of *S.* Typhimurium ATCC to CARV 0.6 μL/mL (62 μg/mL) in meat broth caused a shift towards the synthesis of UFAs and *cis–trans* isomerization and sub-lethal damage to the cytoplasmic and outer membrane. Moreover, CARV also showed strong inhibitory effects against *St.* aureus (Luz et al., 2013) and inhibitory activity against the growth of *P. aeruginosa* ATCC (Luz et al., 2015).

Previously numerous studies have been carried out on the *in vitro* and different food matrix antibacterial activity of CARV such as vegetables (Oliveira et al., 2015), fruit juices (Ait-Ouazzou et al., 2013), peanut paste (Chen et al., 2015), incorporated to alginate films (Matiacevich et al., 2015), CARV incorporated packaging films on ground beef (Wang et al., 2020), marinated fresh chicken (Karam et al., 2019), cheese (Honório et al., 2015), poultry (Du et al., 2012; Luna et al., 2010; Mastromatteo et al., 2009), CARV with HHP in fresh ground chicken meat (Chuang et al.,

2020) (Table 2-Appendix), meat-based broth (Luz et al., 2014), broiler chicken feed (Galli et al., 2020), sliced bologna sausages (Churklam et al., 2020). CARV exhibit antioxygenic, antifungal, antiparasitic, and insecticidal activities (Veldhuizen et al., 2006). Lambert et al. (2001) studied the effect of thymol and CARV and oregano EO against *Pseudomonas aeruginosa* and *St. aureus*. They found these BACs cause total inhibition of microorganisms due to damage in membrane integrity which increased the permeability of cells to the nuclear stain (ethidium bromide), which further affects homeostasis of pH and equilibrium and leakage of inorganic ions. It has been reported that the antimicrobial activity of the CARV derivatives CARV methyl ether and *p*-Cymene was much lower than CARV, this could be due to the exchanging hydroxyl group with methyl ether that influences the hydrophobicity CARV (Dorman & Deans, 2000). It has been reported that CARV and thymol showed synergistic and antagonistic effects against *Staphylococcus* sp., *Micrococcus* sp., *Bacillus* sp., and *Enterobacter* sp. (Burt, 2004).

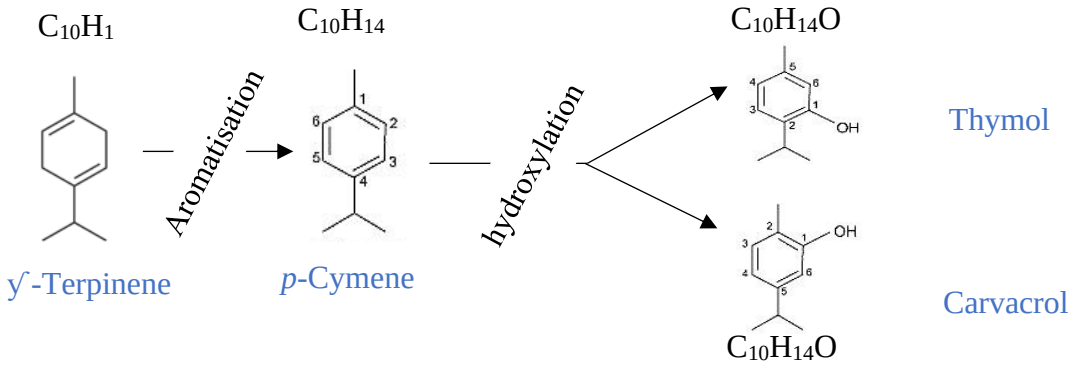


Figure 13: Pathway for the biosynthesis of thymol and CARV from γ-Terpinene and *p*-Cymene (adapted from Nhu-Trang et al., 2006).

2.8.3. α-Terpineol,

α-Terpineol (alpha-Terpineol) (αTPN) is a volatile monoterpene relatively nontoxic alcohol. There are three isomers of terpineol, alpha-, beta-, and gamma-terpineol and is the main isomers is αTPN ((*S*)-*p*-Menth-1-en-8-ol) that comprises up to 30 % of some EOs of different plant species and trees, such as eucalyptus globulus, pine oil, marjoram, oregano, thyme, Ravensara aromatica, cajuput oil, Melaleuca qinquenervia and Croton sonderianus (Oliveira et al., 2016). αTPN is a relatively cheap and abundant aroma BACs which is widely used in cosmetics, and household products (Bicas et al., 2011). The antioxidant activity of αTPN very low using the ferric reducing antioxidant power (FRAP) and DPPH assay, however using the Oxygen Radical Absorbance Capacity (ORAC) assay the αTPN possesses a strong antioxidant activity could be compared to commercial antioxidants, however, this antioxidant is less compare to other

oxygenated monoterpenes BACs such as thymol and CARV (Bicas et al., 2011; Zengin & Baysal, 2014). *In vitro* study on the antimicrobial activity of αTPN reported that due to the presence of OH this BACs interact with intracellular components and cause the change in the permeability of the outer membrane, change the function of the cell membrane and leads to the leakage of intracellular materials (Zengin & Baysal, 2014). The EOs that are rich in αTPN, has been used widely in folk medicine for aromatherapy due to its anti-spasmodic, antinociceptive, and immunostimulant properties. Several studies have been conducted on αTPN for different aspects including; antimicrobial, anticonvulsant (Sousa et al., 2007), a potential anticancer agent which acts through suppressing NF-κB signalling (Hassan et al., 2010), promising insecticidal activities (Pandey et al., 2013), anti-inflammatory and anti-nociceptive central effects (Oliveira et al., 2012), anti-hyperalgesic effect in the animal model (Oliveira et al., 2016). To the best of our knowledge, no studies can be seen that dealing with the preservative potential of αTPN in a food matrix, as is reported for some of its monoterpene counterparts.

2.8.4. Linalool

Linalool (LIN) (3,7- dimethylocta-1,6-dien-3-ol) is acyclic monoterpene alcohol, which constitutes approximately 69 % of basil (Ocimum basilicum) composition (Kéita et al., 2000; Predoi et al., 2018), 22.35- 23.78 % of *Lavandula angustifolia*, 66.3-75 % bergamot mint (*Mentha citrate*), and Coriandrum sativum L. (Seeds) (Marín et al., 2016; Oussalah et al., 2007; Samojlik et al., 2010). LIN is highly soluble in organic solvents (alcohol, ether, etc), while it is poorly soluble in water due to the hydrocarbon a polar structure (Pereira et al., 2018). This BAC possesses antifungal activity (Pattnaik et al., 1997), antioxidant properties (Liu et al., 2012), anti-inflammatory (Peana et al., 2002), anticancer activities in animal models (Jana et al., 2014), and it is commonly used as a food additive (fragrance and flavour agent) (Aprotosoaie et al., 2016). Duarte et al. (2016) reported used LIN as antibacterial in anti-biofilm against pathogens that contaminate food. They observed antibacterial activity against *C. jejuni* and *C. coli*. Besides using DPPH method and found that LIN exhibits radical scavenging and lipid peroxidation inhibition, this property could make LIN potential alternatives to synthetic antioxidants (e.g. BHT). Zengin and Baysal, (2014) stated that LIN, 1,8-cineole (Eucalyptol) possess very weak antioxidant activity using FRAP and DPPH methods compare to other BACs such as αTPN, thymol, and CARV, however they found that LIN caused permeability alteration of the outer membrane, alteration of cell membrane function and leakage of intracellular materials. Besides, LIN with MIC value of 1 % caused inhibition to *St. aureus, L. innocua* and *S. liquefaciens*, while *E. coli* O157:H7 had a MIC value of 0.6 % following by *S.* Typhimurium with 0.7 % MIC. Zakarienė et al. (2015) found

that *C. jejuni* reduced by 1.09 $\log^{10}$ CFU/g after treatment with 2 % LIN, however, LIN did not show a significant reduction of total aerobic bacterial count in broiler breast fillet. Fisher and Philips, (2006) found linalool with a concentration of 0.06 % to be effective against pathogenic bacteria and linalool vapours produced 6 log reductions *Campylobacter spp., St. aureus, L. monocytogenes* and *Bacillus cereus* on cabbage leaf after 8-10 h exposure, they also found that these bacteria were less susceptible in food models than *in vitro*. Nevertheless, there is only limited information on the antimicrobial, antioxidant, and organoleptic effect of linalool in meat and food preservation.

2.8.5. Piperine

Piperine (PIP), the major organic alkaloid BAC that accounts for up to 9-33 % of black pepper (Calo et al., 2015). The high spectrum of beneficial physiological, pharmaceutical, and biological activities of PIP has been reported, PIP is responsible for the aroma of pepper and exhibits sedating, detoxification, hypotensive, chemopreventive, and anticarcinogenic properties (Gorgani et al., 2017), therapeutic potential, decreases the total plasma cholesterol, LDL cholesterol, very-low-density lipoprotein (VLDL) (Stojanović-Radić et al., 2019), antiulcer (Bai & Xu, 2000), antifungal (Pattnaik et al., 1997), anti-inflammatory, anticancer (Peana et al., 2002; Tasleem et al., 2014) and antimicrobial activities (Yang et al., 2002), antidiabetic and antioxidant activities in an animal model (Arcaro et al., 2014). Additionally, it has been reported that these BACs exhibit hydroxyl radical scavenger activity at low concentrations in *in vitro* experiments by quenching free radicals and cause reduced lipid peroxidation (Mittal & Gupta, 2000; Srinivasan, 2007). The low water solubility can limit the pharmaceutical activities of PIP besides using the high concentrations of PIP can be toxic for the central nervous and reproductive systems (Gorgani et al., 2017). Along with the mentioned spectrum activities of PIP has, as the main ingredient of the most known spice, particularly pepper been used traditionally as a food additive for centuries (Stojanović-Radić et al., 2019). Martinez et al. (2006) found that levels of black pepper of 0.5 % or above, caused a reduction in TBARS values under 1 mg MDA/kg after 16 days of storage of fresh pork sausages, they state that this antioxidant activity of black pepper depends upon the amount of PIP. However, the best of our knowledge, no scientific research has been devoted in food models to the putative physicochemical, antioxidant, antimicrobial, and sensory (E-nose) based effects of PIP on chicken meat (Stojanović-Radić et al., 2019), although both black pepper and PIP are commonly used as antimicrobials *in vitro* in many studies.

2.8.6. Other BACs (α-Pinene, *p*-Cymene, Citronellol, Geraniol, Eugenol, α-Bisabolol, γ-Terpinene, 1,8-Cineole, Camphor, Limonene, Cuminaldehyde)

Other BACs have been used in various broad studies, some of them may have the potential to be applied in the food preservation industry and can have a single target or multiple targets of their activity. α-Pinene is a colourless bicyclic organic BAC that belongs to the water-insoluble but oil- and ethanol-the soluble terpenoid hydrocarbon, α- and β-pinene are two isomers. α-Pinene has been detected in EO of pine (coniferous trees) and at least 40 others different EOs. In a recent review by Salehi et al. (2019) they mentioned various aspects in which α-Pinene assessed such as preclinical pharmacological, anticoagulative/antiplatelet, anti-inflammatory, anti-tumor, preclinical antioxidant, neuroprotective and gastroprotective activity, and other biological activity including fungicidal, flavours, fragrances, antiviral, and antimicrobial activity. It has been reported that the presence of BACs such as camphor, camphene, *α*-pinene, 1,8-cineole, borneol, and *β*-pinene in thyme oil exhibited effective antibacterial activity against *St. aureus, S. epidermidis, Streptococcus* sp., *Pantoa* sp., and *E. coli* (Amatiste et al., 2014; Santurio et al., 2014). However, in a study by Zengin and Baysal, (2014), they found that α-Pinene showed no activity with the MIC values above the concentration of 2 % against G+VeB (*St. aureus, Carnobacterium divergens* and *Listeria innocua*) and G-veB (*E.coli* O157:H7, *S.* Typhimurium, *Serratia liquefaciens* and *Shewanella putrefaciens*).

Thymol is a phenolic monoterpenoid that is found in the EOs and constitutes about 10-64 % of thyme (*Thymus vulgaris* L.) (Boskovic et al., 2017; Burt, 2004; Cosentino et al., 1999; Herman et al., 2016) and 16.22-64 % of oregano (*Origanum vulgare*) (dos Santos Rodrigues et al., 2017; Fasseas et al., 2008; Oussalah et al., 2007; Radha krishnan et al., 2014). Thymol is part of a naturally occurring class of compounds known as biocides. The structure of thymol (5-methyl-2-isopropylphenol) is similar to its isomer CARV, and it has hydroxyl groups occupying different positions on the phenolic ring (Sikkema et al., 1995). It has strong antioxidant/antimicrobial characteristics when used alone or in combination with other BACs or EOs (Palaniappan & Holley, 2010). The mechanisms of action of thymol are believed to be similar to the antimicrobial activity of CARV as both possess hydroxyl group at various locations (Nazzaro et al., 2013). Previously thymol has been studied in broiler chicken feed (Galli et al., 2020; Luna et al., 2010), feed supplementation in grass carp (*Ctenopharyngodon idella*) (Morselli et al., 2019), microencapsulation (Guarda et al., 2011), incorporating in antimicrobial active films (Ramos et al., 2012), food-packaging films (López et al., 2007), antioxidant enzyme activity and its effect on phospholipid fatty acid composition of the ageing in rat brain (Youdim and Deans, 2000), and in

fresh minced beef patties packed using a high barrier film and stored under normal atmosphere packaging and MAP conditions (Del Nobile et al., 2009).

p-Cymene is a monoterpene which considered as a precursor to CARV and thymol. *p*-Cymene (4-isopropyltoluene) constitute about a trace to 65 % of oregano and thyme EO (Gutierrez et al., 2008; Predoi et al., 2018; Radha krishnan et al., 2014; Rodríguez-Lázaro et al., 2017), it has a benzene ring without any functional groups on its side chains. This BAC through hydroxylation contributes to as precursor in biosynthesize and aromatization of thymol and CARV (Nhu-Trang et al., 2006). *p*-Cymene shows a high affinity for microbial cell membranes and can perturb the membranes. It did not exhibit an efficient antimicrobial compound when used alone but it potentiates the activity of other compounds like CARV and thymol (Ultee et al., 2002).

Citronellol is an alcoholic monoterpene found in EOs such as lemongrass (*Cymbopogon citratus)* and citronella (*Cymbopogon nardus* (*L)* Rendle), it constitutes about 7.7 to 14.40 % while citronellal is about 27.55 to 45.4 % of citronella composition (Pontes et al., 2018; Victoria et al., 2012). Citronellol is important commodities in the fragrance industry. Victoria et al. (2012) reported the antimicrobial potential of citronellol through measuring AU/mL using the agar diffusion method, they found that alcohol (*R*)-citronellol showed antimicrobial activity against *L. monocytogenes* and the best result was against *St. aureus* and *S.* Typhimurium. No studies reported the potential preservative effect of this BAC in meat mode. Santos et al. (2019) reviewed the pharmacological activities attributed to citronellol, they observed that citronellol had low toxicity and possess activities such as *in vitro* antibiotic and antifungal effects and *in vivo* analgesic and anticonvulsant effects.

Geraniol, (3,7-dimethylocta-*trans*-2,6-dien-1-ol) is an acyclic monoterpene alcohol constitute about 19 % to 33.88 % in citronella (*Cymbopogon nardus* (*L)* Rendle), also extracted from palmarosa, ninde, and rose EOs (Pontes et al., 2018; Syed et al., 2020; Victoria et al., 2012). This BAC is commonly used in fragrance industries due to its pleasant odour, geraniol has shown antimicrobial, antioxidant, anti-inflammatory, insecticidal, and repellent properties with low toxicity (Chen & Viljoen, 2010). Geraniol considered as GRAS by FDA (FDA, 2015; Syed et al., 2020), it has shown to be promising antibiotics against *St. aureus* and led to the formation of inhibition halos 19 mm in diameter (Pontes et al., 2018). Syed et al. (2020) used geraniol, and CARV in oil-in-water emulsions on raw goat meat surface during extended storage at 4° C. They found that the geraniol and CARV emulsion-entrapped formulations could extend antimicrobial efficacy on the goat meat model to 9 days as compared to samples with oil only, non-emulsion formulations. These authors also confirmed the results obtained by Yegin et al. (2016) associated with *in vitro* and on spinach surface growth inhibition of *Salmonella enterica* Typhimurium and *E.*

coli O157:H7 at 0.25 and 0.2 wt. %, respectively using geraniol-loaded polymeric nanoparticles prepared by flash nanoprecipitation. Guimarães et al. (2019) found that BACSs such as L-Carveol, β-citronellol, and *trans*-geraniol were not effective at killing *E. coli* at the MIC. While this BAC killed bacteria at 4× MIC; carveol and geraniol decreased the number of CFUs by 6 $\log^{10}$ in 2 h, and citronellol decreased them by 4 $\log^{10}$. They also observed that the scanning electron microscopy (SEM) showed morphological changes in *E. coli, St. aureus*, and *S.* Typhimurium by using L-carveol, β-citronellol, and *trans*-geraniol.

(−)-α-Bisabolol is monocyclic sesquiterpene alcohol, it constitutes about 90 % of *Salvia runcinata* EO its also isolated from Matricaria chamomilla (Asteraceae) (Viljoen et al., 2006). Bisabolol has been granted by the FDA as GRAS due to the low toxicity. Despite the poor antioxidant activity that has been exhibited by *in vitro* studies showed using DPPH radical value [450 lg/mL), it has been claimed that a-bisabolol to show antioxidant activity on chemical and/or biological tests (Zyl et al., 2006). Additionally, this BAC has several applications particularly in the pharmaceutical sector as an ingredient in dermatological and cosmetic formulations as well as its anti-inflammatory, antispasmodic, anti-allergic, and drug permeation properties have been reported (de Souza et al., 2008; Kamatou & Viljoen, 2009).

Eugenol (4-allyl-2-methoxyphenol) present in various plants such as clove (70–90 %) (Herman et al., 2016; Oussalah et al., 2007; Radha krishnan et al., 2014), this BAC is active against fungi, viruses and different pathogenic bacteria such as *E. coli, L monocytogenes, P. fluorescens, C. jejuni, S. enterica, St. aureus, Lactobacillus sakei, B. thermosphacta,* and *Helicobacter pylori* (Ali et al., 2005). Eugenol plays a prominent role in health materials such as dental and oral hygiene preparation (Saeed et al., 2019). Eugenol has the ability to cause non-specific permeabilization of the cytoplasmic membrane (Kumar et al., 2010), also it causes cell wall degradation and cell lysis, interact with proteins, affects the transport of ions and ATP and changes the fatty acid profile of bacteria, inhibit the activity of different bacterial enzymes (ATPase, histidine carboxylase, amylase, and protease) (Gill & Holley, 2006; Nazzaro et al., 2013). Interestingly, these BACs exhibit higher activity against G-veB than G+veB (Hyldgaard et al., 2012). BACs can be divided into two groups: slow-acting BACs and fast-acting BACs. Guimarães et al. (2019) considered terpineol, eugenol, geraniol, carveol, and citronellol as fast-acting BACs since they inactivated organisms such as *E. coli* and *S.* Typhimurium in a 2 h period.

γ-Terpinene belongs to terpenes that contain a hydrocarbon backbone that possesses the cyclic structure (Nazzaro et al., 2013). It constitutes about 12.9-16.86 % of cumin (*Cuminum cyminum*) (Pajohi et al., 2011; Patil et al., 2016), 2-31 % of thyme (Boskovic et al., 2017; Gouveia et al., 2017; Marino et al., 1999), and 2.14-52 % of oregano (dos Santos Rodrigues et al., 2017;

Karabagias et al., 2011; Radha krishnan et al., 2014). It has been reported that γ-Terpinene play the main role in the aromatization process for biosynthesizing thymol and CARV (Nhu-Trang et al., 2006). It has been reported that γ-Terpinene exhibits very low or no antimicrobial activity *in vitro* against 25 genera of bacteria (Dorman & Deans, 2000).

1,8-Cineole (eucalyptol) is a bicyclic mono-terpenoid colourless. It is the main component of eucalyptus EO and may also be present in high quantities in rosemary EO and laurel leaf (Mihara & Shibamoto, 2015; Zengin & Baysal, 2014). Various biological properties of 1,8-Cineole have been reported including antimicrobial (Sato et al., 2007), antioxidant (Nam et al., 2012), anti-inflammatory (Juergens et al., 2004), antiviral (Astani et al., 2010), anti-cancer (Murata et al., 2013). However, 1,8-Cineole in some studies showed weak antioxidant activity by the FRAP method and almost no free radical scavenging activity with the DPPH method. 1,8-Cineole, αTPN, and LIN terpene components caused permeability alteration of the outer membrane, alteration of cell membrane function, and leakage of intracellular materials. This was also supported in the results obtained by Zengin & Baysal, (2014) using the cell constituent release tests.

Camphor is a cyclic monoterpene ketone obtained through distillation of the wood from the camphor laurel tree (*Cinnamomum camphora*), also found in basil (*Ocimum kilimandscharicum*) (Zuccarini, 2009). There is no evidence on the developmental toxicity by oral administration of camphor as it has been tested in an animal model (EFSA, 2008). Camphor shows biological properties such as antimicrobial and antiviral effects (Chen et al., 2013). It has been reported that camphor has been widely used as an additive for food flavouring and as a preservative in confectionary good, besides it is for fragrance in cosmetics, and artificial mint flavours and some medicinal purpose (Chen et al., 2013; Mihara & Shibamoto, 2015).

Limonene (p-Mentha-1,8-diene) is considered as a colourless aliphatic hydrocarbon BACs that classified as a cyclic monoterpene. Limonene is the major BAC of EOs of Citrus plants, lemon 60 %, and orange 85 % (Sangkasanya et al., 2018; Teixeira et al., 2018). This BAC is widely used as a flavouring agent in food and beverage industry. Besides, D-limonene has been reported to exhibit a wide spectrum of antimicrobial activities and is considered as GRAS. Zahi et al. (2017) noticed that using the checkerboard method exhibited that d-limonene show strong synergistic and useful additive effects against *E. coli, St. aureus*, *Bacillus subtilis,* and *Saccharomyces cerevisiae*. It has been documented that the main antimicrobial mechanism of D-limonene against the microorganisms is through causing loss of the cytoplasmic membrane integrity, inhibition of the respiratory enzymes, and dissipation of the proton-motive forces (Vuuren & Viljoen, 2007). Depending on alkyl group limonene can be considered to be more effective than *p*-Cymene (Dorman & Deans, 2000). In a study using DPPH and b-carotene/linoleic acid the *in vitro*

antioxidant activity of the BACs were evaluated, thymol and CARV, terpinene-4-ol, 1,8-cineole, camphor, borneol, *p*-Cymene, α-pinene, and β-pinene showed no activity, thymol and CARV showed strong antioxidant activity, while the others exhibited no activity (Tepe et al., 2005).

Cuminaldehyde (4-isopropylbenzaldehyde) is one of the major natural organic flavoring components of EO of cumin and eucalyptus it constitutes about 22.34 % of Bunium persicum Boiss and 27.10 % of *Cuminum cyminum* L. that can induce different biological activities (Aminzare et al., 2017; Oroojalian et al., 2010). Hernández-Ochoa et al. (2014) investigated the effect of cumin EO in red meat. They observed that the cumin EO produced a reduction of 3.78 log UFC/g with the application of 750 μL, they mentioned that this prolongs meat's shelf life by cumin is due to BACs like cuminaldehyde that can inhibit growth and production of some bacteria toxins on meat. In the same study, they found that cumin EO showed MIC levels (750 mg/L) against *E. coli* and *S. enteritidis* Typhimurium. Moreover, Wongkattiya et al. (2019) noticed that the EO of *Cuminum cyminum* L. which is rich in cuminaldehyde exhibited antibacterial activity against food-borne pathogenic bacteria *B. cereus, St. aureus, E. coli*, and *S.* Typhimurium.

This review was an attempt to illustrate the key concerns causing quality deterioration of fresh chicken meat with identifying the most recent approaches to combat these concerns. At the beginning, the physicochemical properties of meat were explained. Then major issues in meat preservation were elucidated including LO, microbiological deterioration, and sensorial changes. Subsequently, the high hydrostatic pressure as a mild preservative technology in meat industry were explained. Finally, the application of BACs from EOs and plant extracts in meat preservation was reviewed.

3. MATERIALS AND METHODS

3.1. Chemicals and bioactive compounds

Chemicals; XLD agar (Xylose Lysine Deoxycholate Agar), PALCAM (Listeria Selective Agar and PALCAM Listeria Selective Supplement), Cetrimide agar (*Pseudomonas* Selective Agar), Tryptic-Soy agar (TSA, Biokar Diagnostics BK046HA), thiobarbituric acid (TBA), and trichloroacetic acid (TCA) were obtained from (SIGMA, Germany).

Bioactive compounds; α-Terpineol (>95 %), β-citronellol (≥95 %), geraniol (>96 %), (−)-α-Bisabolol, Carvacrol (98 %), Linalool (97 %), Allyl isothiocyanate (95 %), Piperine (≥97 %), Thymol (5-Methyl-2-isopropylphenol) ≥ 98.5 %, Eugenol (99 %), γ-Terpinene (97 %), α-Pinene (98 %), 1,8-cineole (98 %), *p*-Cymene (99 %), Camphor (96 %), cuminaldehyde (98 %), limonene (97 %) and BHT (butylated hydroxytoluene) (≥99 %) were obtained from (SIGMA, Germany).

3.2. Preparation of raw meat samples

Fresh chicken breast meat 24 hours post-mortem were obtained from a local slaughterhouse and transported to the laboratories at the Department of Refrigeration and Livestock Product Technology (Faculty of Food Science - Hungary). The meat was skin-off minced then homogenized using a meat grinder (BOSCH-Slovenia), and divided into treatment groups. The samples were then placed in polyethylene bags, vacuum packaged and stored at 4 ± 0.5 °C. Experiments were conducted at room temperatures between 22 and 25 °C.

3.3. Experimental design and meat treatments

Experiments were performed to study the preservation effect of BACs alone/ or in combination with other BACs and with HHP to extend the shelf life of fresh minced chicken meat. In the first experiment, meat samples were mixed with 500 and 1000 ppm of AITC, CARV, LIN, and PIP (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 (Figure 14-Appendix). 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.

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.

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 + 3.45 + 1.25 g of BAC + DW + ethanol, respectively, in MIC-1 was used in 100 g meat (Table 3-Appendix), in MIC-2 the ratio of αTPN was twofold and in MIC-4 the ratio of αTPN was 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.* Typh-imurium, and *P. lundensis*) and myoglobin content of chicken meat were monitored.

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 0.0008 + 0.004 + 4.993 g of BAC + DW + ethanol, respectively, in MIC-1 was used in 100 g meat (Table 4-Appendix), in MIC-2 the ratio of AITC was twofold and in MIC-4 the ratio of AITC was 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.* Typh-imurium, and *P. lundensis*) myoglobin content of chicken meat were monitored

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. The samples were grouped as follow: control (No BACs + No HHP), Inoculated control, MIC-1 of αTPN, MIC-1 of AITC, MIC-1 αTPN + MIC-1 AITC, HHP 300 MPa, HHP 600 MPa, MIC-1 αTPN + HHP 300, MIC-1 αTPN + HHP 600, MIC-1 AITC + HHP 300, MIC-1 AITC + HHP 600, MIC-1 αTPN + MIC-1 AITC + HHP 300, and MIC-1 αTPN + MIC-1 AITC + HHP 600. The meat was vacuum packed, treated with HHP at 300 and 600 MPa, and stored at 4 ± 0.5 °C for up to 21 days. 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 (TBARS), odour detection (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.

3.4. High hydrostatic pressure treatment

For the HHP treatment, meat samples were packed in polyethylene bags and subjected to the high-pressure vessel and pressurize at 300 and 600 MPa for 5 minutes at room temperature in a RESATO FPU-100-2000 HHP unit (Resato International B.V., the Netherlands) (Figure 15). As a pressure transmitting medium glycol-oil mixture was used (Resato PG fluid, Roden, Holland). The pressure build-up rate was 100 MPa/min, build-up and decompression times were not included in the treatment time. The initial temperature of the pressure transmission fluid was 20.5 °C, the adiabatic temperature change of the system (samples and the pressure transmission fluid) was under 12 °C. Samples were stored at 4 ± 0.5 °C.

Figure 15: High hydrostatic pressure unit (Resato International)

3.5. Procedures and measurements

3.5.1. Physicochemical properties

3.5.2. Measurement of pH

The pH value of meat samples was measured in different experiments (starting 24 h post-mortem) of storage, and the readings were recorded in triplicate by immersing a pH electrode (Testo 206; Testo-AG, Germany) about 1 cm into the minced samples.

3.5.3. Colour measurement

The colour values of minced meat were measured using CIELAB (CIE, 1986) scoring system. The following parameters were obtained: *L** (lightness), *a** redness (+*a*, red; –*a*, green), and *b** yellowness (+*b*, yellow; –*b*, blue) by using Konica Minolta CR-400 colourimeter (Konica Minolta Sensing Inc., Japan) making sure calibration was carried out before taking a reading from

each treatment (Dias et al., 2013). Results from L^*, a^*, and b^* were recorded as the mean of five random readings and from the measured values relative colourfulness or chroma magnitude (C^*) and hue angle (h^*) were calculated as the following:

- Chroma: $C^* = [(a^*)^2 + (b^*)^2]^{1/2}$
- Hue angle: $h^* = \tan^{-1}$ (arctangent) (b^*/a^*)

3.5.4. Measurement of water holding capacity

Measurement of water holding capacity was performed using the filter paper press technique. A sample (0.25-0.32 g) was placed on a filter paper (Whatman no. 10) set between 2 Plexiglas plates and pressed for 5 min by a 500 g weight. The filter paper then placed in an oven for 10 min followed by 5 min in a desiccator. WHC was calculated as the ratio of meat film area-to-total liquid outlined area (Grau & Hamm, 1953). Samples were measured in triplicates.

WHC (%) = [1 − {(meat weight before pressing – meat weight after pressing) / (meat weight before pressing × moisture content in gram)}] × 100

3.5.5. Measurement of water activity

Water activity (a_w) in meat was measured using a LabMaster (Neo, Neutec Group, United States). Samples of 3-5 grams were weighed into a plastic container and placed in the a_w meter.

3.5.6. Determination of metmyoglobin, deoxymyoglobin, and oxymyoglobin pigments

Meat pigment content was measured using the method applied by (Utama et al., 2017) with minor modifications, by which myoglobin determined from absorbance measurements of the sarcoplasmic extract, dissolved in mM phosphate buffer (pH 6.8), from the reflex attenuance at 503, 525, 557, 572, and 582 and 700 nm. Briefly, 2 g of sample was homogenized with 20 ml phosphate buffer using a homogenizer (Digital Ultra-Turrax, Germany) at 10,000 rpm for 20 s. The homogenate was centrifuged at 5,500×g for 30 min. The supernatant was filtered through filter paper. The presented values are the mean of triplicate measurements per sample. The relative proportions (%) each myoglobin form: oxymyoglobin (OxyMb), metmyoglobin (MetMb), and deoxymyoglobin (DeoMb) were calculated according to the method updated by Tang et al. (2004), the calculation performed as below:

$$\% \text{ MetMb} = (-0{,}159R1 - 0{,}085R2 + 1{,}262R3 - 0{,}520) * 100$$

$$\% \text{ DeoMb} = (-0{,}543R1 + 1{,}594R2 + 0{,}552R3 - 1{,}329) * 100$$

$$\% \text{ OxyMb} = (0{,}722R1 - 1{,}432R2 - 1{,}659R3 + 2{,}599) * 100$$

*$R1 = A582/A557$, $R2 = A557/A525$, and $R3 = A503/A525$

3.5.7. Determinations of thiobarbituric acid-reactive substances (TBARS)

In 1st experiment: lipid oxidation was measured by analysing TBARS using the method described by Tarladgis et al. (1960), slightly modified as follows. Chicken meat of 4 g was dispensed in mixing glass tubes and homogenized (Digital Ultra-Turrax Disperser, Germany) with 15 ml of distilled water. Then 5 ml of 25 % trichloroacetic acid (TCA) was added to the mixture and centrifuged in 50-ml polypropylene conical centrifuge tubes at 5000 rpm for 10 min. After the filtration 3.5 ml of this solution was added to 1.5 ml of 0.6 % w/v thiobarbituric acid (TBA) (0.02 M). The tubes were then kept in a water bath at 100 °C for 30 minutes. After cooling, absorbance readings were taken with a Spectrophotometer (U-2900 Hitachi Ltd., Japan) at 532 nm against a blank. TBARS were expressed as mg malonaldehyde (MDA equivalent)/1000 g sample.

In experiment 3, 4 and 5: lipid oxidation was determined by analysing the thiobarbituric acid reactive substances (TBARs) index according to Dias et al. (2013). Five-gram portions of chicken breast meat samples were combined with 20 mL of 5 % trichloroacetic acid (TCA) and to prevent oxidation during the preparation 0.5 mL of 0.15 % BHT antioxidant (2,6-ditert- butyl-4-methylphenol) (Sigma Aldrich) was used, and homogenized (Digital Ultra-Turrax Disperser, Germany) for 2 min. The homogenates were then centrifuged (5000 g for 10 min) and the supernatant filtered through filter paper into 25 mL volumetric flasks, and 5 % TCA was added to reach a final volume of 25 mL. Two mL of filtrate was combined with 2 mL of 0.08 % w/v TBA (0.02 M) reagent and the tubes were then sealed and placed in a water bath (95 °C) for 30 min. After cooling the samples were vortexed and absorbance of the resulting solution was measured at 532 nm using Spectrophotometer (U-2900 Hitachi Ltd., Japan) (Figure 16-Appendix) against a blank containing all of the reagents except the sample, and the TBARs values were expressed as mg of malondialdehyde (MDA equivalent) per kg sample (Oliveira et al., 2015; Ganhão et al., 2011).

3.5.8. Microbiological properties

3.5.8.1. *In vitro* anti-microbial activity of BACs

3.5.8.2. Bacterial strains

Six bacterial strains, three Gram-positive (G+veB) (*Listeria monocytogenes* CCM 4699, *Staphylococcus aureus* ATCC 6538 and *Bacillus cereus* T1) and three Gram-negative (G-veB) (*Escherichia coli* O157:H7 BO1909, *Salmonella typhimurium* B1310 and *Pseudomonas lundensis* CCP5) were used as target bacteria in antimicrobial tests. Each strain was grown on a plate

containing 25 ml sterile TSA (Biokar Diagnostics BK046HA) at 37 °C for 24 h (except *Pseudomonas lundensis*, which was incubated at 30 °C for 24 hours).

3.5.8.3. Agar well diffusion assay

The *in vitro* anti-microbial activity of BACs (CARV, AITC, LIN, and PIP) in experiment 1 was examined using the method applied by Fernández-López et al. (2005) with minor modifications. BACs prepared as a mixture solution of BACs and sunflower oil in various ratios (v/v) (Table 5). The culture was diluted with MRD (Maximum recovery diluent) solution (0.5 g peptone + 4.25 g sodium chloride in 500 ml distilled water) adjusted to the desired concentration of 0.5 optical density (OD) by using a Densitometer (DEN-1B; McFarland, Latvia). Test strains were pour plated a final cell density of approximately 10^6 CFU/ml, after solidification of the inoculated agar (each Petri dish contained 20 ml agar), they were prepared 4 wells per sterile Petri dish (Kord-Valmark Labware, Bioplast, L.L.C. USA) with a diameter of 8 mm using special sterilized metal cork. Wells were filled with 100 µl of the appropriate dilution of the BACs. Ethanol (96 %) and sterile DW was pipetted into the negative control wells. The thickness of the inhibition zone around the holes was measured using a Digital Vernier Caliper (Workzone-Caliper, Japan) in millimetres and data were recorded after 24, 48, and 72 h of incubation (Balouiri et al., 2016).

Table 5: Serial dilutions of BACs in sunflower oil used for the microbiological assessment

Dilution ratio (v:v)	BACs mg/5g Oil
1:640	7.81
1:320	15.62
1:158	31.25
1:80	62.5
1:40	125
1:20	250
1:16	312.5
1:10	500
1:8	625
1:5	1000
1:4	1250
1:2.5	2000
1:2	2500
1:1.25	4000
1.6:1	8000

3.5.8.4. Disc diffusion assay

The test was performed in a sterile Petri dish (90 mm diameter) containing 20 ml TSA. Plates were inoculated with 1 ml of the target bacterium (approximately 10^6 CFU/ml, set by measuring OD) on the agar surface. After a few minutes, the plates were sloped, and the access

inoculum was removed by pipetting. Then, a sterile 5-mm diameter disc-shaped filter paper (Whatman no. 1, ≥10.5 cm in diameter) was put on the middle of the agar surface, and 4 µl of BACs {AITC, Thymol, CARV, αTPN, eugenol, LIN, PIP, camphor, γ-Terpinene, *p*-Cymene, limonene, α-Pinene, 1,8-cineole, cuminaldehyde, β-citronellol, geraniol, (−)-α-Bisabolol, and synthetic compound (BHT)} were applied on it (undiluted or diluted BACs in ethanol (96 %) or DW). For control, 4 µl of a sterile solution of ethanol (96 %) was used. Each plate was sealed well with parafilm to prevent evaporation from the samples as well as the loss of volatile components of BACs (Dussault et al., 2014). Plates were incubated for 24, 48, and 72 h at either 30 or 37 °C according to the growth temperature requirement of the bacteria. The inhibition zone (mm) (colony-free perimeter) around the disc (starting from the edge of the disc) was measured using a Digital Vernier Caliper (Workzone-Caliper, Japan). The experiments were repeated in triplicate for all the tested strains.

3.5.8.5. Minimal inhibition concentration (MIC) - Micro-dilution method

The MIC was determined using microdilution of Tryptic-Soy Broth (TSB) in 96-well plates. The stock solution was prepared by diluting 200 µl/ml of BACs in absolute ethanol in order to enhance their solubility. Non-liquid BACs were also diluted in ethanol (thymol 30 mg/600 µl, camphor 200 mg/200 µl, PIP 12 mg/200, and BHT 50 mg/500 µl).

The MIC was determined using the resazurin microtiter plate-based antibacterial assay as described by Semeniuc et al. (2017) with minor modifications. 100 µl of TSB with 100 µl of sterile DW was pipetted into each well, and 100 µl of appropriately diluted BACs (from the stock solution) were placed in the well of the first column. Micro dilution and mixing did via a pipette 2-3 times for homogenization, then serial 11-fold dilutions were performed by transferring 50 µl into the right well and continued to the last well of the plate row. Then 30 µl of the cell bacterial suspensions (10^6 cells/ml) were pipetted into the appropriate well. Ethanol (96 %) was also used as a control. After incubation 10 µl aqueous mixture of resazurin (see later) was pipetted to each well. The final volume in each well was 290 µL. Microbial growth was indicated by colour change. If the colour stayed blue, it means there were no growth. If the colour changed to pink that means there were growth, and the intensity of the colour depends on the amount of growth. Microplates were incubated at 37 °C for 24 h (except *P. lundensis*, which was incubated at 30 °C). The concentration that completely inhibited bacterial growth designated as MIC. Three replicates were run for each BAC.

Resazurin solution made by diluting 0.025 g of resazurin sodium salt in 1 mL sterile DW and added to pre-weighed medium of 8 mL TSB (double Tryptic-Soy Broth) and distributed in

Eppendorf tubes (in each tube 900 µL). Then 0.014 g of menadione was diluted in 1 mL of DMSO, separately, then menadione added to previously made resazurin solutions to prepare the stock solution. The stock solution was stored in a freezer at -20 ºC. 10 µL of this stock solution was added to each well of the plate after incubation.

3.5.8.6. Determination of aerobic mesophilic counts (AMCs) in meat

In the second trial on the BACs (CARV, AITC, LIN, and PIP) the microbiological analysis of chicken meat was carried out through analysing a population of aerobic mesophilic counts (AMCs), with the method applied by Jridi et al. (2015). Briefly, ten grams of each sample were obtained aseptically, Stomachered for 2 min with 90 ml of diluent, and 10-fold serial dilutions were made. The appropriate dilutions were plated on Nutrient count agar. Plates were incubated for 48 h at 37 °C, and the colonies were counted by a colony counter. The results were given as logarithms of colony-forming units per gram of sample (log CFU/g).

3.5.8.7. Determination of aerobic mesophilic counts (AMCs), *Pseudomonas lundensis, Listeria monocytogenes,* and *Salmonella* Typhimurium in meat

3.5.8.8. Preparation of bacterial strains and inocula

The microbiological challenge testing was carried out as a useful method in determining the potential shelf life of refrigerated meat. Both G+veB (*L. monocytogenes* CCM 4699) and G-veB (*S.* Typhimurium B1310 and *P. lundensis* CCP5) were used as target bacteria in antimicrobial tests which obtained from the Department of Microbiology and Biotechnology, Faculty of Food Science, Szent István University. Cultures were streaked on TSA plates and incubated for 24 h at 37 °C (except *P. lundensis* incubated 30 °C). The inocula of the test organisms were prepared by transferring a single colony from culture plates into 100 mL TSB and culturing at 37 °C for 24 h. These cultures were further used for testing the antimicrobial activities and for the inoculation of chicken breasts.

3.5.8.9. Bacterial inoculation on chicken meat

The meat samples (approximately 10 g/bag) were then inoculated with 10 µL mixtures of *L. monocytogenes, S.* Typhimurium, and *P. lundensis* bacterial solution from 300 mL TSB (100 ml/strain) with an initial cell count of 6 - 7 Log CFU/ml for each inoculated bacterium (inoculated control and treated samples). The meat was then vacuum packaged and some of them HHP treated depending on the experiment. This meat (10 g/bag) was stored at 4 °C until the day of measurement depending on the experiment.

3.5.8.10. Microbial enumeration

Each sample (10 g/bag) was suspended aseptically with 40 mL of sterile saline solution, and the samples were homogenised in a sterile filter containing Stomacher bag for 2 min (Inter-science, France). Decimal serial dilutions were performed with sterile 0.1 % peptone water. The microbial populations were quantified by spreading 100 µL from the homogenized meat bag and plated using the following media: Xylose Lysine Deoxycholate Agar (XLD) for *Salmonella*, PALCAM (Polymyxin acriflavine lithium chloride ceftazidime aesculin mannitol) for *L. monocytogenes*, Cetrimide agar for *Pseudomonas*, and for AMCs 1 mL for Tryptone Glucose Extract (TGE) (to one litre of sterile DW 0.5 % peptone, 0.1 % Glucose, 0.25 % yeast extract and 1.5 % Bacteriological agar). *Listeria* and *Salmonella* plates were thoroughly shacked before solidification was then incubated for 24 h at 37 °C, and TGE and *Pseudomonas* were incubated at 30 °C before enumeration. The results are expressed as the log CFU/g.

3.5.9. Electronic nose analysis

Electronic nose determinations were performed with an NST 3320 instrument (Applied Sensor Technologies, Linköping, Sweden) (Figure 17-Appendix) as described by Friedrich et al. (2008). This instrument has a built-in headspace sampler for 12 samples, a detector unit containing 23 different sensors, and software for collecting and processing data from the sensors. NST 3320 is equipped with 10 MOSFET (metal-oxide semiconductor field-effect transistor) sensors, 12 MOS (metal oxide semiconductor) sensors, and a humidity sensor for measuring relative humidity. The response of the MOS sensors is measured as the change in resistance between the electrodes as a result of the chemical reactions occurring at the surface of the metal oxide semiconductor. The MOSFET sensors are based on a change in electrostatic potential. Eight-gram meat samples (Three replicates each) were filled to special glass vials which were closed by a septum. The standby temperature, at which the samples were kept until their incubation phase started, was 20 °C. Before analysis, the samples were equilibrated at 60 °C for 30 min (incubation phase). The total cycle time per sample was 430 s. The difference of sensor signals between the baseline and the signal value at the end of the sampling time was used for multivariate statistical analysis as a sensor response.

3.5.10. Texture profile analysis (TPA) spreadability of meat

The texture spreadability of minced chicken meat extrusion and adhesiveness was measured with the conical measuring head of TPA (Stable Micro Systems, Great Britain). The cross-head pushed the 90° cone probe of spreadability rig with 2 mm/s speed into the sampling

holder. The meat samples were tempered to 12 °C and the measuring time was 90 s. Three replicates of each samples were evaluated using the official software of the instrument called Texture Exponent 32.

3.5.11. Sensory quality of ground chicken meat treated with bioactive compounds

Sensory evaluation of raw chicken meat samples (ca. 60 g) (Figure 18-Appendix) was conducted for sensory attributes: the intensity of colour, aroma, appearance, and acceptability to buy. The panel consisted of 10 researchers, teachers, and technicians of Szent István University (50 % male/female, aged between 25 and 57 years) they were familiar with chicken meat consumption. The assessment was conducted using a 9-point hedonic scale (Meilgaard et al., 1999): 1, dislike extremely; 2, dislike very much; 3, dislike moderately; 4, dislike slightly; 5, neither like nor dislike; 6, like slightly; 7, like moderately; 8, like very much; 9, like extremely. All raw meat samples were coded with 3-digit random codes and offered to the panellist in the random order. The sequence in which treatments were offered to each panelist was randomized.

3.6. Statistical analysis

The experimental data were analysed using SPSS (Version 23.0, SPSS Inc.). The data were subjected to analysis of variance (ANOVA) and General Linear Model (GLM), then the level of significance was established using Tukey test at ($P<0.05$). In physiochemical and lipid oxidation analysis the mean data ± standard deviation was presented. Microbiological data were converted to Log CFU/g. In the case of E-nose measurements, canonical discrimination analysis (CDA) was applied to distinguish between different meat groups. In sensorial measurement 10 experienced panellists were selected for the sensory scores the mean data ± standard deviation was presented.

4. RESULT AND DISCUSSION

4.1. USE OF ALLYL-ISOTHIOCYANATE, CARVACROL, LINALOOL, AND PIPERINE TO PRESERVE FRESH CHICKEN MEAT DURING CHILLING STORAGE

4.1.1. Physicochemical properties

4.1.1.1. pH of meat

The result from the physicochemical properties of chicken meat treated with AITC, CARV, LIN, and PIP are shown in Table 6. Both AITC and CARV were active in reducing the pH of meat and significant differences were observed within groups containing BACs and compared to control ($P<0.05$). LIN showed activity towards maintaining the pH, while PIP had more activity in reducing pH values of the meat, and both LIN and PIP had higher pH than the control group at the end of storage. Decrease of pH may be attributed to the inhibitory effect of BACs on the growth and proliferation of spoilage microorganisms that metabolize basic nitrogen compounds.

4.1.1.2. Colour values

The colour of chicken meat shows significant differences with intensifying drifts in lightness during the storage period. The L^* values of samples treated with AITC were significantly higher than those treated with other BACs and of the control group (Table 6). In contrast to AITC, the higher concentration of CARV (1000 ppm) reduced the lightness of meat significantly compared to the control ($P<0.05$). The causes of decreasing in L^* values to a statistically significant level by a higher concentration of CARV could be explained by a possible absorption of free water within the product, thereby decreasing lightness of the meat (Fernández-López et al., 2005). The addition of LIN was effective in keeping the L^* values close to the initial L^* values, and both LIN and PIP reduced the L^* values compared to control meat. The a^* values of control increased gradually in the last day of storage and a similar trend with significant rise was observed in meat with CARV. However, AITC indicated a significant reduction of redness of the meat. The a^* values in meat containing LIN-500 ppm were significantly high, however, this increase in the a^* values was lower compared to the meat treated with 1000 ppm of PIP. The data from b^* values showed that AITC, CARV, and PIP had a significant effect in increasing the yellowness of the meat compared to control over the storage period. The b^* value of the control decreased at day 8 of storage, unlike and reverse the trend with no significant difference were observed in meat treated with LIN.

An increasing trend in *C** was noticed in all meat groups, while CARV and PIP were more active to cause a significant increase in *C** of the meat. Besides, AITC, CARV, and PIP and particularly at PIP-1000 showed an increase in the hue values (*h**) compared to LIN and not treated meat, which exhibits a decrease in *h** values of meat. Mastromatteo et al. (2009) evaluated the combined effect of CARV and thymol (0-300 ppm) in non-conventional poultry patties packaged in air and MAP. Similar to the current study they noticed a slight increase in *b** and *C** values at the end of storage, contrariwise they observed an increase in *L** and decreasing *a** values. In accordance to current finding Olaimat et al. (2014) coated the chicken breast with AITC, they noticed that 100 µl/g AITC was able to reduce pH value and has the potential to give a yellowish colour to the coating at day 11 of storage. An increase in *C** properties and *a** values indicate that CARV and PIP has a great contribution towards the final redness of the meat. An increase in the *L** value particularly in control meat refers to the paleness of meat and may be due to an increase in oxidation this may develop the rancidity and influence the consumers' acceptability for the meat (Karabagias et al., 2011). Our result agreed to the finding by Martínez et al. (2006) who observed that an increase in the concentration of red-sweet, cayenne (0.1 %, 0.5 %, or 2 %) and black pepper powders (0.1 %, 0.5 %, or 1 %) in pork sausages with MAP (80 % O_2+ 20 % CO_2) resulted in lower *L** and a significant increase in *a** and *b** values. The current results of redness of meat appeared with an inconsistent relationship to an increase of *L** and decreases in *b**. This resulted in a decrease of *C** particularly in the control group that could be due to gradual oxidation of myoglobin that could build-up metmyoglobin and develop discoloration attributes of meat. Simultaneously, 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.

4.1.1.3. Water holding capacity

WHC as an important quality parameter directly affects appearance, profitability, and consumption of meat and meat products. In the current study LIN, CARV 500 ppm, and PIP 1000 ppm increased WHC of meat with no significant differences (Table 6). While AITC showed a significant decrease, particularly in the last days of storage. Decreasing of WHC in AITC treated meat might also associate with the increased lightness of the meat. On the other hand, PIP-500 exhibited almost stabilized efficiency in WHC. Based on the storage time only significant WHC was detected with LIN-1000 compared to an increased drip loss in control.

Table 6: The influence of different concentrations of AITC, CARV, LIN and PIP (500 and 1000 ppm) on physicochemical properties: pH, Colour values (*L**, *a**, *b**, *C** and *h**), and WHC of fresh chicken meat stored up to 8 days at 4 °C.

Meat parameters	Storage (d)	Bioactive compounds								
		No-BAC	**AITC-500**	**AITC-1000**	**CARV-500**	**CARV-1000**	**LIN-500**	**LIN-1000**	**PIP-500**	**PIP-1000**
pH	0	5.89±0.02 ^{bA}	5.89±0.01 ^{bA}	5.90±0.01 ^{bA}	5.9±0.02 ^{bA}	5.92±0.01 ^{cA}	5.93±0.01 ^{bB}	5.91±0.01 ^{cAB}	5.91±0.01 ^{cA}	5.89±0.01 ^{cA}
	3	5.93±0.01 ^{cA}	6.08±0.01 ^{dB}	6.10±0.01 ^{dB}	5.99±0.01 ^{cB}	6.04±0.01 ^{dC}	5.98±0.01 ^{cB}	5.98±0.01 ^{dB}	5.95±0.01 ^{dA}	5.96±0.01 ^{dA}
	6	5.78±0.01 ^{aA}	5.89±0.01 ^{bB}	5.93±0.02 ^{cC}	5.73±0.01 ^{aB}	5.79±0.02 ^{bA}	5.81±0.01 ^{aB}	5.83±0.01 ^{bC}	5.70±0.02 ^{aA}	5.71±0.02 ^{aB}
	8	5.76±0.01 ^{aA}	5.83±0.02 ^{aB}	5.86±0.00 ^{aB}	5.71±0.02 ^{aB}	5.75±0.01 ^{aAB}	5.79±0.01 ^{aA}	5.80±0.02 ^{aA}	5.78±0.01 ^{bA}	5.77±0.02 ^{bA}
L*	0	49.23±0.29 ^{aA}	49.29±0.76 ^{aA}	49.44±0.55 ^{aA}	48.83±0.74 ^{aA}	48.89±0.67 ^{aA}	48.41±0.38 ^{aB}	48.30±0.42 ^{aB}	48.47±0.80 ^{aA}	49.15±0.81 ^{aA}
	3	51.40±0.29 ^{bA}	52.82±0.54 ^{bB}	54.82±0.54 ^{bC}	49.80±0.60 ^{aB}	49.78±0.32 ^{aB}	51.07±1.09 ^{bA}	50.48±0.11 ^{bA}	50.79±0.24 ^{bA}	50.76±0.56 ^{bA}
	6	53.47±0.38 ^{cA}	56.22±0.47 ^{cB}	58.62±0.84 ^{cC}	51.94±0.50 ^{bB}	52.03±0.78 ^{bB}	51.95±0.42 ^{bB}	51.72±0.45 ^{cB}	52.58±0.28 ^{cA}	53.01±0.89 ^{cA}
	8	52.70±0.42 ^{cA}	56.14±0.38 ^{cB}	58.06±0.57 ^{cC}	51.12±0.93 ^{bB}	51.15±0.90 ^{bB}	50.89±0.55 ^{bB}	50.50±0.62 ^{bB}	51.40±0.78 ^{bA}	51.9±0.32 ^{bAB}
a*	0	3.84±0.77 ^{aA}	5.08±0.41 ^{aB}	4.76±0.73 ^{aAB}	4.32±0.18 ^{aA}	4.11±0.39 ^{aA}	4.31±0.58 ^{aA}	4.07±0.22 ^{aA}	4.32±0.34 ^{aA}	4.16±0.30 ^{aA}
	3	4.66±0.62 ^{aA}	5.05±0.33 ^{aA}	4.44±0.13 ^{aA}	4.62±0.24 ^{aA}	4.37±0.16 ^{aA}	4.64±0.28 ^{aA}	4.50±0.38 ^{abA}	4.65±0.15 ^{abA}	5.00±0.36 ^{bA}
	6	4.57±0.18 ^{aAB}	4.86±0.36 ^{aA}	4.40±0.16 ^{aB}	4.68±0.15 ^{abA}	4.39±0.28 ^{aA}	5.18±0.50 ^{aB}	5.27±0.24 ^{cB}	4.95±0.60 ^{abA}	5.20±0.30 ^{bA}
	8	4.25±0.18 ^{aA}	4.98±0.19 ^{aB}	4.20±0.44 ^{aA}	5.22±0.56 ^{bB}	4.26±0.27 ^{aA}	5.09±0.55 ^{aB}	4.95±0.51 ^{bcA}	5.03±0.20 ^{bB}	5.19±0.41 ^{bB}
b*	0	2.60±0.52 ^{aA}	2.41±0.50 ^{aA}	1.87±0.41 ^{aA}	2.24±0.61 ^{aA}	2.40±0.50 ^{abA}	2.36±0.58 ^{aA}	2.51±0.22 ^{aA}	2.78±0.09 ^{aA}	2.93±0.27 ^{aA}
	3	2.34±0.35 ^{aA}	2.38±0.11 ^{aA}	2.03±0.25 ^{aA}	2.43±0.27 ^{abA}	2.19±0.36 ^{abA}	2.26±0.40 ^{aA}	2.57±0.37 ^{aA}	2.70±0.40 ^{aAB}	3.16±0.36 ^{abB}
	6	2.58±0.42 ^{aA}	2.39±0.21 ^{aA}	2.12±0.60 ^{aA}	2.23±0.21 ^{aAB}	1.93±0.41 ^{aB}	2.53±0.38 ^{aA}	2.56±0.29 ^{aA}	2.66±0.18 ^{aA}	3.23±0.33 ^{abB}
	8	2.46±0.28 ^{aA}	3.38±0.39 ^{bB}	3.08±0.31 ^{bB}	3.08±0.60 ^{bA}	3.14±0.92 ^{bA}	2.60±0.31 ^{aA}	2.66±0.55 ^{aA}	3.07±0.36 ^{aAB}	3.95±0.96 ^{bB}
C*	0	4.66±0.75 ^{aA}	5.64±0.41 ^{aA}	5.13±0.68 ^{aA}	4.88±0.42 ^{aA}	4.77±0.54 ^{aA}	4.94±0.63 ^{aA}	4.79±0.22 ^{aA}	5.14±0.30 ^{aA}	5.09±0.34 ^{aA}
	3	5.24±0.43 ^{aAB}	5.58±0.31 ^{aA}	4.88±0.20 ^{aB}	5.23±-0.22 ^{aA}	4.90±0.23 ^{aA}	5.17±0.42 ^{aA}	5.19±0.49 ^{abA}	5.39±0.31 ^{abAB}	5.93±0.34 ^{abB}
	6	5.26±0.31 ^{aA}	5.42±0.36 ^{aA}	4.90±0.38 ^{aA}	5.19±0.12 ^{aAB}	4.81±0.21 ^{aB}	5.78±0.50 ^{aA}	5.87±0.28 ^{bA}	5.63±0.54 ^{abAB}	6.13±0.24 ^{bB}
	8	4.92±0.13 ^{aA}	6.03±0.26 ^{aB}	5.23±0.29 ^{aA}	6.06±0.78 ^{bB}	5.32±0.72 ^{aAB}	5.72±0.58 ^{aB}	5.64±0.53 ^{bAB}	5.90±0.12 ^{bB}	6.55±0.85 ^{bB}
*h**	0	0.89±0.36 ^{aA}	0.52±0.15 ^{aAB}	0.43±0.14 ^{aB}	0.57±0.16 ^{aA}	0.66±0.13 ^{abA}	0.62±0.19 ^{aA}	0.71±0.10 ^{aA}	0.75±0.09 ^{aA}	0.86±0.11 ^{aA}
	3	0.58±0.18 ^{aA}	0.51±0.04 ^{aA}	0.49±0.06 ^{aA}	0.58±0.10 ^{aA}	0.55±0.11 ^{aA}	0.53±0.08 ^{aA}	0.64±0.07 ^{aA}	0.65±0.11 ^{aA}	0.74±0.14 ^{aA}
	6	0.64±0.13 ^{aA}	0.53±0.06 ^{aA}	0.52±0.15 ^{aA}	0.51±0.07 ^{aA}	0.48±0.14 ^{aA}	0.53±0.11 ^{aA}	0.52±0.07 ^{aA}	0.60±0.10 ^{aA}	0.72±0.12 ^{aA}
	8	0.66±0.13 ^{aA}	0.81±0.13 ^{bA}	0.95±0.29 ^{bA}	0.66±0.09 ^{aA}	0.93±0.32 ^{bA}	0.56±0.08 ^{aA}	0.61±0.19 ^{aA}	0.71±0.14 ^{aA}	0.97±0.29 ^{aA}
WHC (%)	0	1.38±0.03 ^{aA}	1.38±0.08 ^{aA}	1.46±0.06 ^{aA}	1.44±0.01 ^{aA}	1.30±0.20 ^{aA}	1.49±0.00 ^{aB}	1.49±0.19 ^{abB}	1.42±0.03 ^{aA}	1.52±0.19 ^{aB}
	3	1.57±0.19 ^{aA}	1.48±0.20 ^{aA}	1.41±0.08 ^{aA}	1.48±0.12 ^{aA}	1.55±0.02 ^{aA}	1.66±0.02 ^{aA}	1.58±0.04 ^{aA}	1.60±0.05 ^{aA}	1.35±0.13 ^{aA}
	6	1.34±0.04 ^{aA}	1.27±0.04 ^{aA}	1.50±0.01 ^{aB}	1.38±0.16 ^{aA}	1.57±0.03 ^{aA}	1.40±0.00 ^{aA}	1.48±0.21 ^{abA}	1.73±0.02 ^{aC}	1.58±0.17 ^{aB}
	8	1.44±0.11 ^{aA}	1.72±0.12 ^{aA}	1.62±0.15 ^{aA}	1.43±0.10 ^{aA}	1.34±0.09 ^{aA}	1.27±0.02 ^{aA}	1.26±0.16 ^{bA}	1.43±0.03 ^{aA}	1.35±0.03 ^{aA}

^{a,b,c}means in the same column with different superscript are significantly different regarding the days of storage; ^{A,B,C}means in the same row with different superscript are significantly different regarding the concentrations of BACs (P<0.05). Colour values: L^*, lightness; a^*, redness; b^*, yellowness; h^*, $\mathrm{Tan}^{-1}\, b^*/a^*$; C*, $(a^{*2} + b^{*2})^{1/2}$; and WHC, water holding capacity.

4.1.2. Thiobarbituric acid-reactive substances (TBARS)

TBARS analysis determines the formation of secondary products of lipid oxidation, i.e. as a result of UFA oxidation, mainly MDA, which may contribute to the off-flavour in stored meat products (Šojić et al., 2017). In the current study at the end of storage, the control group showed higher TBARS values compared to the rest of the samples, while meat containing CARV and 1000 ppm of AITC and PIP showed significantly lower TBARS values ($P<0.05$) (Figure 19). It has been reported that 1-2 mg MDA/kg meat could be considered as a threshold limit value for rancidity in meat (Tarladgis et al., 1960). Martinez et al. (2006) studied the shelf life of fresh pork sausages using MAP with *Capsicum annuum* (red sweet and hot cayenne) and *Piper nigrum* (black and white) pepper powders stored samples for 16 d in the dark at 2 °C. They found that these PIP rich spices were effective in inhibiting lipid oxidation, chiefly at the highest concentration used (2 %), which resulted in a delay of off-odour formation and all those spices inhibited microbial growth when added at the highest concentration (1 % *Piper* and 2 % *Capsicum*). Our result was in accordance to the finding by Martínez et al. (2006) who treated sausage with 0.5 % of different types of pepper and noticed TBARS level of 1.5 mg MDA/kg at day 10 of storage compared to 6.5 mg MDA/kg for control at the same day. Results from the present study are particularly meaningful because BACs mainly CARV and LIN had a clear protective effect against LO by keeping TBARS scores lower than 2 mg MDA/kg. This fact could be attributed to the strong antioxidant activity of LIN and CARV that interferes with free radical propagation process and it can react with lipid and hydroxyl radicals to convert them into stable products (Sharma et al., 2017). Additionally, this could be attributed to the strong potential antioxidant activity of selected BACs in inhibiting the formation of secondary products of LO that may contribute to the off-flavour in stored meat products.

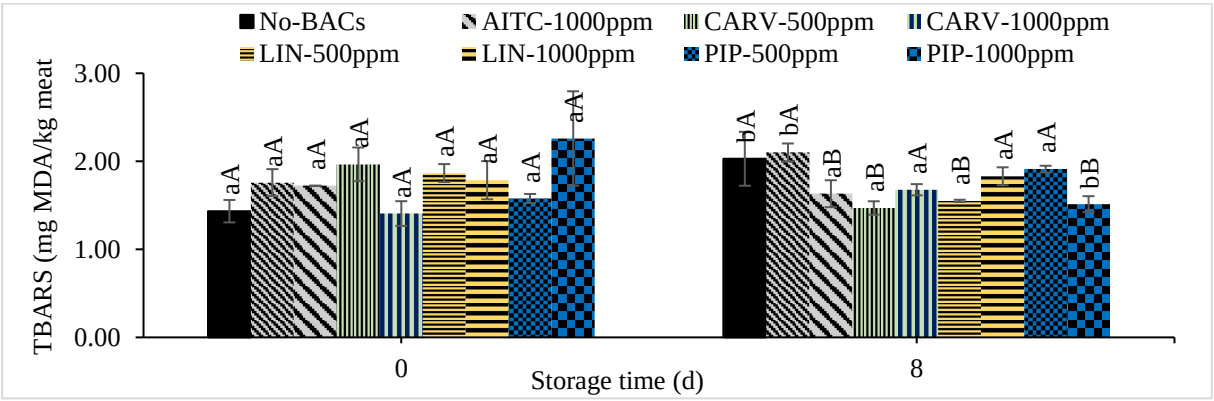


Figure 19: Effect of different concentrations of AITC, CARV, LIN, and PIP on TBARS values of fresh chicken meat stored up to 8 days at 4 °C. * ^{a,b,c}means with different superscript are significantly different regarding the days of storage; ^{A,B,C}means with different superscript are significantly different regarding the concentrations of BACs ($P<0.05$).

4.1.3. Microbiological characteristics

Figure 20 shows that the total AMCs increased from 3.96 to 6.59 log^{10} CFU/g in control at the end of storage ($P<0.05$). The AITC was more active in reducing the AMCs compared to other BACs and 1000-ppm AITC caused about 3 logs reduction in AMCs. CARV 1000-ppm exhibit less activity than AITC, but higher activity than LIN and PIP in reducing AMCs in meat. In accordance with our findings, Olaimat et al. (2014) noted that aerobic bacterial numbers in chicken breast treated with 25 to 100 µl/g AITC were reduced by 1.72 to 3.75 log^{10} CFU/g during 21 days of storage at 4 °C. Additionally, Mastromatteo et al. (2009) found the final cell load of total viable count for poultry patties stored at 0-3 °C was decreased about 1-1.5 log^{10} CFU/g with 150-ppm CARV. Moreover, it has been reported that TVC of 7 log^{10} CFU/g^{-1}, considered as the upper microbiological limit for acceptable quality meat (Karabagias et al., 2011). Such high populations of bacteria were not recorded in the present study. Luz et al. (2015) studied the efficacy of Origanum vulgare L. essential oil and CARV in inhibiting the growth of *P. aeruginosa*. They found a decreased number of viable cells of *P. aeruginosa* in meat-based broth by approximately 2 log CFU/mL, while the lethal effect is established when observed 3 log reduction of the initial inocula, i.e. 99.9 % killed. Surprisingly, less effectiveness of PIP and LIN was noticed, thus indicating a small prolongation of the lag phase of microbial growth. Comparable findings were highlighted by Martinez et al. (2006) who observed lower values of psychrotrophic counts as they recorded 6.10 and 5.06 log^{10} CFU/g^{-1} for control and 1 % black pepper at day 8 of storage, respectively. Poor solubility in aqueous and reaction with constituents of meat may reduce the antimicrobial activity of BACs. During our study further *in vitro* work was carried out to confirm the antibacterial activity of these BACs. No data on the antimicrobial effect of LIN and PIP on chicken meat has been found thus far.

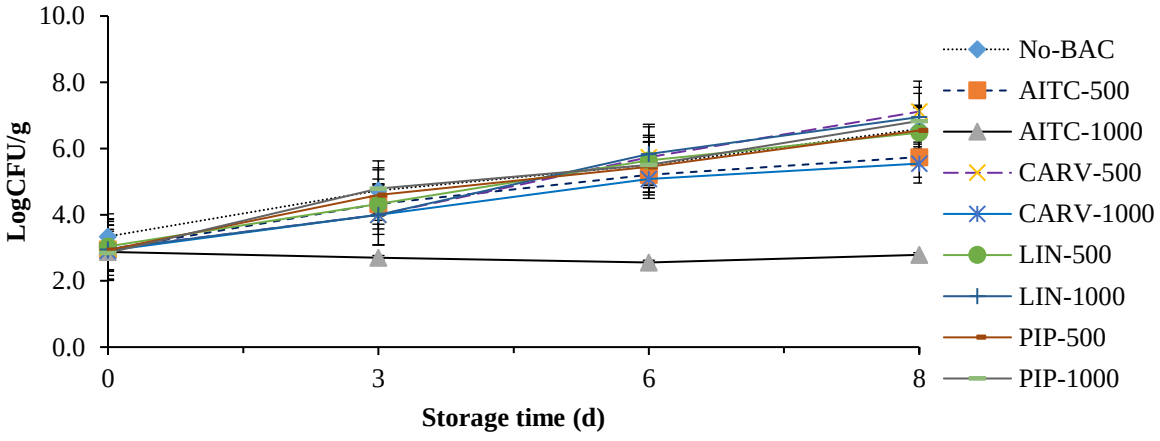


Figure 20: Effect of different concentrations of AITC, CARV, LIN and PIP on aerobic mesophilic counts of chicken meat stored up to 8 days at 4 °C**.**

4.1.4. Evaluation of *in-vitro* antimicrobial activity of allyl-Isothiocyanate, carvacrol, linalool, and piperine using agar well method

The result from the antibacterial activity of BACs (AITC, CARV, LIN, and PIP) using the agar well method is summarized in Table 7. The selected BACs in this study showed the growth inhibition of both food pathogenic and food spoilage bacteria. The result from AITC compared to control showed complete inhibition (CI) of *P. lundensis, St. aureus,* and *B. cereus* with partial inhibition of *E. coli, L. monocytogenes,* and no inhibition of *S.* Typhimurium. CARV did not show CI of any of the studied strains, however, partial inhibition of *E. coli, L. monocytogenes, S.* Typhimurium, and *B. cereus* was observed, and no inhibition noticed for *P. lundensis* and *St. aureus*. Current findings agreed with the results by Guarda et al. (2011) who coated films with microcapsules containing 10 % of CARV and thymol at 4 °C for 28 days and observed 9.0 ± 0.8 mm zone of inhibition in *E. coli*. While their findings do not agree to ours regarding the *St. aureus* as it was observed 11.3 ± 1.3 mm zone of inhibition. Ward et al. (1998) used a volatile distillated extract from fresh horseradish root contained about 90 % AITC in cooked beef. They noted that the growth of *St. aureus, E. coli*, *S.* Typhimurium, and *L. monocytogenes* on agar was completely inhibited for 7 days in aerobic storage at 12 °C. Helander et al. (1998) found the CARV and thymol showed a more inhibitory effect against *E. coli* and *S*. Typhimurium than (+)-carvone and *trans*-Cinnamaldehyde. They also found that CARV and thymol decreased the intracellular ATP pool of *E. coli* and disintegrated the cytoplasmic membrane and released outer membrane-associated material from the cells. LIN with the ratio of 1:10 (v:v) during 24-72 h of storage exhibited inhibition zone for *E. coli*, *St. aureus*, *S.* Typhimurium, and *B. cereus*. While *L. monocytogenes* required 1:80 (v:v) LIN to show inhibition, concomitantly no inhibition was detected for *P. lundensis* (Table 7). Our results are in accordance with Dorman & Deans (2000) work that showed *in vitro* activity of LIN and recorded inhibitory zone of 13.8 ± 0.3, NI, 7.5 ± 0.5 and 9.0 ± 0.4 mm for each of *E. coli, Pseudomonas spp., S.* Pullorum, and *St. aureus* respectively. In the current study, LIN inhibited both foodborne pathogenic and food spoilage bacteria. Thus, these data can be evidently served as well confirmatory and complementary data to the previously published work. PIP at different concentrations, did not exhibit inhibitory activity against the studied bacteria. This could be due to its poor solubility in aqueous and oily environments that limit it is biological applications (Gorgani et al., 2017). Shivarani et al. (2013) used 100 µl of PIP *in vitro* and observed high susceptibility of G+veB bacteria (*St. aureus* and *B. subtilis*) and less susceptibility of G-veB bacteria (*Pseudomonas spp.* and *E. coli*) to PIP. Thus, less antibacterial activity of PIP leads us to carry out further *in vitro* studies to find alternative BAC to be applied with AITC besides the HHP in meat preservation.

Table 7: *In vitro* antibacterial activity using agar well method estimated by inhibition zone of AITC, CARV, LIN, and PIP against *P. lundensis*, *E. coli* O157:H7, *St. aureus, L. monocytogenes, S.* Typhimurium, and *B. cereus*.

Bacterial strains	**Storage time (h)**	**Bioactive compounds**																
			AITC				**CARV**				**LIN**				**PIP**			
***Pseudomonas lundensis* CCP5**		No BAC	1:10 (v:v)	1:20 (v:v)	1:40 (v:v)	1:80 (v:v)	1:10 (v:v)	1:20 (v:v)	1:40 (v:v)	1:80 (v:v)	1:10 (v:v)	1:20 (v:v)	1:40 (v:v)	1:80 (v:v)	1:2 (v:v)	1:4 (v:v)	1:8 (v:v)	1:16 (v:v)
	24	NI	CI	CI	CI	CI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	48	NI	CI	CI	CI	CI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	72	NI	CI	CI	CI	CI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
***Escherichia coli* O157:H7 BO1909**		No BAC	1:80	1:158	1:320	1:640	1:2	1:4	1:8	1:16	1:10	1:20	1:40	1:80	1:2	1:4	1:8	1:16
	24	NI	2.98±1.26	1.36±0.50	NI	NI	3.88±0.54	2.42±0.40	NI	NI	1.56±0.38	NI	NI	NI	NI	NI	NI	NI
	48	NI	1.59±1.57	0.55±0.96	NI	NI	4.03±0.64	2.23±0.20	NI	NI	1.44±0.34	NI	NI	NI	NI	NI	NI	NI
	72	NI	1.39±1.42	0.48±0.83	NI	NI	3.83±0.41	2.39±0.29	NI	NI	1.33±0.04	NI	NI	NI	NI	NI	NI	NI
***Staphylococcus aureus* ATCC 6538**		No BAC	1.6:1	1:1.25	1:2.5	1:5	1:10	1:20	1:40	1:80	1:10	1:20	1:40	1:80	1:2	1:4	1:8	1:16
	24	NI	CI	CI	CI	CI	NI	NI	NI	NI	2.24±0.25	0.81±0.70	NI	NI	NI	NI	NI	NI
	48	NI	CI	CI	CI	CI	NI	NI	NI	NI	1.50±0.25	0.26±0.45	NI	NI	NI	NI	NI	NI
	72	NI	CI	CI	CI	CI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
***Listeria monocytogenes* CCM 4699**		No BAC	1:80	1:158	1:320	1:640	1:2	1:4	1:8	1:16	1:80	1:158	1:320	1:640	1:2	1:4	1:8	1:16
	24	NI	3.05±2.18	1.91±1.01	1.09±0.16	NI	3.87±0.48	2.16±0.13	NI	NI	1.28±0.26	0.22±0.38	NI	NI	NI	NI	NI	NI
	48	NI	1.78±0.74	NI	NI	NI	3.69±0.39	2.13±0.10	NI	NI	1.45±0.33	NI	NI	NI	NI	NI	NI	NI
	72	NI	1.75±0.30	NI	NI	NI	3.66±0.18	2.14±0.10	NI	NI	0.35±0.61	NI	NI	NI	NI	NI	NI	NI
***Salmonella* Typhimurium B1310**		No BAC	1:80	1:158	1:320	1:640	1:2	1:4	1:8	1:16	1:10	1:20	1:40	1:80	1:2	1:4	1:8	1:16
	24	NI	NI	NI	NI	NI	3.39±0.62	2.00±0.53	NI	NI	1.43±0.64	NI	NI	NI	NI	NI	NI	NI
	48	NI	NI	NI	NI	NI	3.10±0.54	1.69±0.51	NI	NI	0.99±0.18	NI	NI	NI	NI	NI	NI	NI
	72	NI	NI	NI	NI	NI	3.10±0.29	1.97±0.10	NI	NI	0.93±0.10	NI	NI	NI	NI	NI	NI	NI
***Bacillus cereus* T1**		No BAC	1.6:1	1:1.25	1:2.5	1:5	1:10	1:20	1:40	1:80	1:10	1:20	1:40	1:80	1:2	1:4	1:8	1:16
	24	NI	CI	CI	CI	CI	0.50±0.17	NI	NI	NI	1.74±0.10	0.26±0.46	NI	NI	NI	NI	NI	NI
	48	NI	CI	CI	CI	CI	0.69±0.60	NI	NI	NI	1.31±0.25	NI	NI	NI	NI	NI	NI	NI
	72	NI	CI	CI	CI	CI	0.36±0.62	NI	NI	NI	1.10±0.29	NI	NI	NI	NI	NI	NI	NI

NI: No inhibition, CI: complete inhibition. Thickness of inhibition zone was calculated in (mm ± SD).

4.1.5. Electronic nose

The E-nose was able to show proper discrimination between untreated and treated meat based on the type of BAC and storage time. Additionally, overlapping between CARV, PIP and control groups were noticed, while AITC and LIN yield the biggest mean differences compared to control and CARV and PIP (Figure 21: A, B and C). Moreover, the high concentration of BACs (1000 ppm) showed clear separations compared to other groups. In this study, it was noted that BACs and clearly AITC and LIN produced spicy odour, this odour was perceived abundantly just after opening the packages, which might produce pleasing flavour attributes for some foods such as meat and increase the acceptance by consumers. Alongside, reduced TBARS was noticed with some BACs (e.g. CARV, LIN and PIP) indicating that the instrument can classify the chicken meat as either fresh or spoiled with rancid flavour. However, a very low quantity of AITC and LIN can be applied to foods due to its potential to produce a strong aroma that can modify odour properties of meat. Olaimat et al. (2014) also noted that slight odour was detected through informal sensory analysis by using AITC in coatings at 50 µl/g. Chacon et al. (2006) used 500-ppm AITC in dry fermented sausages and resulted in an acceptable level of spiciness although slightly spicy by panellists. Boskovic et al. (2017) stated that based on their finding of sensory data pork packaged under MAP with 0.3 % thyme EO added was most acceptable compared to the meat BACs treated meat. Mastromatteo et al. (2009) also observed that the application of CARV in poultry patties had a distinctive but pleasant flavour and showed no modification for off-odour perception during the storage period. Concomitantly, reduced AMCs were noticed with AITC 1000 indicating that the E-nose can distinguish the meat as either fresh or spoiled (Edita et al., 2018). Rokaityte et al. (2016) found that after one day of storage the minced meat treated with LIN exhibited a higher score of flavour and overall acceptability than control. Martínez et al. (2006) noticed that the off-odour formation was significantly delayed in all fresh pork sausages with added black pepper ($P<0.05$). Additionally, several factors can support the changes in aroma profile of meat during storage such as: progress of LO, fat content, liberation of fatty acids and increased microbial load during storage.

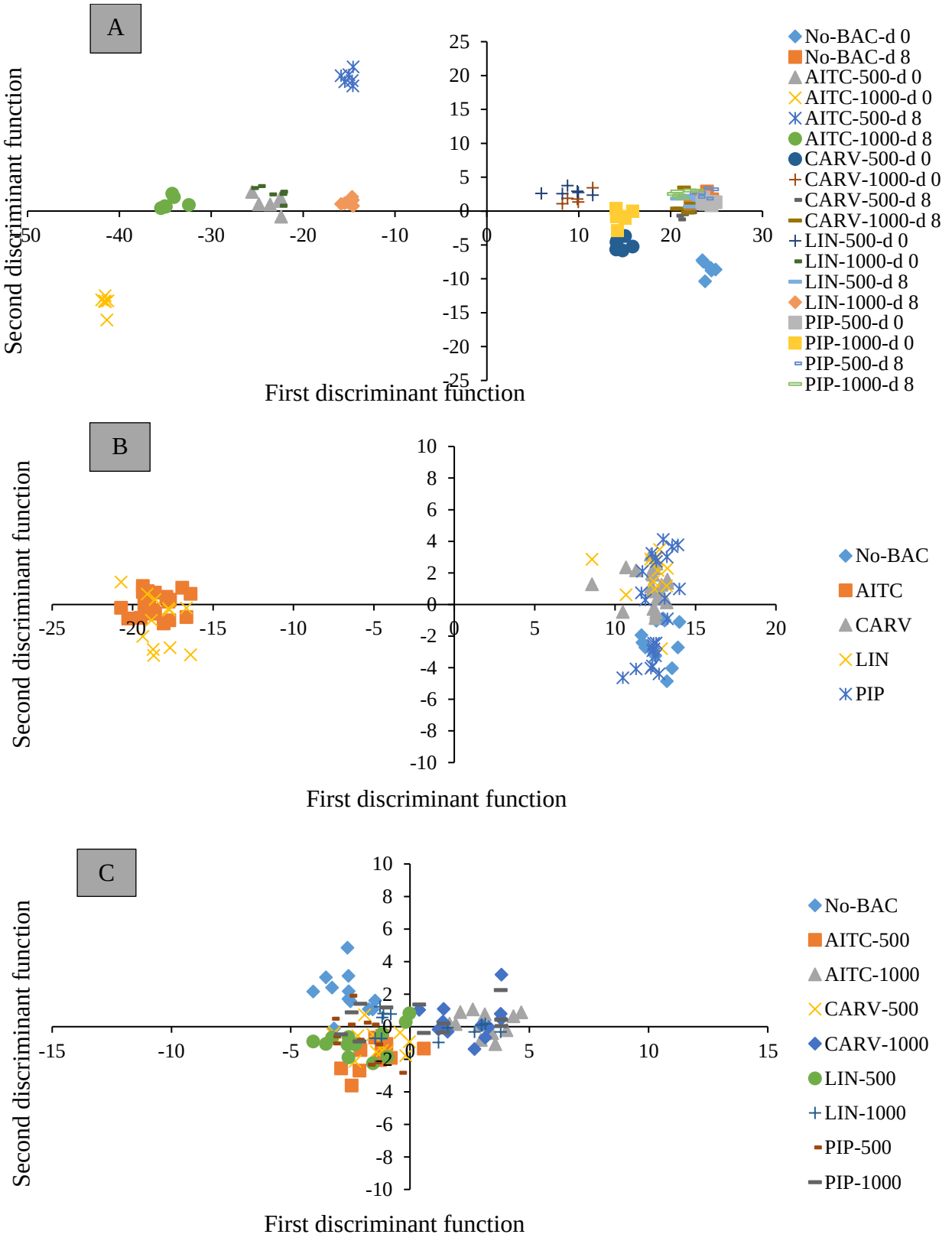


Figure 21: Effect of different concentrations of AITC, CARV, LIN and PIP on smell detection by an E-nose in chicken meat stored up to 8 days at 4 °C, Canonical discriminant analysis score plot of A: The separation based on storage days and concentration of BACs, B: The separation based on BACs type, and C: The separation based on the concentration of BACs.

4.2. EVALUATION OF THE *IN-VITRO* ANTIMICROBIAL ACTIVITY OF BIOACTIVE COMPOUNDS AGAINST *LISTERIA MONOCYTOGENES*, *STAPHYLOCOCCUS AUREUS*, *BACILLUS CEREUS*, *ESCHERICHIA COLI*, *SALMONELLA* TYPHIMURIUM, AND *PSEUDOMONAS LUNDENSIS*

4.2.1. Evaluation of the *in-vitro* antimicrobial activity of BACs using disc method

The antibacterial activity of the individual BACs using the filter paper disc method is summarized in Figure 22 and Table 8. The BACs evaluated were: AITC, Thymol, CARV, αTPN, eugenol, LIN, PIP, camphor, γ-Terpinene, *p*-Cymene, limonene, α-Pinene, 1,8-cineole, cuminaldehyde, β-citronellol, geraniol, (−)-α-Bisabolol, and synthetic compound (BHT). From this, the components with the widest spectrum of antibacterial activity against the studied bacteria was found to be CARV, followed by thymol, eugenol, LIN, AITC, cuminaldehyde, αTPN, geraniol, β-citronellol, α-Pinene, limonene, 1,8-cineole, γ-Terpinene, camphor, and *p*-Cymene. After 24 h incubation CARV showed 5.19 ± 0.02, 20.14 ± 0.73, 16.70 ± 0.29, 17.27 ± 1.00, 15.15 ± 0.27, and 17.60 ± 0.39 mm inhibition zone for each of *P. lundensis, E. coli, St. aureus, L. monocytogenes, S.* Typhimurium and *B. cereus*, respectively (Table 8). Moreover, AITC, thymol, LIN, cuminaldehyde, eugenol, αTPN, β-citronellol, and geraniol showed a zone of inhibition against all the studied strains. Whereas AITC showed a complete inhibition against *St. aureus,* α-Pinene also did not show inhibitory activity against *L. monocytogenes and B. cereus*. Additionally, camphor only was active against *E. coli* and *p*-Cymene against *St. aureus,* and the PIP did not exhibit antimicrobial activity using the disc method. Whereas BHT showed less than 1.09±0.51 mm zone of inhibition against only *P. lundensis, St. aureus,* and *S.* Typhimurium. Kim et al. (1995) studied the antimicrobial effect of some BACs against four G-veB bacteria (*E. coli*, *E. coli* 0157:H7, *S.* Typhimurium, and *Vibrio vulnificus*) and one G+veB bacterium (*L. monocytogenes*). Using disk diffusion method, they ranked BACs effect against *E. coli* as citronellal > perillaldehyde > citral > geraniol > linalool > eugenol > terpineol > CARV and against *S.* Typhimurium as citronellal > citral > geraniol > perillaldehyde > linalool > eugenol > terpineol > CARV. They found that CARV (MBC 250 μg/mL) was most active against all the tested strains. Their finding was not agreed to ours they also found that αTPN and LIN were least potent against the studies strain (1000 μg/mL), whereas geraniol, perillaldehyde and citral (500 μg/mL) completely killed *E. coli*, *E. coli* 0157:H7, and *S.* Typhimurium, while citronellal (250 μg/mL) killed *Vibrio vulnificus*, and limonene were mostly inactive. Lin et al. (2000) tested liquid AITC *in vitro* against G+veB bacteria (*L. monocytogenes*) and G-veB bacteria (*Salmonella* Montevideo

and *E. coli* O157:H7) their result indicates that *Salmonella* Montevideo and *E. coli* O157:H7, were more sensitive to AITC than *L. monocytogenes*. In their study they found that 500 μg/mL (approximately 0.5 μL/mL) was needed to achieve a 3 to 4 log reduction of *E. coli* O157:H7 and *Salmonella* Montevideo, however, 2500 μg/mL of AITC were required for *L. monocytogenes*. AITC can cause cell membrane damages to *E. coli* and *Salmonella* Montevideo, it did not show cell lysis but lead to the leakage of cellular metabolites, besides these bacteria are more susceptible to AITC during the early and late exponential growth stages in comparison to the lag and stationary phases. Guimarães et al. (2019) observed that thymol, CARV, and eugenol presented strong antimicrobial action against *B. cereus, S.* Typhimurium, *E. coli* and *St. aureus,* On the other hand, the compounds *m*-Cymene, (±)-linalool, camphor, *trans*-Geraniol, terpineol, (±)-citronellal, (+)-borneol and *R*-(+)-limonene demonstrated the least action and BACs such as *p*-Cymene, (+)-α-Pinene, γ-Terpinene, (−)-α-Bisabolol, eucalyptol showed no activity against these evaluated strains. Thymol and eugenol inhibited the growth of *S.* Typhimurium, and *St. aureus* and were considered potent antimicrobials.

4.2.2. Evaluation of *in-vitro* antimicrobial activity of BACs using the MIC method

The antibacterial effects of various BACs against six foodborne and spoilage bacteria in a liquid phase (MIC values) using micro-dilution are presented in Table 9. AITC showed the best activity among all the BACs followed by geraniol, β-citronellol, CARV, αTPN, 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. Among the BACs AITC and αTPN were chosen as the most effective BACs in liquid phase against *P. lundensis, E. coli, St. aureus, L. monocytogenes, S.* Typhimurium and *B. cereus* due to their overall lower MIC values against these pathogenic bacteria as compared to the other BACs such as geraniol, CARV, thymol and β-citronellol which showed less activity against *P. lundensis.*

Guarda et al. (2011) found that Thymol and CARV showed significant antimicrobial activity against the *E. coli* O157:H7, *St. aureus*, *Listeria innocua*, Saccharomyces cerevisiae, and Aspergillus niger with MIC of 125-250 ppm and 75-375 ppm for thymol and CARV respectively. They noticed the synergistic effect of thymol and CARV at a concentration of 50 % and 50 %. López et al. (2007) found that thymol and CARV showed similar results against yeast (Candida albicans), moulds (Aspergillus flaVus), and G+veB (*L. monocytogenes*), but thymol was significantly (p<0.05) more effective than CARV against G-veB (Salmonella choleraesuis), linalool were active against *Salmonella* choleraesuis and Candida albicans while other BACs

camphor, esragol, 1.8-cineole, *p*-Cymene, and limonene did show any inhibitory activity against these microorganisms. Zengin and Baysal, (2014) also determined the MIC values of αTPN which was 0.6 % for *E. coli* O157:H7, *S. liquefaciens*, *C. divergens* and *L. innocua*, while 0.7 % of αTPN needed to inhibit *St. aureus* and *S.* Typhimurium. They also observed that αTPN and LIN showed synergistic effects and αTPN/eucalyptol showed additive effects against *S.* Typhimurium, *E. coli* O157:H7 and *St. aureus*. In accordance with this study, Lopez-Romero et al. (2015) found that citronellol was an effective molecule against *E. coli* and *St. aureus*, followed by citronellal, carveol, and carvone. That caused changes in the hydrophobicity, surface charge, and disruption membrane integrity with the subsequent K^+ leakage from *E. coli* and *St. aureus*. Boskovic et al. (2017) treated minced pork with thyme EO (Thymus vulgaris) (0.3, 0.6, and 0.9 %) packaged under vacuum or MAP. They found MIC was greatest for thymol and CARV followed by thyme EO against four serovars of *Salmonella* (*S.* Enteritidis, *S.* Typhimurium, *S.* Montevideo, and *S.* Infantis). In accordance with the current study, they found that thymol and CARV showed greater antimicrobial activity than thyme EO and BACs such as *p*-Cymene, cinnamaldehyde, and eugenol. Additionally,the synergistic interaction of CARV and nisin against *L. monocytogenes* was investigated also *in vitro* by checkerboard assay (Churklam et al., 2020). Li et al. (2015b) demonstrated that the MIC and MBC values of αTPN against *E. coli* (CMCC (B) 44102 were 0.78 μL/mL. It means that αTPN might inhibit the growth of *E. coli* by killing bacteria directly.

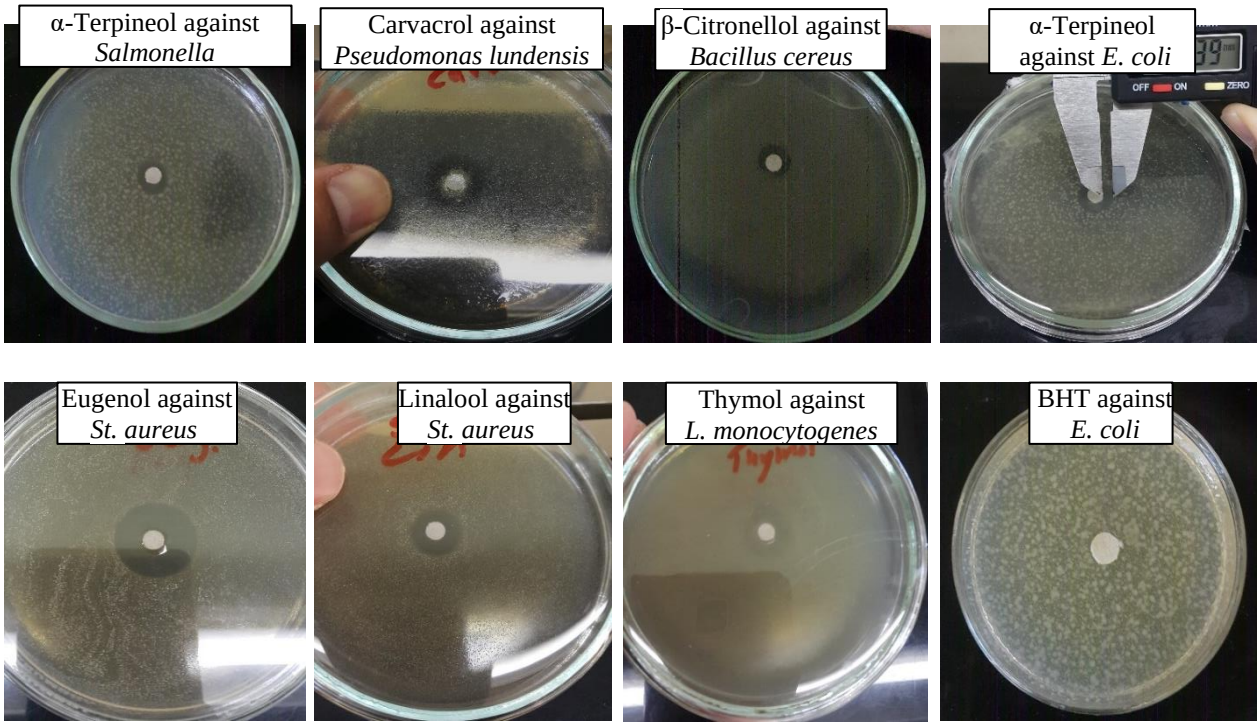


Figure 22: Some examples of bacterial inhibition zones using the disc method.

Table 8: Antibacterial activity using filter paper disc diffusion estimated by inhibition zone of different BACs against *P. lundensis*, *E. coli* O157:H7, *St. aureus*, *L. monocytogenes*, *S.* Typhimurium, and *B. cereus*.

Bacterial strains	Storage time (h)	Bioactive compounds																		
		D.W.	Ethanol	Thymol	CARV	LIN	Eugenol	Camphor	PIP	γ-Terpinene	p-Cymene	Limonene	α-Pinene	cuminaldehyde	AITC	1,8-cineole	α-Terpineol	β-Citronellol	Geraniol	(−)-α-Bisabolol	BHT
Pseudomonas lundensis	24	NI	NI	3.33±0.78	5.19±0.02	1.10±0.06	1.38±0.00	NI	NI	NI	NI	NI	0.93±0.06	1.22±0.30	CI	NI	1.83±0.01	1.59±0.27	1.70±0.36	1.45±0.11	0.58±0.14
	48	NI	NI	2.95±1.13	5.16±0.17	1.10±0.15	1.72±0.00	NI	NI	NI	NI	NI	0.84±0.18	1.25±0.33	CI	NI	1.41±0.38	1.58±0.28	1.25±0.41	NI	NI
	72	NI	NI	2.50±0.67	5.17±0.06	1.03±0.00	1.68±0.00	NI	NI	NI	NI	NI	0.89±0.00	0.80±0.01	10.80±0.5.28	NI	0.94±0.01	1.40±0.25	1.10±0.49	NI	NI
Escherichia coli	24	NI	NI	7.38±0.62	20.14±0.73	7.12±0.07	5.57±0.21	1.02±0.00	NI	NI	NI	1.48±0.01	0.77±0.78	4.80±0.25	1.63±0.49	2.50±0.60	3.02±0.15	1.15±0.38	2.69±0.44	NI	NI
	48	NI	NI	7.21±0.66	16.94±0.66	7.42±0.66	4.63±0.10	1.20±0.00	NI	NI	NI	1.09±0.40	0.53±0.53	3.74±0.69	2.0±0.91	1.75±0.22	2.84±0.03	1.15±0.21	2.67±0.50	NI	NI
	72	NI	NI	6.68±0.53	16.76±0.92	6.66±0.30	4.70±0.36	1.04±0.00	NI	NI	NI	1.20±0.54	0.63±0.63	3.69±0.41	1.72±0.70	2.02±0.18	2.24±0.57	0.91±0.14	2.33±0.42	NI	NI
Staphylococcus aureus	24	NI	NI	7.15±0.98	16.70±0.29	4.01±0.23	6.91±0.00	NI	NI	1.02±1.03	0.88±0.89	0.85±0.10	2.62±0.77	9.42±2.22	CI	NI	2.83±0.01	1.82±0.04	2.05±0.05	0.47±0.02	1.09±0.51
	48	NI	NI	5.77±0.51	16.20±0.60	3.65±0.23	6.67±0.00	NI	NI	0.73±0.74	0.78±0.78	0.86±0.00	1.61±0.26	7.17±0.10	CI	NI	2.42±0.72	1.73±0.19	2.02±0.15	0.42±0.06	0.84±0.25
	72	NI	NI	5.76±0.29	15.86±0.09	3.38±0.25	6.49±0.00	NI	NI	0.64±0.64	0.65±0.65	0.87±0.00	1.44±0.20	6.85±0.68	CI	NI	1.76±0.25	0.99±0.38	1.94±0.02	0.39±0.09	0.80±0.30
Listeria monocytogenes	24	NI	NI	5.41±0.68	17.27±1.00	3.43±0.15	2.75±0.00	NI	NI	NI	NI	NI	NI	1.64±0.05	1.53±0.12	NI	1.86±0.76	1.86±0.10	0.82±0.02	NI	NI
	48	NI	NI	5.34±0.41	17.01±1.57	3.10±0.02	2.62±0.00	NI	NI	NI	NI	NI	NI	1.03±0.09	1.41±0.18	NI	1.13±0.03	1.01±0.16	NI	NI	NI
	72	NI	NI	5.28±0.62	17.07±0.99	2.83±0.36	2.61±0.00	NI	NI	NI	NI	NI	NI	1.07±0.06	1.28±0.06	NI	1.31±0.40	1.10±0.31	NI	NI	NI

***Salmonella* Typhimurium**	24	NI	NI	6.62 ±0. 19	15.1 5±0. 27	5.19 ±0.3 7	3.35 ±0.0 0	NI	NI	1.06±0 .06	NI	0.00	1.22 ±0.0 2	2.52±0.1 7	CI	1.25± 0.25	2.33± 0.04	1.85± 0.24	0.82 ±0.1 6	NI	0.51 ±0.5 2
	48	NI	NI	6.61 ±0. 51	15.4 5±0. 34	4.86 ±0.9 1	3.17 ±0.0 0	NI	NI	1.32±0 .02	NI	0.00	0.87 ±0.0 4	1.92±0.0 8	7.03±4.09	1.10± 0.40	2.09± 0.46	1.42± 0.12	0.77 ±0.7 7	NI	NI
	72	NI	NI	5.55 ±0. 70	15.8 0±0. 24	5.22 ±0.4 0	3.37 ±0.0 0	NI	NI	0.80±0 .30	NI	0.00	0.83 ±0.1 5	1.96±0.2 4	5.33±3.75	1.12± 0.45	1.99± 0.38	1.25± 0.07	0.67 ±0.7 8	NI	NI
Bacillus cereus	24	NI	NI	7.30 ±0. 01	17.6 0±0. 39	4.30 ±0.5 2	5.10 ±0.0 0	NI	NI	NI	NI	NI	NI	6.54±0.1 4	2.48±0.97	NI	2.52± 0.60	2.15± 0.09	2.04 ±0.3 9	0.44± 0.45	NI
	48	NI	NI	5.03 ±0. 01	14.1 4±0. 12	3.84 ±0.6 1	5.56 ±0.0 0	NI	NI	NI	NI	NI	NI	3.08±0.5 8	0.99±0.06	NI	2.07± 1.10	2.05± 0.16	1.11 ±1.1 1	0.30± 0.30	NI
	72	NI	NI	5.77 ±0. 38	15.3 5±0. 09	3.55 ±0.8 2	4.20 ±0.0 0	NI	NI	NI	NI	NI	NI	3.02±0.5 3	1.24±0.35	NI	1.50± 1.02	1.09± 0.69	1.07 ±1.0 7	0.30± 0.30	NI

NI: No inhibition, CI: complete inhibition, BHT: butylated hydroxytoluene, Thickness of inhibition zone was calculated in (mm ± SD).

Table 9: Minimum inhibitory concentration (MIC µl/ml) of various BACs against *P. lundensis, E. coli* O157:H7, *St. aureus, L. monocytogenes, S.* Typhimurium, and *B. cereus*.

Bacterial strains	**Ethanol**	**Bioactive compounds**																	
		Thymol	**CARV**	**LIN**	**Eucgenol**	**Camphor**	**PIP**	**γ-Terpinene**	**p-Cymene**	**Limonene**	**α-Pinene**	**cuminaldehyde**	**AITC**	**1,8-cineole**	**αTPN**	**β-Citronellol**	**Geraniol**	**(−)-α-Bisabolol**	**BHT**
Pseudomonas lundensis	NI	0.25	0.25	0.125	0.125	0.5	0.5	0.5	0.125	1	1	0.25	0.063	0.5	0.125	0.5	0.5	1	0.25
Escherichia coli	NI	0.063	0.063	0.125	0.125	0.5	0.5	1	0.5	0.5	1	0.25	0.008	0.5	0.125	0.25	0.063	0.5	0.5
Staphylococcus aureus	NI	0.063	0.063	0.125	0.125	0.5	0.5	1	0.5	0.5	1	0.25	0.004	0.125	0.25	0.063	0.063	0.25	0.063
Listeria monocytogenes	NI	0.125	0.125	0.125	0.125	0.5	0.5	0.5	0.5	0.5	0.5	0.25	0.008	0.25	0.25	0.063	0.063	0.25	0.5
***Salmonella* Typhimurium**	NI	0.25	0.25	0.125	0.125	0.5	0.5	0.5	0.5	0.5	0.5	0.125	0.004	0.5	0.25	0.25	0.063	0.5	0.5
Bacillus cereus	NI	0.125	0.125	0.25	0.125	0.5	0.5	1	0.5	1	0.5	0.25	0.031	0.5	0.25	0.063	0.125	0.25	0.5

NI: No inhibition.

4.3. EFFECT OF A-TERPINEOL ON CHICKEN MEAT QUALITY DURING REFRIGERATED CONDITIONS

4.3.1. Physicochemical properties

4.3.1.1. pH of meat

The result of the physicochemical properties of chicken meat treated with αTPN is listed in Table 10. Different concentrations of αTPN were able to alter the pH values of chicken meat during 14-day storage. At the end of the storage, the pH value of treated meat was increased significantly except for αTPN-MIC-4 which remain at high values 6.09 to 6.12 at day 0 and 14 respectively, compared to a significant decline in pH of control samples 6.02 and 6.01 at the same days ($P<0.05$). Regarding the concentration of αTPN, significant differences were observed within groups containing αTPN and compared to untreated meat ($P<0.05$). It has been reported that if the boiler meat is very dark, pH will be high and if the meat is very light, it will have a low pH (Mir et al., 2017), this was not witnessed in our result for αTPN treated meat.

4.3.1.2. Colour values

The colour of chicken meat shows significant changes (except redness values) during the 14 days storage period (Table 10). The increase rates were observed in all meat samples, however, the trend was most abundant in intensifying drifts in the lightness of the sample contained a high level of αTPN (MIC-2 and MIC-4) compared to control and MIC-1. At the end of the storage period, no significant differences were found with the addition of a low level of αTPN (MIC-1) compared to control and was effective in keeping the L^* values close to the initial L^* values, whereas the significant difference was noticed in MIC-2 and MIC-4 compared to untreated meat. It is known that muscles at pH ≤ 6.0 undergo greater protein denaturation and lead to an increase in light scattering and opaqueness properties of the meat (Mir et al., 2017). The a^* values in meat containing higher-level αTPN decreased at the end of storage compared to an increasing trend in control but no significant changes were noticed. The decrease of a^* value during storage is due to the accumulation of MetMb pigment (Mancini & Hunt, 2005). However, this decrease in the a^* values were less in the meat treated with αTPN-MIC-1, which was close to the initial a^* values at the beginning of storage. In general, it has been reported that a* values decreased with increasing storage period in the absence of oxygen in the package, while at 2 ºC and in vacuum or MAP storage a^* values can increase (Mancini & Hunt, 2005). The b^* value of the control and αTPN-

MIC-1 decreased at day 14 of storage, unlike and reverse trend with a significant difference were observed in meat treated with αTPN MIC-2 and MIC-4. Regarding the concentration of αTPN the meat containing MIC-2 and MIC-4 resulted in significantly higher *b** compared to MIC-1 and control (Table 10). Similar to yellowness, increasing trends of colour intensity (*C**) 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. The *C** values in MIC-2 and MIC-4 were 13.18 ± 0.68, and 15.40 ± 1.57 at the first day and increased to 15.08 ± 1.16 and 16.71 ± 1.06 at day 14, while for control and MIC-1 it was 12.87 ± 0.68 and 12.67 ± 1.18 at the first day and decreased to 11.81 ± 0.33 and 12.63 ± 0.57 at day 14 of storage. On the other hand, throughout the storage period, the steadiness was detected in the hue values (*h**) despite a slight decrease in control and a slight rise in treated meat with no significant difference. However, regarding the concentrations of BACs significant differences were noticed only at day10 and 14, and only between treated meat and control. To the best of our knowledge, no studies have seen that dealing with the colour changes and the preservative potential of αTPN in meat.

4.3.1.3. Water holding capacity

During the 14 d storage period, different levels BACs particularly αTPN MIC-2 and αTPN MIC-4 were able to show a significant effect on decreasing WHC (Table 10). Whereas, no significant variation was witnessed in MIC-1 and control at end of the storage. Simultaneously the variation noticed between the treated meat with a higher concentration of BAC comparing to control. Increase in the water content of muscles leads to improve the quality and economical value of meat due to enhancing the tenderness, juiciness, firmness, and appearance (Mir et al., 2017). It has been known that changes in meat pH can affect the WHC and meat quality, hence a decrease in meat pH can lead to decreased WHC of muscle proteins (Shirzadegan & Falahpour, 2014). In contrast, in our study the increase in pH was observed with decreased WHC in meat treated with higher levels of αTPN.

Table 10: The influence of different concentrations of αTPN on pH, colour values and WHC of fresh chicken meat stored up to 14 days at 4 °C.

Parameters	**Storage time (d)**	**Treatments**			
		No-BAC	**αTPN-MIC-1**	**αTPN-MIC-2**	**αTPN-MIC-4**
pH	0	6.02±0.02 ^{aA}	6.02±0.02 ^{abA}	6.04±0.01 ^{aAB}	6.09±0.03 ^{aB}
	3	6.00±0.02 ^{aA}	6.01±0.02 ^{aAB}	6.04±0.00 ^{aB}	6.11±0.01 ^{aC}
	7	6.00±0.01 ^{aA}	6.02±0.01 ^{abA}	6.04±0.02 ^{aA}	6.12±0.00 ^{aB}

	10	6.01±0.01 ^{aA}	6.03±0.01 ^{abAB}	6.05±0.01 ^{abB}	6.11±0.02 ^{aB}
	14	6.01±0.01 ^{aA}	6.04±0.01 ^{bB}	6.06±0.01 ^{bB}	6.12±0.01 ^{aC}
L*	0	46.75±1.01 ^{aA}	49.82±0.59 ^{aB}	50.44±0.67 ^{aB}	55.33±1.24 ^{aC}
	3	47.69±0.69 ^{aA}	49.73±1.75 ^{aA}	52.25±1.24 ^{abB}	58.56±0.91 ^{bC}
	7	47.23±1.39 ^{aA}	49.02±0.96 ^{aA}	51.95±0.57 ^{abB}	58.66±1.64 ^{bC}
	10	47.46±0.66 ^{aA}	49.32±0.40 ^{aB}	52.58±1.32 ^{bC}	59.40±1.00 ^{bD}
	14	48.30±1.32 ^{aA}	50.02±0.66 ^{aA}	52.47±1.28 ^{bB}	59.00±0.79 ^{bC}
a*	0	1.41±0.46 ^{aA}	1.36±0.38 ^{aA}	1.66±0.22 ^{aA}	1.75±0.46 ^{aA}
	3	1.66±0.74 ^{aA}	1.27±9.78 ^{aA}	1.67±0.39 ^{aA}	1.87±0.74 ^{aA}
	7	1.69±0.74 ^{aA}	1.26±.039 ^{aA}	1.61±0.44 ^{aA}	1.79±0.57 ^{aA}
	10	1.80±0.18 ^{aA}	1.36±0.18 ^{aA}	1.52±0.25 ^{aA}	1.72±0.21 ^{aA}
	14	1.87±0.44 ^{aA}	1.37±0.64 ^{aA}	1.52±0.30 ^{aA}	1.72±0.35 ^{aA}
b*	0	12.78±0.72 ^{aA}	12.59±1.16 ^{aA}	13.08±0.68 ^{aA}	15.29±1.56 ^{bB}
	3	12.05±2.11 ^{aA}	12.58±1.14 ^{aAB}	14.79±1.16 ^{abBC}	16.90±0.86 ^{bC}
	7	11.34±1.12 ^{aA}	12.42±0.30 ^{aA}	14.71±0.94 ^{abB}	16.98±1.52 ^{bC}
	10	11.19±0.81 ^{aA}	12.26±0.51 ^{aA}	14.51±0.46 ^{abB}	16.82±1.35 ^{bC}
	14	11.66±0.28 ^{aA}	12.55±0.53 ^{aA}	15.00±1.18 ^{bB}	16.62±1.08 ^{bC}
C*	0	12.87±0.68 ^{aA}	12.67±1.18 ^{aA}	13.18±0.68 ^{aA}	15.40±1.57 ^{aB}
	3	12.18±2.13 ^{aA}	12.66±1.19 ^{aAB}	14.89±1.14 ^{abBC}	17.02±0.82 ^{aC}
	7	11.49±1.06 ^{aA}	12.48±0.31 ^{aA}	14.80±0.93 ^{abB}	17.09±1.47 ^{aC}
	10	11.33±0.85 ^{aA}	12.34±0.50 ^{aA}	14.59±0.45 ^{abB}	16.91±1.34 ^{aC}
	14	11.81±0.33 ^{aA}	12.63±0.57 ^{aA}	15.08±1.16 ^{bB}	16.71±1.06 ^{aC}
h*	0	1.46±0.04 ^{aA}	1.46±0.02 ^{aA}	1.44±0.02 ^{aA}	1.46±0.03 ^{aA}
	3	1.43±0.05 ^{aA}	1.47±0.05 ^{aA}	1.46±0.03 ^{aA}	1.46±0.05 ^{aA}
	7	1.42±0.07 ^{aA}	1.47±0.03 ^{aA}	1.46±0.03 ^{aA}	1.46±0.04 ^{aA}
	10	1.41±0.03 ^{aA}	1.46±0.02 ^{aB}	1.47±0.02 ^{aB}	1.47±0.02 ^{aB}
	14	1.41±0.03 ^{aA}	1.46±0.05 ^{aB}	1.47±0.02 ^{aB}	1.47±0.02 ^{aB}
WHC (%)	0	2.02±0.74 ^{aA}	1.96±0.48 ^{aA}	1.81±0.12 ^{aA}	2.45±0.39 ^{aA}
	3	1.74±0.26 ^{aA}	2.01±0.20 ^{aA}	1.88±0.15 ^{aA}	2.70±0.22 ^{abB}
	7	1.80±0.13 ^{aA}	2.44±0.43 ^{aAB}	3.07±0.55 ^{bB}	2.89±0.17 ^{abB}
	10	1.90±0.19 ^{aA}	2.10±0.17 ^{aAB}	2.92±0.37 ^{bBC}	3.56±0.51 ^{bC}
	14	1.96±0.50 ^{aA}	2.58±0.40 ^{aAB}	2.77±0.06 ^{bAB}	3.46±0.29 ^{bB}

^{a,b,c}means in the same column with different superscript are significantly different regarding the days of storage; ^{A,B,C}means in the same row with different superscript are significantly different regarding the concentrations of BACs (P<0.05). Colour values: L^*, lightness; a^*, redness; b^*, yellowness; h^*, $\text{Tan}^{-1}\, b^*/a^*$; C*, $(a^{*2} + b^{*2})^{1/2}$; and WHC, water holding capacity.

4.3.2. Meat pigments (Metmyoglobin, deoxymyoglobin, and oxymyoglobin)

The results from the αTPN on the profile of Mb pigments in chicken meat are presented in Figure 23. The initial percentage of MetMb in control, MIC-1, MIC-2, and MIC-4 was 66, 65, 64, and 63 % and decreased to 62, 64, 63, and 62, respectively on day 14. Besides the initial percentage of DeoMb in control, αTPN MIC-1, MIC-2, and MIC-4 were 18, 19, 19, and 18 % and becomes

18, 17, 16, and 16 % on the end of storage. On the other hand, the percentage of OxyMb increased during the storage period. No considerable changes were noticed in control in DeoMb while decrease rate in MetMB and increase rate observed in OxyMb. Myoglobin is commonly found in three forms: MetMb, DeoMb, and OxyMb and the relative proportions of these determine the colour of fresh meat (Bekhit & Faustman, 2005). Some studies demonstrate that natural privatives can reduce oxidation of meat colour and retard colour loss by increasing the *a** values and delaying MetMb formation (Velasco & Williams, 2011). In the current study, compared to control the meat contained higher level αTPN significantly increased *L** and *b** value, while *a** values decreased (Table 10). The increase in fresh meat lightness is attributed to the increased auto-oxidation of OxyMb and the formation of reactive oxygen species (Bekhit et al., 2007).

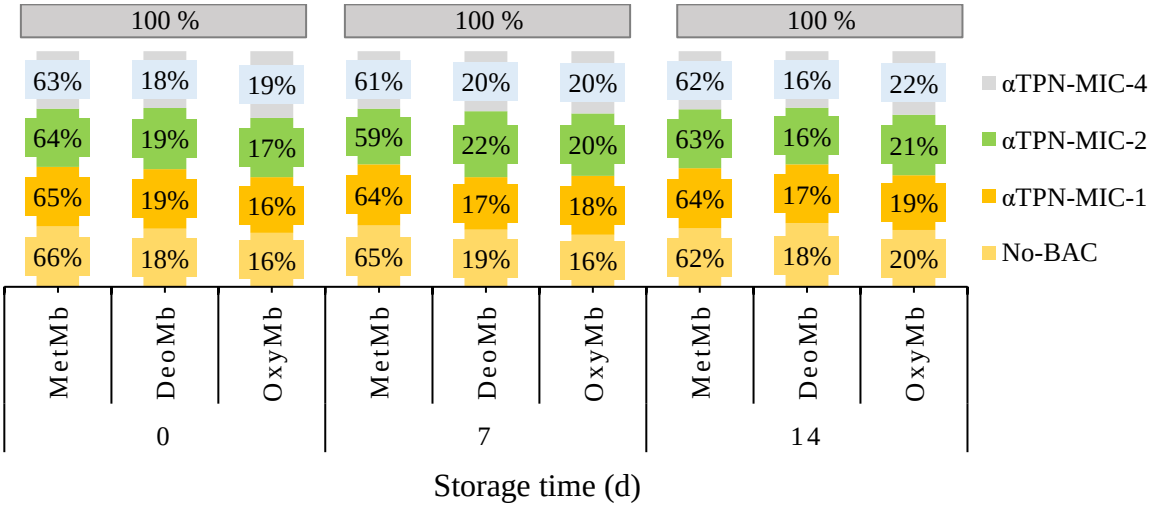


Figure 23: The influence of αTPN on fresh chicken meat pigments {metmyoglobin (MetMb), deoxymyoglobin (DeoMb), and oxymyoglobin (OxyMb)} stored up to 14 days at 4 °C.

4.3.3. Thiobarbituric acid-reactive substances (TBARS)

In this study, at the end of storage, 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 (Figure 24). The reduction was more pronounced in meat treated with MIC-4 which had a positive effect in inhibiting oxidation and resulted in controlling the TBARS value from 0.094 on the first day of storage to 0.112 mg MDA/kg at day 14 comparing to control that was increased from 0.101 to 0.141 mg MDA/kg. This could be attributed to the strong potential antioxidant activity of this BAC in inhibiting the formation of secondary products of LO that may contribute to the off-flavour in stored meat products. It has been investigated that using ferric reducing antioxidant power (FRAP) and DPPH assays indicated that the αTPN possesses a strong antioxidant activity, this antioxidant is less compare to other

oxygenated monoterpenes BACs such as thymol and CARV, however using the ORAC assay the αTPN (2.72 μmol Trolox equiv./μmol) could be compared to commercial antioxidants (Bicas et al., 2011; Zengin & Baysal, 2014). In a study by Bicas et al. (2011) revealed that a range of 181-588 μM αTPN acts as a natural preservative with an antioxidant potential similar to BHA (butylated hydroxyanisole). Thus, αTPN attracts the interest for further research that can culminate in its use as a functional additive in food. To the best of our knowledge, no studies can be seen on the preservative potential of αTPN in controlling TBARS in chicken meat.

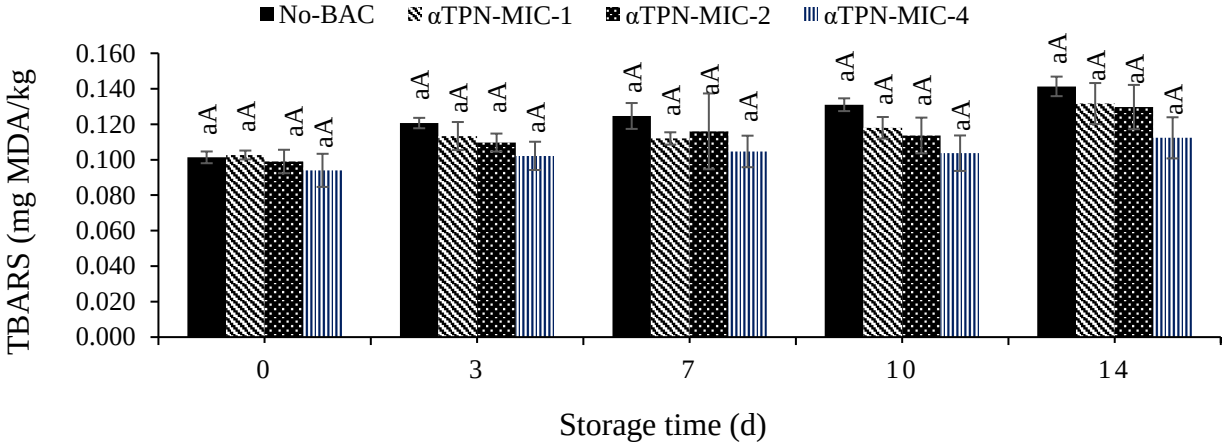


Figure 24: Effect of different concentrations of αTPN on TBARS values of fresh chicken meat stored up to 14 days at 4 °C. * ^{a,b,c}means with different superscript are significantly different regarding the days of storage; ^{A,B,C}means with different superscript are significantly different regarding the concentrations of BACs (P<0.05).

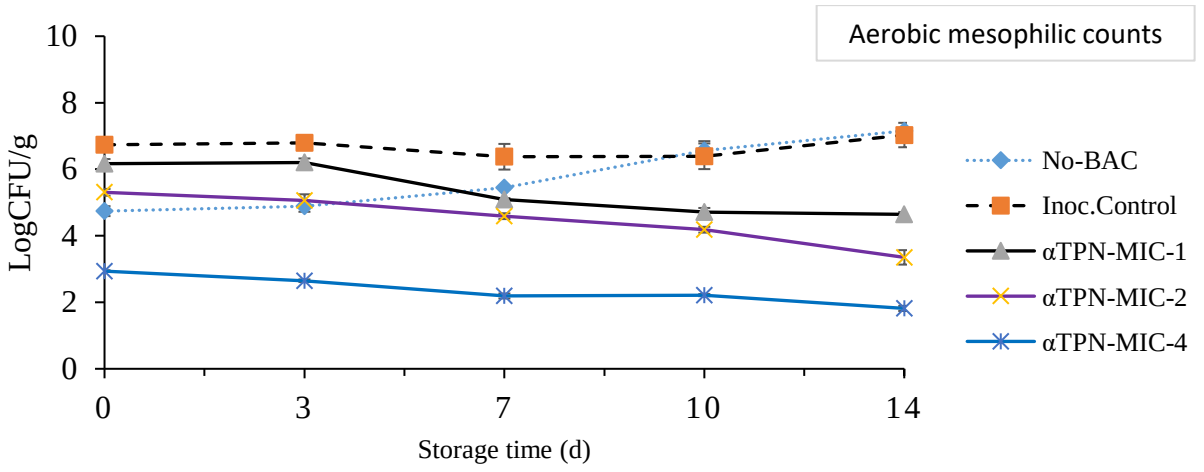
4.3.4. Microbiological characteristics

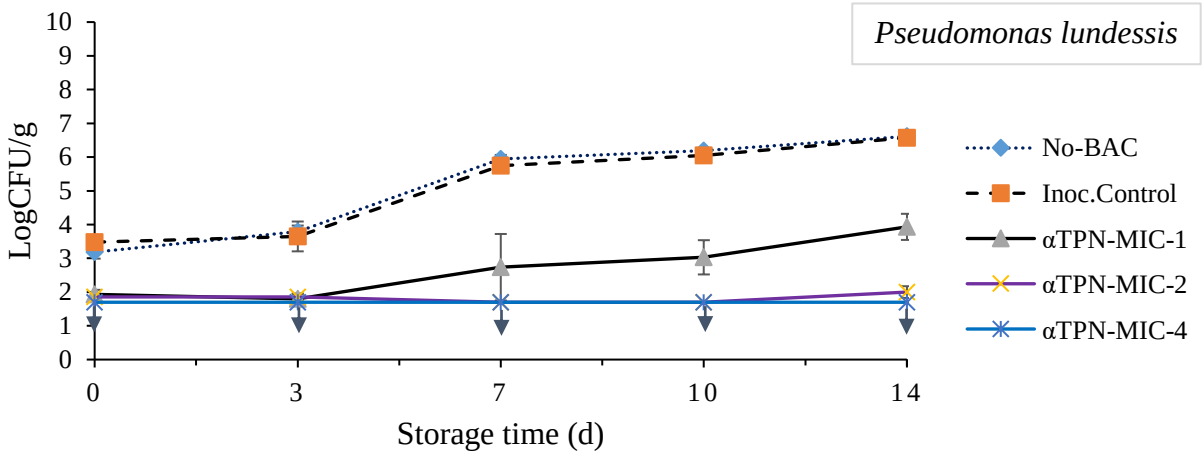
The results from the antimicrobial efficacy of αTPN against aerobic mesophilic counts (AMCs), *L. monocytogenes*, *S.* Typhimurium, *Pseudomonas lundessis* in chicken meat are presented in Figure 25. The initial AMCs population (day 0) in control was 4.74 log CFU/g as a characteristic of acceptable quality chicken meat. The highest count was in control and inoculated control on day 21 which was 7.15 and 7.03 log CFU/g, respectively. It is clear that αTPN had a profoundly higher effect on the inhibition of AMCs, as the concentration of αTPN increased, the surviving count of AMCs decreased, αTPN MIC-1, MIC-2, and MIC-4 caused 2.5, 3.8, and 5.3 log CFU/g reduction in AMCs respectively, for 2 week storage (Figure 25).

To determine the microbiological effect of αTPN, cell counts of *L. monocytogenes* and *S.* Typhimurium and *P. lundessis* are also determined. During storage, the cell count of *P. lundessis* increased in all meat groups except for the sample that contains αTPN MIC-4, which did not

exhibit the growth during the storage (Figure 25). The highest cell count of *P. lundessis* was seen in both controls and inoculated control, both that showed 6.6 log CFU/g at day 14. Whereas meat treated with MIC-1 and MIC-2 the 1.9 log CFU/g cell count of *P. lundessis* was detected at day 0 which increased gradually to 3.9 and 2.0 log CFU/g on day 14 in meat that contains MIC-1 and MIC-2, respectively. On the other hand, cell counts of *L. monocytogenes* in chicken meat samples slightly increased in meat contained αTPN, and even decrease numbers noticed for samples with αTPN MIC-4 which reduced the cell count of *L. monocytogenes* from 2.8 to 1.7 log CFU/g at day 14. In control samples, *L. monocytogenes* started to show the growth at day 7, while the inoculated control showed the highest counts, increased from 5.1 to 6.9 log CFU/g and meat treated with αTPN MIC-1 relatively remained stable throughout the storage. The counts of *S.* Typhimurium were not detected in control meat. The highest growth was observed in inoculated control that reached 6.5 log CFU/g at day 14 (Figure 25). However, the cell counts of *S.* Typhimurium were decreased in meat treated with αTPN and the high concentration of MIC-2 and MIC-4 caused total inhibition to the pathogen at the end of the storage. Additionally, MIC-1 of αTPN reduced the cell count from 5.3 to 4.8 log CFU/g at day 14. Based on the effect of αTPN on AMC and *P. lundessis* in meat the MIC-1 produced less than 7 log CFU/g. This indicates that the meat shelf life was increased by to 2-week storage time. However, for the safety of meat regarding the pathogens *L. monocytogenes* and *S. Typhimurium* further accurate presence/absence detection test needed. Zengin and Baysal, (2014) determined the MIC values of αTPN which was 0.6 % for *E. coli* O157:H7, *S. liquefaciens, C. divergens* and *L. innocua,* while 0.7 % of αTPN needed to inhibit *St. aureus* and *S.* Typhimurium. *In vitro* study on the antimicrobial activity of αTPN reported that due to the presence of OH this BAC interacts with intracellular components and causes the change in the permeability of the outer membrane, change the function of the cell membrane, and leads to the leakage of intracellular materials. The EOs that are rich in αTPN, has been used widely in folk medicine for aromatherapy due to its anti-spasmodic, antinociceptive and immunostimulant properties (section 12 α-Terpineol). Li et al. (2015b) used transmission electron microscopy (TEM) and found that morphostructural alterations in *E. coli* induced MIC levels of αTPN and exhibited decreased cell size and irregular cell shape, cell wall and cell membrane were ruptured, plasmolysis, nucleus cytoplasm was reduced and nuclear area gathered aside. From the control experiment on chicken meat (without inoculation), no *L. monocytogenes* and *S.* Typhimurium were found at the beginning of the period of storage. That demonstrates these pathogens were likely not initially present in the meat used. Park et al. (2012) noticed that αTPN has strong antibacterial activities against *S. enteritidis* and *St. aureus,* in which the MIC and MBC values were 1.56, and 3.13 μL/mL respectively. They also found that LIN and αTPN also exhibited strong

antimicrobial activity against periodontopathic and cariogenic bacteria. They suggested that the concentration of BACs (LIN and αTPN) should be kept below 0.4 mg/ml for use as components of toothpaste or gargling solution. In another study Park et al. (2009) studied the antifungal activity of terpenes with the concentrations of 0.09 and 0.2 mg/ml for citral, 0.4 mg/ml for eugenol, 0.4 mg/ml for nerolidol, and 1 mg/ml for αTPN. In contrast to current findings, they found αTPN had the lowest antifungal activity among all the evaluated terpenes. Besides, It is known that the presence of free hydroxyl group is essential for antimicrobial activity of BACs and that compound could act as a protonophore (Ben Arfa et al., 2006), which is applicable for αTPN as it was noticed in our study.





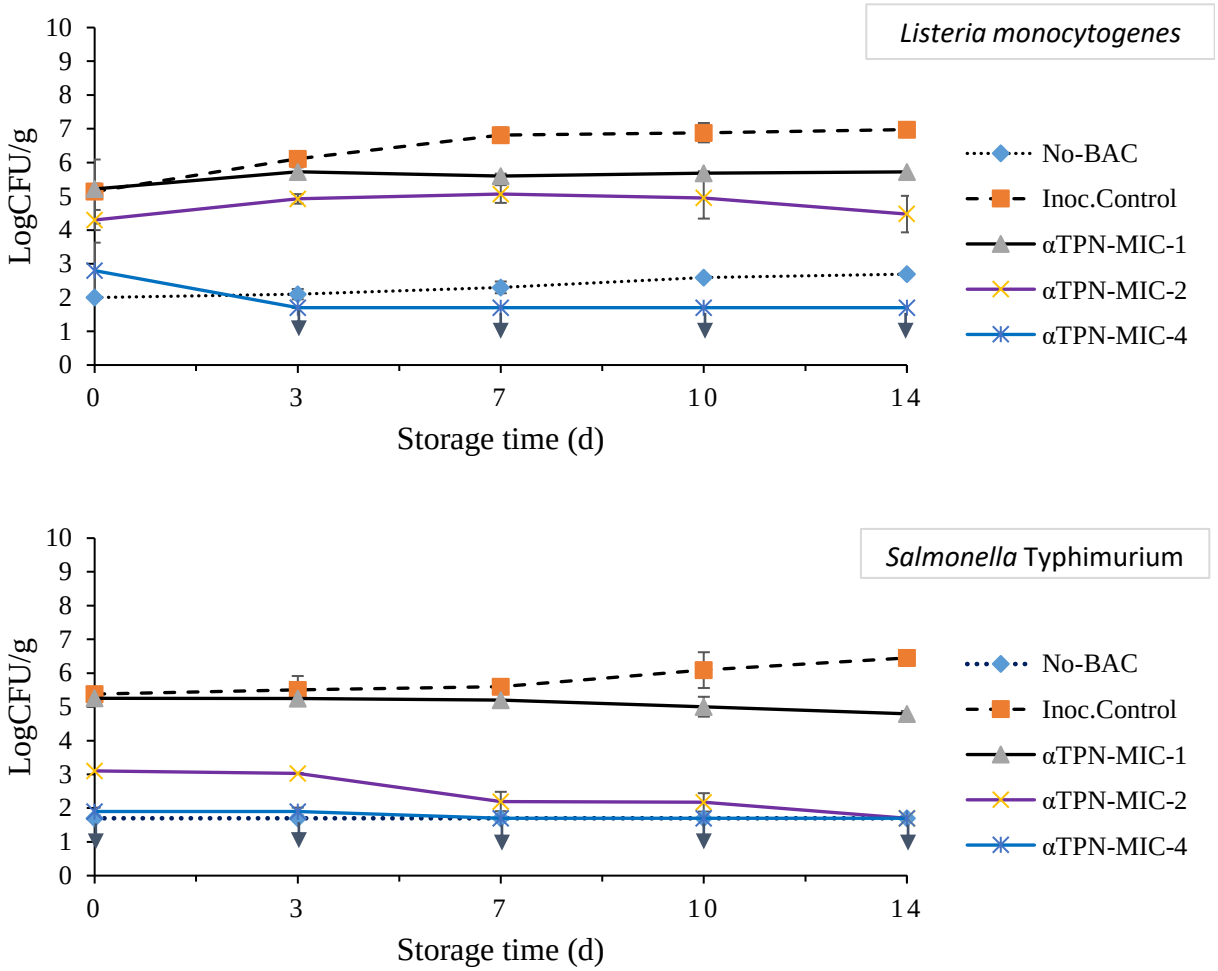
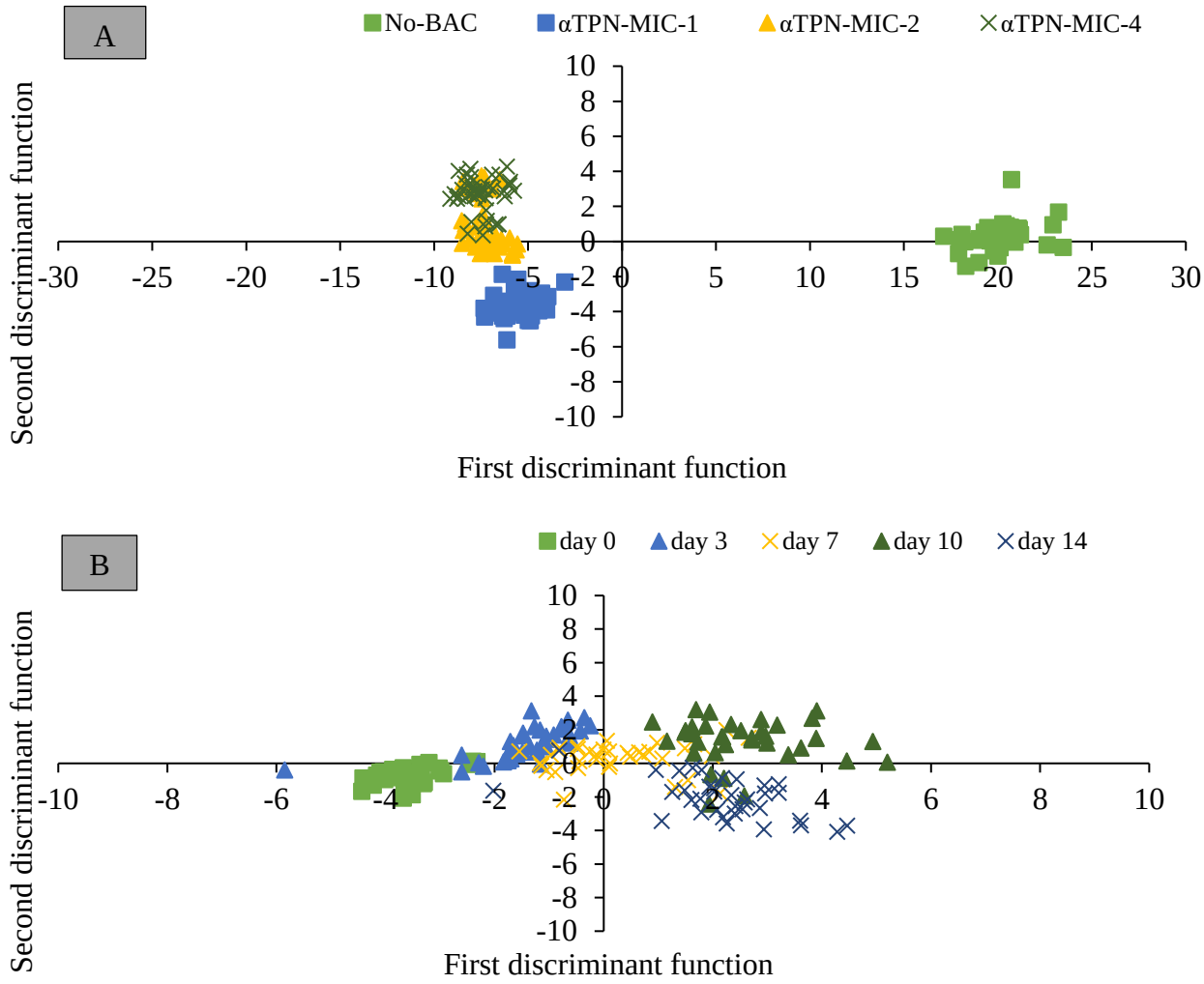


Figure 25: Effect of different concentrations of αTPN on aerobic mesophilic counts-AMCs, *Pseudomonas lundessis, Listeria monocytogenes*, and *Salmonella* Typhimurium in chicken meat stored up to 14 days at 4 °C. Arrow line (↓) represents the lower detection limit.

4.3.5. Electronic nose

The E-nose was applied to examine the group separation of meat sample treated with/without αTPN (Figure 26: A, B and C). Correct distinguish between untreated and treated meat based on the type of BAC and storage time was observed using E-nose. Comparing the different concentrations of αTPN the treated groups exhibit entirely different directions compared to untreated meat, and overlapping was only seen between MIC-2 and MIC-4 (Figure 26: A). Additionally, different concentration of αTPN shows the separation of treated meat on day 0 and day 14 of storage with a clear tendency toward second discriminant function. Whereas comparing the concentration of αTPN and the storage time the E-nose had shown overlapping between the treated meat with clear pattern recognition and a tendency toward second discriminant function compared to control that has remained at first discriminant function. It has been known that the

single compound that is primarily responsible for aroma of meat has not been identified yet, while an aroma profile (fingerprint) which is a combination of volatile compounds may be used as an indicator of spoilage or to differentiate between types of meat (Wojnowski et al., 2017). Generally, chicken meat becomes spoiled in a short time, and despite the storage at 4 °C in refrigerator condition, the shelf life of chicken meat is very short (almost 3 days) (Li et al., 2015a). In the current study, after opening the bags intense odour of eucalyptus globulus, pine oil and marjoram were noticed that could be pleased to some consumers. Alongside higher pH and *L** values with a reduction in TBARS and in the bacterial count was noticed with MIC-2 and MIC-4 indicating that the instrument can classify the chicken meat as either fresh or spoiled with rancid flavour.



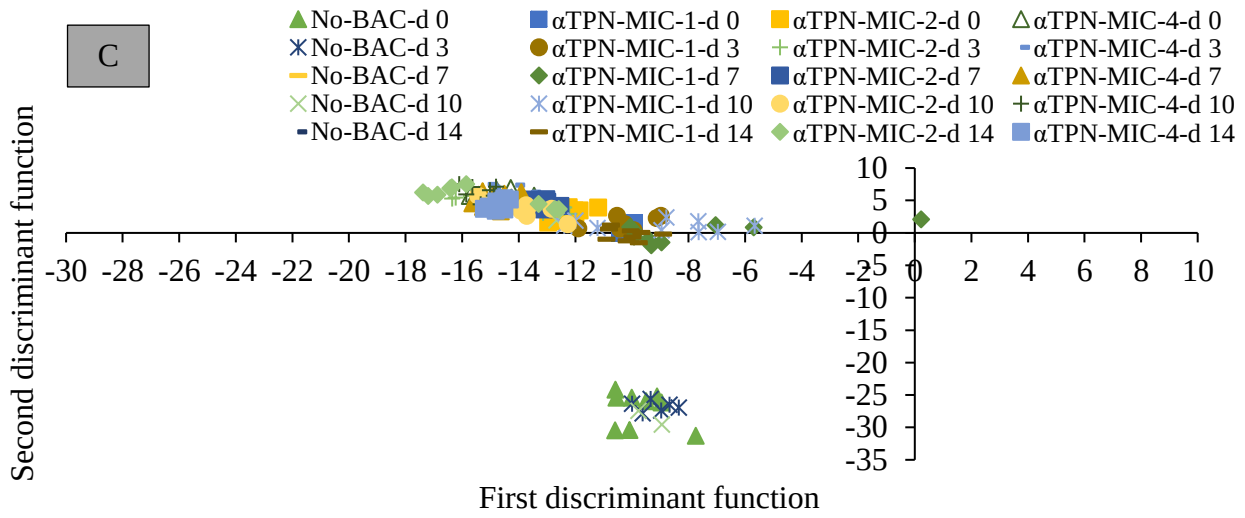


Figure 26: Efficacy of different concentrations of αTPN on smell detection by E-nose in chicken meat stored up to 14 days at 4 °C, Canonical discriminant analysis score plot of A: The separation based on the concentration of BACs, B: The separation based on storage days, and C: The separation based on storage days and concentration of BACs.

4.4. EFFECT OF ALLYL-ISOTHIOCYANATE ON CHICKEN MEAT QUALITY DURING REFRIGERATED CONDITIONS

4.4.1. Physicochemical properties

4.4.1.1. pH of meat

The pH values of the chicken meat treated with AITC showed significantly decreased rate ($P<0.05$). At day 14 of storage and compared to the first day of storage the different concentration of AITC showed significant differences in pH decline compared to control. The higher the concentration of AITC the greater the decline in pH value with slightly decreasing rate in control, the pH of MIC-4 and control was 6.04 and 6.02, respectively at the beginning of storage and decreased to 5.72 and 5.82 at day 14 (Table 11). Current finding not agreed with the results by (Chacon et al., 2006) who noticed no effect of AITC on meat pH reduction. It has been reported that the enzyme activity can determine the rate of pH decline in postmortem glycolysis, a pH reduction of 1 unit increases the rate of protein denaturation by 12 times (Mir et al., 2017).

4.4.1.2. Colour values

The colour of chicken meat shows significant changes during the 14 days storage period (Table 11). The increase rates were significant in all meat samples including control; however, the trend was most abundant in intensifying drifts in the lightness of sample contained a high level of AITC (MIC-2 and MIC-4) compared to control. The addition of a low level of AITC (MIC-1) compared to control was effective in maintaining the L^* value. Whereas at the end of the storage significant difference were seen in MIC-2 and MIC-4 which were 58.09 ± 0.72 and 62.82 ± 0.33, respectively compared to control that reached 52.34 ± 0.56 at day 14. In accordance to our results, Shin et al. (Shin et al., 2010) found that a release rate of 0.6 μg/h of AITC has no effect on chicken meat colour, whereas at 1.2 μg/h of AITC the surface of the chicken was discoloured. The a^* values in meat containing AITC was significantly decreased at the end of storage compared to an increasing trend in control. The a^* values increased amply to day 10 of storage, and started to decline more intensely at day 14, less decrease rates were in the meat treated with MIC-1 which was close to the initial values at the beginning of storage and did not vary significantly from untreated meat. In contrast, the MIC-4 has largely decreased the redness compared to other samples including control meat. The b^* value of all meat samples was significantly increased. Although the yellowness of meat samples declined until day 10, it increased sharply at day 14 of storage, except for MIC-4 which remained on the stable increase until the end of the storage period.

The increase *b** value was significant in meat treated with AITC MIC-2 and MIC-4. The meat containing MIC-4 resulted in double *b** value compared to its initial values at the beginning of the storage. Similar to yellowness, increasing trends of *C** colour intensity were detected in treated and untreated meat. The *C** values at day 0 for control, MIC-1, MIC-2, and MIC-4 was 4.84 ± 0.58, 5.16 ± 0.56, 5.03 ± 0.94, and 4.87 ± 0.40 increased to 6.62 ± 0.87, 6.73 ± 0.59, 7.54 ± 0.45, and 8.97 ± 0.53 respectively, at day14. The decreasing trend was noticed on day 10 for MIC-1 and MIC-2 whereas it increased again on day 14 of the storage, in control and MIC-4 the increasing trend was stable to the end of the storage. Additionally, an increasing rate of *h** was detected in all meat samples at the end of the storage time. The influence of AITC on *h** values was noticed by increasing the level of this BAC in meat, the higher the AITC concentration the higher the increase in *h** values.

4.4.1.3. Water holding capacity

The result from the influence of AITC on WHC in chicken meat (Table 11) shows that MIC-2 and MIC-4 slightly increased the amount of released water at the end of the storage. In contrast to MIC-1 and control that showed an increase in WHC, while no significant effect was noticed in separate meat groups throughout the storage period (p=0.074). Regarding the concentration of AITC only significant variation was witnessed between MIC-2 and MIC-4 compared to MIC-1 and control and day 10 and day14 (p=0.040). The decrease in meat pH that has been observed in this study by adding AITC to meat might associate the stability in WHC of muscle proteins (Shirzadegan and Falahpour 2014).

Table 11: The influence of different concentrations of AITC on pH, colour values and WHC of fresh chicken meat stored up to 14 days at 4 °C.

Parameters	Storage time (d)	Treatments			
		No-BAC	AITC-MIC-1	AITC-MIC-2	AITC-MIC-4
pH	0	6.02±0.01 ^{cA}	6.03±0.03 ^{cA}	6.05±0.04 ^{cA}	6.04±0.04 ^{dA}
	3	5.92±0.05 ^{bA}	5.99±0.01 ^{cAB}	6.01±0.01 ^{cB}	6.01±0.00 ^{dB}
	7	5.85±0.01 ^{abA}	5.89±0.01 ^{bB}	5.88±0.01 ^{bB}	5.91±0.01 ^{cC}
	10	5.83±0.03 ^{aB}	5.77±0.02 ^{aA}	5.78±0.02 ^{aA}	5.83±0.01 ^{bB}
	14	5.82±0.02 ^{aB}	5.76±0.03 ^{aAB}	5.73±0.02 ^{aA}	5.72±0.03 ^{aA}
L*	0	51.15±0.57 ^{aA}	52.40±1.91 ^{aA}	53.26±1.73 ^{aA}	53.83±2.28 ^{aA}
	3	54.13±0.17 ^{cA}	55.39±0.46 ^{bAB}	55.73±0.36 ^{bB}	60.81±1.42 ^{bC}
	7	53.02±1.61 ^{bcA}	56.32±0.51 ^{bcB}	56.60±0.89 ^{bcB}	62.40±0.84 ^{bC}
	10	52.98±0.43 ^{bcA}	58.12±0.39 ^{cB}	58.63±0.16 ^{dB}	62.44±0.59 ^{bC}
	14	52.34±0.56 ^{abA}	55.55±1.36 ^{bB}	58.09±0.72 ^{cdC}	62.82±0.33 ^{bD}
a*	0	2.77±0.32 ^{aA}	2.91±0.62 ^{aA}	2.81±0.68 ^{abA}	2.75±0.18 ^{bA}

	3	3.84±1.11 ^{bA}	3.32±0.32 ^{abA}	3.12±0.22 ^{abA}	3.09±0.12 ^{bA}
	7	3.98±0.65 ^{bC}	3.75±0.35 ^{bBC}	3.20±0.17 ^{bAB}	2.64±0.38 ^{bA}
	10	4.07±1.20 ^{bA}	3.20±0.49 ^{abA}	3.37±0.20 ^{bA}	2.94±0.25 ^{bA}
	14	3.25±0.80 ^{abB}	2.83±0.28 ^{aAB}	2.31±0.60 ^{aAB}	1.87±0.45 ^{aA}
b*	0	3.95±0.64 ^{aA}	4.22±0.57 ^{aA}	4.17±0.70 ^{abA}	4.01±0.46 ^{aA}
	3	4.18±0.88 ^{aA}	4.70±0.67 ^{aA}	4.65±0.51 ^{bA}	5.32±0.50 ^{bA}
	7	3.32±0.52 ^{aA}	3.73±0.50 ^{aA}	4.19±0.57 ^{abA}	5.69±0.65 ^{bB}
	10	3.15±0.90 ^{aA}	3.80±0.57 ^{aA}	3.37±0.20 ^{aA}	5.12±0.38 ^{bB}
	14	5.74±0.75 ^{bA}	6.09±0.72 ^{bAB}	7.17±0.34 ^{cB}	8.76±0.61 ^{cC}
C*	0	4.84±0.58 ^{aA}	5.16±0.56 ^{aA}	5.03±0.94 ^{aA}	4.87±0.40 ^{aA}
	3	5.72±1.17 ^{abA}	5.77±0.56 ^{abA}	5.61±0.38 ^{aA}	6.16±0.37 ^{bA}
	7	5.23±0.37 ^{abA}	5.30±0.52 ^{aA}	5.29±0.38 ^{aA}	6.29±0.51 ^{bB}
	10	5.27±0.79 ^{abAB}	4.97±0.75 ^{aAB}	4.76±0.29 ^{aA}	5.91±0.24 ^{bB}
	14	6.62±0.87 ^{cA}	6.73±0.59 ^{bA}	7.54±0.45 ^{bA}	8.97±0.53 ^{cB}
h*	0	0.96±0.09 ^{abA}	0.97±0.13 ^{bA}	0.98±0.05 ^{bA}	0.97±0.06 ^{aA}
	3	0.84±0.14 ^{abA}	0.95±0.09 ^{bAB}	0.98±0.07 ^{bAB}	1.04±0.06 ^{abB}
	7	0.70±0.14 ^{aA}	0.78±0.06 ^{aAB}	0.91±0.09 ^{bB}	1.13±0.09 ^{bC}
	10	0.67±0.26 ^{aA}	0.87±0.02 ^{abAB}	0.79±0.00 ^{aA}	1.05±0.07 ^{abB}
	14	1.06±0.10 ^{bA}	1.13±0.07 ^{cAB}	1.26±0.07 ^{cBC}	1.36±0.06 ^{cC}
WHC	0	1.71±0.12 ^{aA}	1.78±0.21 ^{aA}	1.87±0.20 ^{aA}	1.96±0.11 ^{aA}
	3	1.61±0.14 ^{aA}	1.75±0.30 ^{aA}	1.78±0.17 ^{aA}	1.84±0.21 ^{aA}
	7	1.72±0.26 ^{aA}	1.75±0.09 ^{aA}	2.05±0.07 ^{aA}	2.13±0.16 ^{aA}
	10	1.63±0.03 ^{aA}	1.72±0.13 ^{aA}	2.12±0.11 ^{aB}	2.21±0.22 ^{aB}
	14	1.34±0.13 ^{aA}	1.67±0.03 ^{aA}	2.15±0.22 ^{aB}	2.22±0.11 ^{aB}

$^{*\ a,b,c}$means in the same column with different superscript are significantly different regarding the days of storage; ^{A,B,C}means in the same row with different superscript are significantly different regarding the concentrations of BACs (P<0.05). Colour values: L^*, lightness; a^*, redness; b^*, yellowness; h^*, $\text{Tan}^{-1}\, b^*/a^*$; C*, $(a^{*2} + b^{*2})^{1/2}$; and WHC, water holding capacity.

4.4.2. Meat pigments (Metmyoglobin, deoxymyoglobin, and oxymyoglobin)

The current result shows that the MetMb and DeoMb in chicken meat treated with AITC decreased particularly in meat contain MIC-4 decreased MetMb from 62 to 61 % and DeoMb from 19 to 15 % on day 14 of storage, whereas the increase in OxyMb noticed from 19 to 24 % during the storage and less changes were observed in control (Figure 27). In the current study AITC especially a high level of AITC (MIC-2 and MIC-4) showed a significant increase in L^* 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. The meat containing and MIC-4 resulted in double b^* value compared to its initial value at the beginning of the storage. The current result confirmed previously as it has been explained the increased lightness in fresh meat attributed to the increased auto-oxidation of OxyMb and the formation of reactive oxygen species (Bekhit et al., 2007; Csehi et al., 2016). Discolouration of fresh meat during the treatment

resulted from the conformational changes that occur Mb, for instance, the denaturation of globin or the oxidation of ferrous ions (Toldrà et al., 2008).

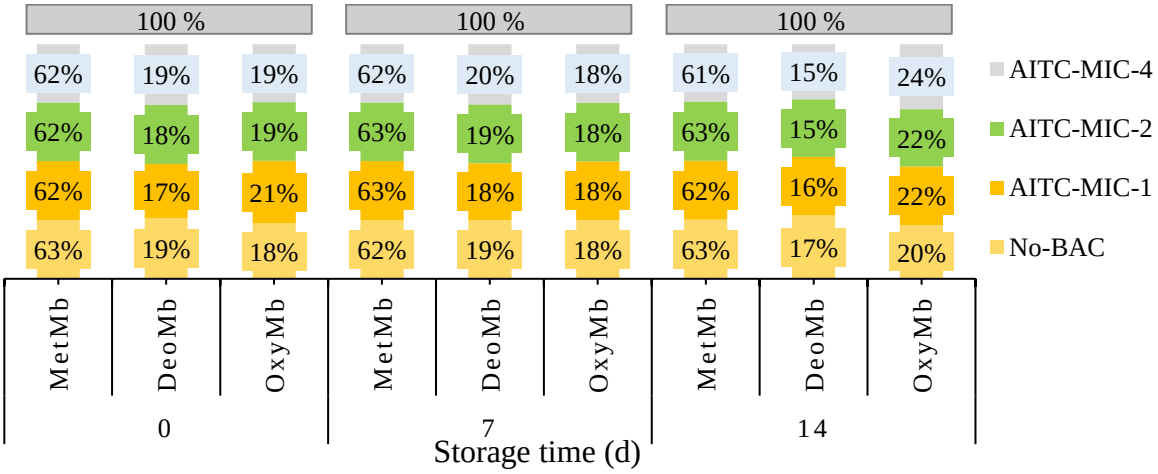


Figure 27: The influence of AITC on fresh chicken meat pigments {metmyoglobin (MetMb), deoxymyoglobin (DeoMb), and oxymyoglobin (OxyMb)} stored up to 14 days at 4 °C.

4.4.3. Thiobarbituric acid-reactive substances (TBARS)

In the current study, at the end of storage, the TBARS values were increased in all meat groups, and the higher rate seen in control (Figure 28). The meat containing AITC showed a reduction in TBARS values ($P<0.05$), the controlling of TBARS was more noticeable in meat treated with MIC-2 and MIC-4 with no significant variation. The TBARS value at day 0 were 0.099, 0.104, and 0.099 in MIC-1, MIC-2, and MIC-4, respectively increased to 0.103, 0.115, and 0.116 mg MDA/kg at day 14, compared a significant increase rate in control from 0.095 to 0.132 mg MDA/kg at the same period. This could be attributed to the strong potential antioxidant activity of this BACs in inhibiting the formation of secondary products of LO that may contribute to the off-flavour in stored meat products. However, some studies depicted that vacuum-packaged chicken meat also produces significantly decreased TBARS value as compared to aerobic storage (Arshad et al., 2019). Karwowska and Dolatowski, (2014) detected lower TBARS values compared to the control in cooked model meat products with mustard seed 12 days (mustard contains a high composition of AITC). In our study, the secondary LO product decrease could be due to the inhibitory effect of AITC. From a nutritional point of view, it is very important to reduce LO because MDA that is one of the main compounds determined as TBARS, the MDA has bifunctional aldehydic property, which gives it the potential to cross-link proteins, thereby reducing their functional capacity and reacts with nucleophilic amine groups such as lysine, arginine (Karwowska & Dolatowski, 2014).

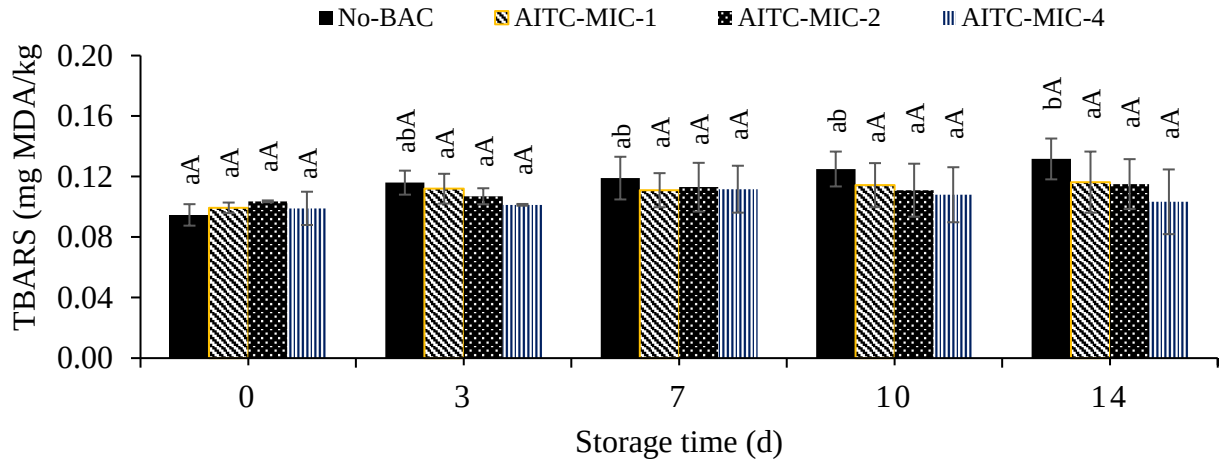


Figure 28: Effect of different concentrations of AITC on TBARS values of chicken meat stored up to 14 days at 4 °C. * [a,b,c]means with different superscript are significantly different regarding the storage days; [A,B,C]means with different superscript are significantly different regarding the concentrations of BACs (P<0.05)

4.4.4. Microbiological characteristics

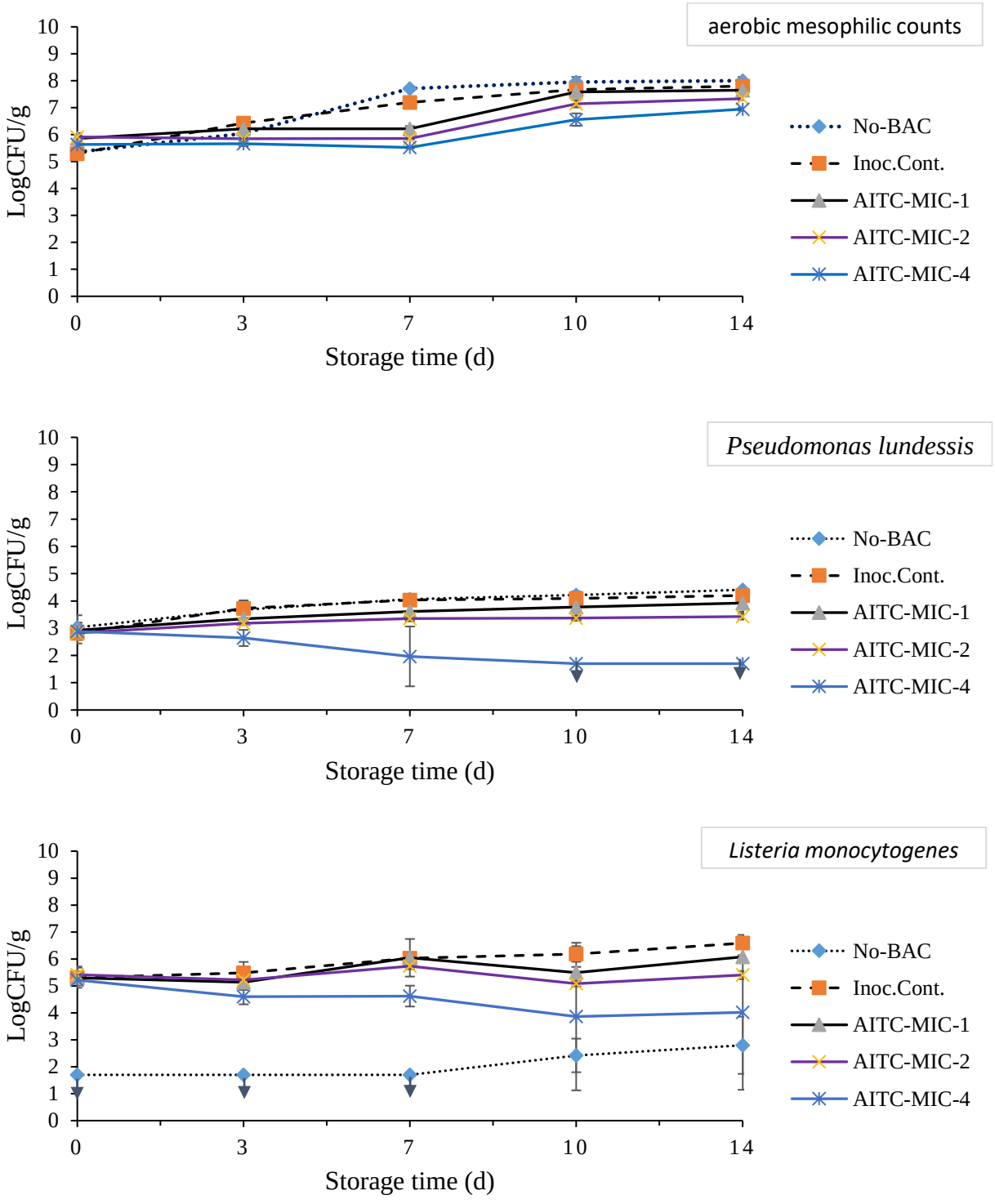
The result from the antimicrobial efficacy of AITC against aerobic mesophilic counts (AMCs), *L. monocytogenes*, *S.* Typhimurium, *P. lundensis* in chicken meat is presented in Figure 29. The initial AMCs population (day 0) in control and treated meat samples was less than was 5 log CFU/g and characteristic of acceptable quality chicken meat. At the end of storage, the highest cell count was observed in control and inoculated control which was 8 and 7.8 log CFU/g, respectively. AITC affected the inhibition of AMCs, as the concentration of αTPN increased, the surviving count of AMCs decreased. The least cell count of AMCs recorded in meat treated with AITC MIC-4, the AMCs count in meat treated with AITC MIC-1, MIC-2 and MIC-4 was 7.6, 7.3 and 6.9 log CFU/g, respectively at day 14 of storage (Figure 29).

The microbiological effect of AITC on cell counts of *L. monocytogenes* and *S.* Typhimurium and *P. lundensis* was also determined in chicken meat. During storage, the cell count of *P. lundensis* increased in all meat groups except for the sample that treated with MIC-4 of AITC which did not exhibit the growth at day 10 onward. At the end of the storage, the highest cell count of *P. lundensis* was seen in both controls and inoculated control that showed 6.4 and 4.2 log CFU/g. Whereas meat treated with MIC-1 and MIC-2 the 2.9 and 2.8 log CFU/g cell count of *P. lundensis* was detected at day 0 which increased gradually to reach 3.9 and 3.4 log CFU/g, respectively on day 14. While MIC-4 of AITC had initial cell counts of 2.8 and decrease to less than 1.7 log CFU/g at the end of the storage. On the other hand, cell counts of *L. monocytogenes* in all meat samples increased except AITC MIC-4 which reduced the cell count of *L. monocytogenes* from 5.2 to 4.0

log CFU/g at day 14 and compared to inoculated control MIC-4 caused 2.3 log reduction. In control samples, *L. monocytogenes* started to show the growth at day 10, while the inoculated control showed the highest counts and the population increased from 5.3 to 6.5 log CFU/g while meat treated with AITC MIC-1 and MIC-2 showed 6.1 and 5.4 log CFU/g respectively, at the end of storage. Regarding the *S.* Typhimurium, the highest cell numbers were observed in inoculated control that reached 6.4 log CFU/g at day 14, while the cell counts of *S.* Typhimurium were not detected in control samples. However, *S.* Typhimurium numbers were decreased in meat treated with AITC and the high concentration of AITC was more effective in eliminating the cell growth (Figure 29). The initial cell count of AITC MIC-1, MIC-2 and MIC-4 at the beginning of storage was 6.2, 6.2 and 5.7 log CFU/g, and reduced to 5.9, 5.7 and 5.4 log CFU/g, respectively at day 14. Based on the effect of AITC on AMCs, the cell count less than 7 log CFU/g was only observed with group of MIC-4, indicating that the meat shelf life was increased to 2-week storage time in this group. With respect to *P. lundessis* less than 7 log CFU/g was noticed in all meat group that contain AITC, implying the increased shelf life of meat to 14 days storage time. Regarding the safety of meat with the association to pathogens *L. monocytogenes* and *S. Typhimurium* further accurate presence/absence detection test needed. Ahn et al. (2001) monitored the AITC-treated cells in *L. monocytogenes* by transmission electron microscopy (TEM) and noticed that AITC reduced the intracellular levels of ATP and altered internal cell structures without causing cell wall damages. Moreover, Shin et al. (2010) applied AITC with a 0.6 and 1.2 μg/h on the fresh chicken breast with MAP and stored at 4 °C for up to 21 days. They observed that the maximum reduction was 0.77 $\log^{10}$CFU/g for *L. monocytogenes* and 1.3 $\log^{10}$CFU/g for *S.* Typhimurium. Outbreaks of food poisoning caused by *L. monocytogenes* and *Salmonella* have been associated with fresh produce. *L. monocytogenes* is considered as an environmental pathogen that can contaminate foods and cause listerial gastroenteritis (a mild, non-invasive illness) or listeriosis (a severe, invasive illness) (Gou et al., 2017).

In a study by Chacon et al. (2006) who microencapsulated AITC at 500, 750, or 1,000 ppm and added to sausage batters (17.59 % beef, 60.67 % pork, and 17.59 % pork fat) at 13 °C for 25 days. In their study AITC with 750 and 1000 ppm reduced *E. coli* O157:H7 by 6.5 $\log^{10}$ CFU/g after 21 and 16 days of processing. Meira et al. (2017) determined the efficiency of AITC and CARV in combination with phenolic acids (PA) ferulic acid (FA), o-coumaric acid (CA), and p-hydroxybenzoic acid (AHB) *in vitro* and in dry-fermented sausages. The MIC values of AITC, CARV, FA, CA and AHB for 5-strain mixture of *E. coli* O157:H7 were 0.25; 1.3; 5.12; 18.27; and 37 mM respectively. AITC showed synergism with all phenolic acids (FA, CA and AHB, while CARV only showed synergism with o-coumaric acid and the combination of AITC and CA had

the strongest synergistic effect which applied in dry-fermented sausages at 10- and 20-fold the FIC. They also found that the presence of *E. coli* O157 was reduced ≥5 log CFU/g after 21 days. Ward et al. (1998) also tested the antimicrobial potential of AITC-rich horseradish distillates contained on a filter paper disk which was packaged with a ground beef patty. They found that after 7 days at 12 °C in aerobic storage (4000 nl distillate/l) completely inhibited the growth of *St. aureus*, *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*.



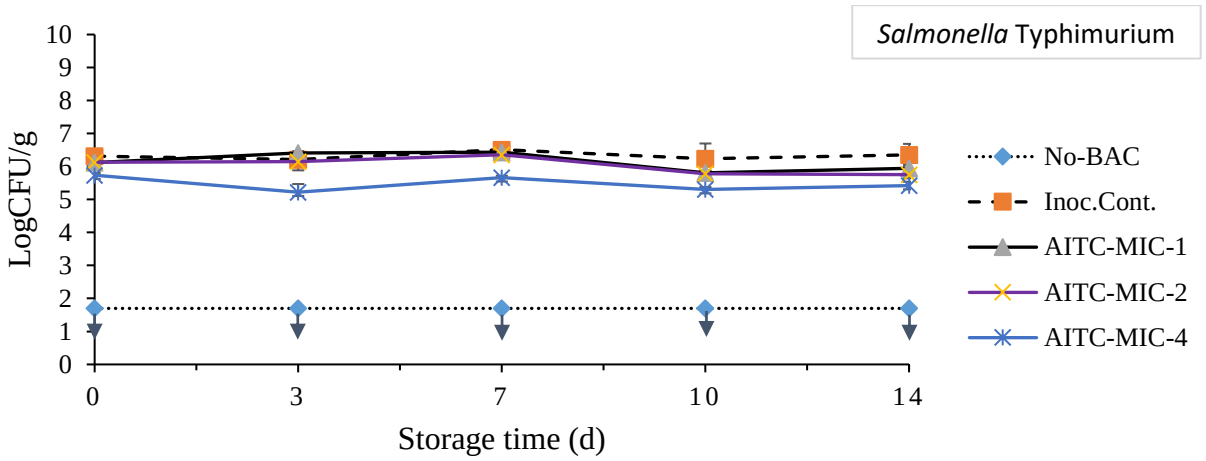


Figure 29: Effect of different concentrations of AITC on aerobic mesophilic counts-AMCs, *Pseudomonas lundessis, Listeria monocytogenes*, and *Salmonella* Typhimurium of chicken meat stored up to 14 days at 4 °C. Arrow line (↓) represents the lower detection limit.

4.4.5. Electronic nose

The result from the effect of AITC on meat and using E-nose to separate the meat groups based on smell detection are presented in Figure 30: A, B, and C. Comparing the different concertation of AITC the treated groups exhibit different direction toward 2nd discriminant function compared to untreated meat. Additionally, E-nose made proper differentiation of meat groups especially at 10 and 14 of storage. Whereas comparing the concentration of AITC and the days of storage the E-nose had shown overlapping between the control, meat treated with AITC MIC-1, and MIC-2, while MIC-4 on different days exhibited a clear tendency toward 1st discriminant function. In the current study, the meat contained MIC-1 produced minor smell after opening the bags, accordingly Chacon et al. (2006) found that beef samples treated with AITC concentrations lower than 1480 ppm only had a faint residual odour. The major challenge for ATIC application in food is its pungent odour, which can significantly affect the taste of foods, however, this sensory impact is also concentration-dependent (Chacon et al., 2006; Delaquis & Mazza, 1995; Kim et al., 2002). Alongside lower pH and *L**, *b**, *C** and *h** values, reduced *a** values with the reduction in TBARS compared to control, besides reduction in *L. monocytogenes*, *S.* Typhimurium, and *P. lundessis* count was noticed with MIC-2 and MIC-4 indicating that the instrument can classify the chicken meat as either fresh or spoiled with rancid flavour.

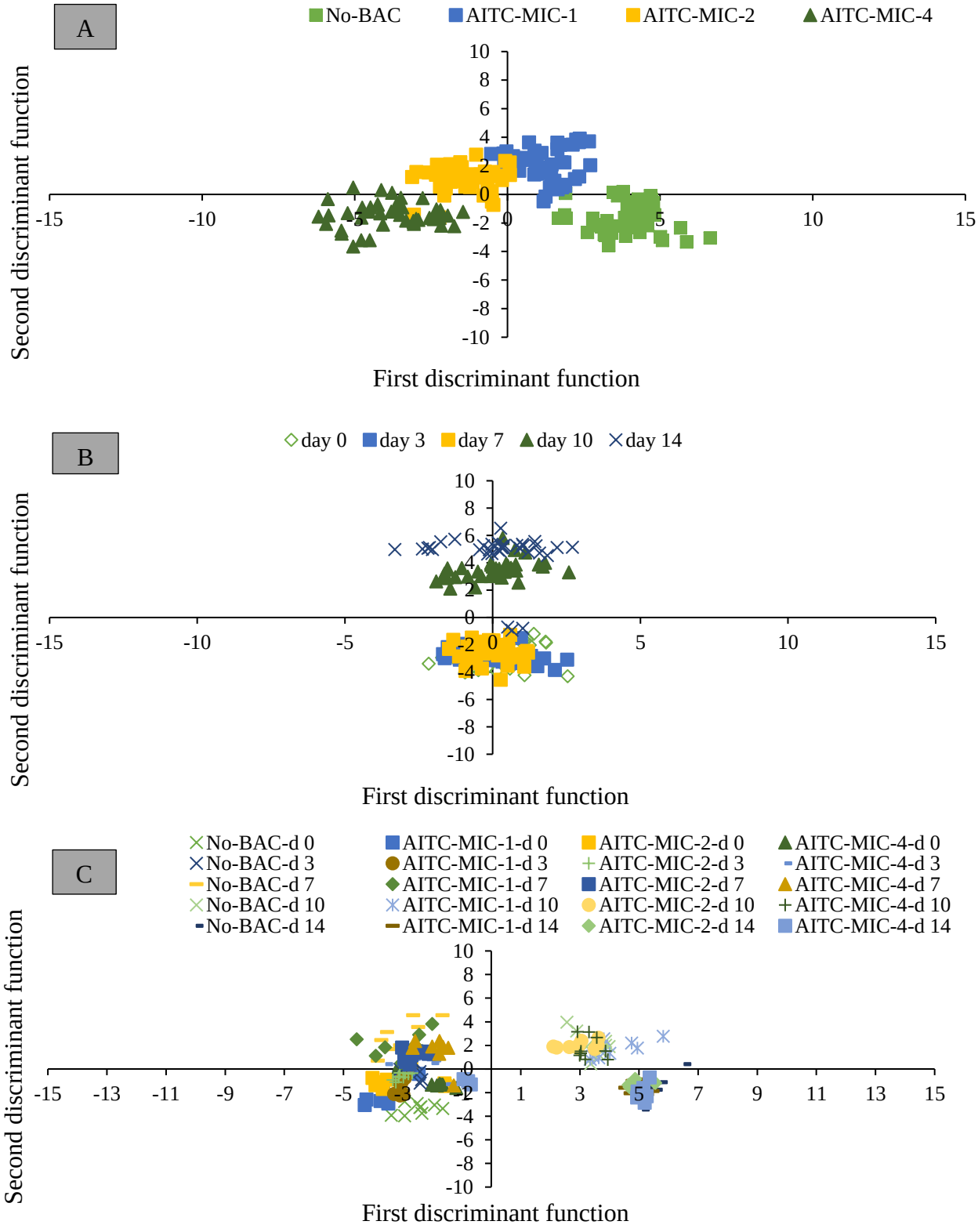


Figure 30: Efficacy of different concentrations of αTPN on smell detection by E-nose in chicken meat stored for up to days at 4 °C, Canonical discriminant analysis score plot of A: The separation based on the concentration of BACs, B: The separation based on storage days, and C: The separation based on storage days and concentration of BACs.

4.5. COMBINED EFFECT OF BIOACTIVE COMPOUNDS (A-TERPINEOL+ALLYL-ISOTHIOCYANATE) WITH HIGH HYDROSTATIC PRESSURE ON QUALITY ATTRIBUTES OF CHICKEN MEAT IN REFRIGERATED CONDITIONS

4.5.1. Physicochemical properties

4.5.1.1. pH of meat

The pH values of the chicken meat did not show significant changes at the beginning of storage in control and samples that treated with only αTPN and AITC, while the HHP treatment increased the pH values significantly, the higher the HHP level the higher the pH values (Table 12). At day 0 the highest pH values were recorded for HHP600, αTPN+HHP600, AITC+HHP600 and αTPN+AITC+HHP600 which was 6.21, 6.20, 6.21 and 6.20 respectively, while the pH values for HHP300, αTPN+HHP300, AITC+HHP300, and αTPN+AITC+HHP300 was 6.14, 6.13, 6.12 and 6.13 compared to lower values in control, αTPN, AITC, αTPN+AITC that was 6.01, 6.0, 6.01 and 6.01. At the end of storage the pH values in control and meat treated with AITC were decreased, in contrast to HHP600 treated samples that showed a significant increase to reach 6.28, and the αTPN was able to control the pH of αTPN+HHP600 and αTPN+AITC+HHP600 at 6.22 and 6.20, while the rest of the samples showed no significant variation in pH over the storage period.

4.5.1.2. Colour values

The colour values of chicken meat treated with BACs and HHP shows significant changes compare untreated meat during the 21 days storage period (Table 12). At the beginning of storage and comparing control (that showed L^* of 49.12 ± 0.31) various treated meat groups showed a significant increase in L^* value, and the highest value was recorded in meat treated with αTPN+HHP600 which was 82.79 ± 0.66. No significant difference was found between meat treated with αTPN, AITC, and αTPN+AITC that showed 51.83 ± 1.01, 51.35 ± 1.19, and 51.35 ± 1.19, respectively, between αTPN+HHP300, AITC+HHP300, and αTPN+AITC+HHP300 showed 75.66 ± 0.30, 73.31 ± 0.76, and 74.89 ± 0.25, respectively and between HHP600, αTPN+HHP600, AITC+HHP600, and αTPN+AITC+HHP600 that showed 80.20 ± 1.54, 82.79 ± 0.66, 81.04 ± 0.88, and 80.88 ± 0.4 respectively. The interaction was significant in L^* values between BACs and HHP (P=0.001)., whereas no significant interaction was noticed between BACs, HHP and storage days (P=0.199). At the end of storage L^* of almost all treated meat with BACs and HHP were increased compared to decreased values in control no significant differences were noticed. The initial of a^*

values of meat were ranged between 3.19 ± 0.66 in control and 2.17 ± 0.28 in meat treated with αTPN+HHP600. The difference was significant between control, AITC, αTPN+HHP600, AITC+HHP600, while the rest groups not showed significant variation. On day 21, the variation between meat groups increased and very significant variation noticed between control and αTPN+AITC+HHP600 that showed 3.85 ± 1.14 and 1.57 ± 0.11, respectively. At the end of storage, the *a** values were increased only in control and meat treated with αTPN+AITC, the rest of the meat groups decreased the redness without significant differences to the initial values. The *b** value of meat at the beginning of storage were ranged between the lowest value of 3.74 ± 0.49 in all of the control, αTPN, and AITC and the highest value of 9.52 ± 0.30 in meat treated with αTPN+AITC+HHP600. The initial *b** value was significantly varying between all groups except between HHP600, αTPN+HHP300, and αTPN+HHP600. At the end of the storage period, the *b** values were increased in all meat samples but the only significant increase was seen in meat treated with αTPN, AITC+HHP300, AITC+HHP600, αTPN+AITC+HHP300, and αTPN+AITC+HHP600, and the highest *b** values were 10.45 ± 0.20 that is found in meat treated with αTPN+AITC+HHP600. Indicating that αTPN alone was effective in increasing yellowness of meat, while AITC was more effective with HHP in increasing yellowness of meat. According to Jouki and Khazaei (2012), the increase in *L** values of meat attributed to the oxidation of haem pigments and the increase in *b** values value could be explained by changes in meat pigmentation during storage.

The *C** values in meat at the beginning of storage were lower in meat that treated with BACs without HHP treatment and in control, and these values were significantly lower than meat treated either with HHP alone or with BACs+HHP. The lowest initial *C** value was 4.69 ± 0.81 recorded in meat treated with AITC and the highest value 9.83 ± 0.34 in meat treated with αTPN+AITC+HHP600. Throughout the storage period, the *C** value increased in all meat samples, and the significant level of increase was only seen in meat treated with αTPN, AITC+HHP300, AITC+HHP600, αTPN+AITC+HHP300, and αTPN+AITC+HHP600. Our findings indicating that αTPN alone was more effective in increasing *C** value of meat, while AITC was more effective with HHP in increasing *C** value of meat. On the other hand, hue values were observed at a higher rate compared to control. The initial *h** value in control was 0.86 ± 0.14 which the lowest and the highest value 1.34 ± 0.02 that was noticed in meat treated with AITC+HHP600. Throughout the storage period, the increasing rate of *h** values was detected in all treated meat samples while in control the stability was noticed at the end of storage. At the end of storage, similar to *C** values the significant level of increase was only seen in meat treated with αTPN, AITC+HHP300, AITC+HHP600, αTPN+AITC+HHP300 and αTPN+AITC+HHP600.

Overall, studies on fresh and cured meat products have reported that high pressure causes changes in colour i.e. an increase in *L** and decrease in *a**, which might be produced from dramatic changes in the conformation and integrity of sarcoplasmic Mb protein (Oliveira et al., 2015)

4.5.1.3. Water holding capacity

The WHC results are presented in Table 12. The initial values of WHC were not significantly varied in control and meat treated with BACs alone while it was significantly varied compared to the meat treated with HHP. This variation between various treated groups was diminished at the end of storage. At the end of storage, the WHC was decreased in all treated meat samples while the significant decrease only seen in meat treated with AITC+HHP600 compared increased WHC in untreated meat from 1.88 ± 0.11 at day 0 to 1.50 ± 0.17 at day 21. Using the vacuum-packaging system can cause an increase in drip loss (Polawska et al., 2014). In accordance with our study, Marco et al. (2010) observed that HPP treatments of sea bream muscle at 300 and 400 MPa resulted in reduced WHC. The WHC in control at day 21 was observed at minimum range compared to the treated sample and the highest value was 2.53 ± 0.18 that is observed in meat treated with αTPN+HHP600. The decrease in WHC with an increase in HHP could be as a result of protein denaturation, protein–protein interaction, that cause the compact of structures being formed (Ezeh et al., 2018).

4.5.1.4. Water activity

Water activity (a_w) results are presented in Table 12. Raw chicken meat showed fluctuation in a_w with statistical variation only between meat treated with BACs and HHP. The a_w in meat treated with BACs and in control was decreased in 21 days storage, while in HHP treated meat it remained stable. In general, low a_w protects cells against high pressure, but pressure can injury the microorganisms and making them more sensitive to low a_w (Garriga et al., 2004). In this study the high level of HHP caused a significant reduction in microorganisms this could be due to the high a_w of meat during the storage. In accordance with our result, Porto-Fett et al. (2010) stated that a reduction of 1.6 to ≥ 5 log CFU/g can be achieved in salami by HHP at 483-600 MPa depending on the a_w of the product and the treatment strength.

Table 12: The influence of combined BACs (αTPN and AITC) with HHP on pH, colour values, WHC and water activity of fresh chicken meat stored up to 21 days at 4 °C

Parameters	Storage time (d)	Treatments											
		No-BAC	αTPN	AITC	αTPN+AITC	HHP300	HHP600	αTPN+HHP300	αTPN+HHP600	AITC+HHP300	AITC+HHP600	αTPN+AITC+HHP300	αTPN+AITC+HHP600
pH	0	6.01±0.03 aA	6.00±0.02 aA	6.01±0.01 aA	6.01±0.01 bA	6.14±0.01 bB	6.21±0.02 aC	6.13±0.01 aB	6.20±0.01 abC	6.12±0.01 aB	6.21±0.02 aC	6.13±0.01 bcB	6.20±0.01 bC
	5	6.00±0.02 aA	5.99±0.01 aA	5.98±0.02 aA	5.98±0.01 aA	6.06±0.02 aB	6.21±0.03 aE	6.13±0.02 aCD	6.19±0.02 aE	6.13±0.01 aC	6.19±0.03 aD	6.06±0.01 aB	6.13±0.02 aC
	10	5.99±0.02 aAB	6.00±0.02 aAB	5.98±0.04 aA	6.03±0.01 bcB	6.13±0.01 bC	6.24±0.02 abD	6.14±0.01 aC	6.20±0.01 abD	6.11±0.01 aC	6.20±0.00 aD	6.12±0.01 bC	6.14±0.00 aC
	15	5.99±0.04 aAB	6.04±0.01 bAB	5.98±0.06 aA	6.05±0.01 cBC	6.16±0.02 bDEF	6.25±0.02 abG	6.16±0.01 aDE	6.23±0.0 cFG	6.12±0.01 aCD	6.22±0.01 aEFG	6.16±0.01 cDE	6.22±0.02 bEFG
	21	5.99±0.08 aA	6.04±0.01 bAB	5.97±0.08 aA	6.05±0.02 cABC	6.13±0.02 bBCDE	6.28±0.01 bF	6.15±0.02 aCDE	6.22±0.01 bcEF	6.11±0.02 aBCD	6.20±0.01 aDEF	6.14±0.02 bcCDE	6.20±0.01 bDEF
L*	0	49.12±0.31 aA	51.83±1.01 aB	51.35±1.19 aB	51.87±0.52 aB	70.21±0.65 aC	80.20±1.54 aF	75.66±0.30 aE	82.79±0.66 aG	73.31±0.76 aD	81.04±0.88 aFG	74.89±0.25 abDE	80.88±0.43 aF
	5	49.66±1.25 aA	52.51±0.62 aB	51.35±0.51 aAB	51.99±0.60 aB	71.40±1.54 aC	80.12±1.35 aF	75.96±0.54 aE	82.87±0.97 aG	73.54±0.52 aD	82.02±0.62 abFG	75.44±0.49 bDE	81.47±0.81 aFG
	10	49.22±1.48 aA	52.83±0.43 aB	52.05±0.66 aB	52.86±0.49 abB	70.82±0.78 aC	80.53±1.18 aF	75.65±0.58 aE	82.73±0.77 aG	73.54±0.78 aD	81.91±0.39 abFG	75.44±0.49 bE	81.03±0.96 aFG
	15	49.71±1.23 aA	52.53±0.86 aB	52.24±0.26 aB	53.33±0.34 bB	71.13±0.84 aC	80.60±0.67 aF	76.00±0.43 aE	82.31±0.95 aG	73.78±0.34 aD	82.22±0.42 bG	74.45±0.47 aDE	81.11±1.07 aFG
	21	48.72±0.43 aA	53.14±0.66 aB	52.61±0.50 aB	53.04±1.02 abB	70.65±0.18 aC	80.32±0.39 aG	76.55±0.80 aF	83.22±0.44 aH	73.14±1.18 aD	81.41±0.22 abG	75.05±0.38 abE	81.23±0.36 aG
a*	0	3.19±0.66 aB	2.81±0.62 aAB	3.01±0.40 aB	2.82±0.22 aAB	2.76±0.26 abAB	2.56±0.37 aAB	2.82±0.28 aAB	2.17±0.28 aA	2.94±0.17 bAB	2.18±0.10 bA	2.90±0.06 bAB	2.44±0.30 cAB
	5	2.98±0.30 aB	2.73±0.38 aAB	2.97±0.25 aB	2.69±0.44 aAB	2.90±0.59 abBC	2.42±0.56 aAB	2.59±0.59 aAB	2.14±0.33 aAB	2.47±0.34 abAB	2.08±0.12 bAB	2.90±0.32 bBC	2.03±0.15 bA
	10	3.17±0.90 aCD	2.73±0.49 aABCD	3.39±0.45 aD	3.26±0.76 aCD	3.00±0.33 bBCD	2.34±0.37 aABC	2.46±0.36 aABCD	2.03±0.21 aAB	2.47±0.42 abABCD	1.83±0.21 abA	2.87±0.23 bBCD	1.78±0.16 abA
	15	3.29±0.97 aCDE	2.68±0.82 aA-E	3.47±0.30 aE	3.44±0.53 aDE	2.80±0.20 abB-E	2.23±0.44 aABCD	2.29±0.87 aA-E	2.17±0.08 aABC	2.09±0.82 abABC	1.78±0.40 abAB	2.34±0.19 aA-E	1.57±0.11 aA
	21	3.85±1.14 aF	2.72±0.44 aCDE	2.93±0.24 aDE	3.29±0.38 aEF	2.26±0.24 aABCD	2.06±0.06 aABCD	2.46±0.31 aBCDE	1.94±0.16 aABC	2.00±0.42 aABC	1.65±0.07 aAB	2.26±0.15 aABCD	1.54±0.05 aA
b*	0	3.74±0.49 aA	3.74±0.64 aA	3.74±0.41 aA	3.97±0.50 aA	7.42±0.57 aB	9.02±0.18 aDE	8.91±0.19 aDE	8.79±0.15 aDE	7.49±0.30 aBC	9.36±0.52 aE	8.39±0.49 aCD	9.52±0.30 aE
	5	4.40±0.49 aA	4.67±0.34 abA	3.73±0.28 aA	4.40±0.36 abA	7.62±0.38 aB	9.42±1.16 aCD	9.57±0.80 aD	9.20±0.1.04 aCD	8.12±0.58 abBC	10.04±0.08 bcD	9.30±0.32 bCD	9.91±0.27 abD
	10	3.86±0.89 aA	5.66±0.38 bcB	3.86±0.62 aA	5.22±0.13 bcB	7.81±0.72 aC	9.75±0.76 aEF	9.83±0.56 aEF	9.44±0.09 aDEF	8.37±0.70 abCD	10.35±0.13 cEF	9.24±0.55 bDE	10.49±0.50 cF
	15	3.65±0.40 aA	6.36±0.44 cB	4.49±0.30 aA	5.55±0.91 cB	8.01±0.38 aC	9.98±0.12 aD	9.88±0.54 aD	9.30±0.27 aD	9.37±0.24 cD	9.80±0.26 abD	10.09±0.15 cD	10.09±0.15 abcD

	21	4.49±0.80 aA	6.55±0.84 cB	3.99±0.69 aA	4.85±0.42 abcA	8.13±0.18 aC	9.99±0.22 aEF	9.90±0.38 aEF	9.32±0.06 aDE	8.81±0.40 bcCD	10.06±0.14 bcEF	10.02±0.21 cEF	10.45±0.20 bcF
C*	0	4.95±0.46 aA	4.69±0.81 aA	4.81±0.46 abA	4.87±0.47 aA	7.91±0.60 aB	9.38±0.19 aC	9.35±0.17 aC	9.05±0.16 aC	8.05±0.21 aB	9.61±0.50 aC	8.87±0.46 aBC	9.83±0.34 aC
	5	5.31±0.52 aA	5.41±0.46 abA	4.77±0.23 aA	5.16±0.48 abA	8.17±0.21 aB	9.75±1.00 aD	9.93±0.71 aD	9.45±1.02 aCD	8.49±0.60 abBC	10.26±0.06 bcD	9.74±0.21 bcD	10.11±0.28 abD
	10	5.10±0.50 aA	6.30±0.44 bcB	5.15±0.59 abA	6.18±0.45 bcAB	8.36±0.73 aC	10.03±0.65 aE	10.14±0.51 aE	9.65±0.11 aDE	8.74±0.63 abCD	10.52±0.12 cE	9.68±0.56 bDE	10.64±0.51 cE
	15	4.92±0.94 aA	6.93±0.63 cC	5.67±0.39 bAB	6.54±0.90 cBC	8.49±0.34 aD	10.23±0.18 aE	10.17±0.61 aE	9.55±0.28 aDE	9.62±0.23 cE	9.96±0.28 abE	10.35±0.14 cE	10.21±0.16 abE
	21	6.01±0.63 aB	7.10±0.84 cC	4.96±0.59 abA	5.87±0.42 abcB	8.44±0.17 aD	10.20±0.22 aFG	10.20±0.42 aFG	9.52±0.09 aFG	9.04±0.33 bcDE	10.20±0.15 bcFG	10.27±0.21 bcFG	10.56±0.20 cG
h*	0	0.86±0.14 aA	0.92±0.08 aA	0.89±0.07 aA	0.94±0.06 aA	1.21±0.02 abBC	1.32±0.04 aBC	1.26±0.03 aBC	1.32±0.03 aC	1.19±0.03 aB	1.34±0.02 aC	1.23±0.02 aBC	1.32±0.03 aBC
	5	0.97±0.05 aAB	1.04±0.04 abB	0.89±0.06 aA	1.02±0.06 aB	1.20±0.08 aC	1.34±0.08 aCD	1.30±0.07 aCD	1.34±0.04 aD	1.27±0.04 abCD	1.36±0.01 abD	1.26±0.04 aCD	1.36±0.01 bD
	10	0.87±0.23 aA	1.12±0.07 bBC	0.84±0.09 aA	1.01±0.10 aAB	1.20±0.04 aCD	1.35±0.05 aDE	1.32±0.04 aDE	1.35±0.02 aDE	1.28±0.06 abCDE	1.39±0.02 bE	1.26±0.02 aCDE	1.40±0.01 cE
	15	0.84±0.09 aA	1.17±0.10 bC	0.91±0.03 aAB	1.01±0.09 aB	1.23±0.03 abCD	1.34±0.04 aDE	1.34±0.08 aDE	1.34±0.00 aDE	1.35±0.09 bDE	1.39±0.04 bE	1.34±0.02 bDE	1.41±0.01 cE
	21	0.86±0.21 aA	1.17±0.06 bB	0.92±0.09 aA	0.97±0.06 aA	1.29±0.03 bBC	1.36±0.01 aC	1.32±0.03 aBC	1.36±0.02 aC	1.34±0.05 bC	1.40±0.01 bC	1.34±0.01 bC	1.42±0.00 cC
WHC	0	1.88±0.11 bA-D	1.80±0.04 aAB	1.90±0.07 aA-E	1.79±0.17 aAB	1.69±0.03 aA	1.82±0.16 aABC	2.37±0.16 aF	2.26±0.24 aEF	2.21±0.03 aDEF	2.08±0.00 aB-F	2.27±0.07 aEF	2.18±0.19 aC-F
	5	1.82±0.13 bA	1.94±0.04 aA	2.06±0.02 aABC	2.26±0.04 bCD	1.98±0.10 aAB	2.58±0.11 bEF	2.40±0.13 aDEF	2.61±0.08 aF	2.20±0.05 aBCD	2.26±0.10 abCD	2.60±0.12 aEF	2.35±0.01 aDE
	10	1.38±0.03 aA	2.19±0.13 aCD	1.91±0.12 aBC	2.34±0.03 bD	1.72±0.13 aB	2.21±0.02 abD	2.16±0.06 aCD	2.63±0.18 aE	2.29±0.12 aD	2.36±0.0 bcDE	2.34±0.07 aDE	2.21±0.02 aD
	15	1.32±0.08 aA	2.11±0.13 aCD	1.92±0.31 aBC	2.23±0.03 bCDE	1.73±0.16 aB	2.08±0.05 abBCD	2.52±0.05 aE	2.49±0.06 aE	2.01±0.00 aBC	2.52±0.11 cE	2.44±0.12 aDE	2.17±0.13 aCDE
	21	1.50±0.17 aA	2.23±0.00 aAB	1.96±0.42 aAB	2.23±0.18 bAB	1.75±0.26 aAB	2.09±0.48 abAB	2.49±0.29 aAB	2.53±0.18 aB	2.22±0.45 aAB	2.30±0.03 bAB	2.43±0.44 aAB	2.29±0.07 aAB
Water activity	0	0.988±0.001 bB	0.987±0.003 bB	0.986±0.005 bB	0.988±0.000 bB	0.988±0.007 bB	0.984±0.004 bB	0.985±0.005 aB	0.981±0.001 aB	0.986±0.007 aB	0.987±0.001 aB	0.987±0.003 aB	0.984±0.004 aB
	5	0.997±0.000 bC	0.980±0.012 bB	0.985±0.004 b	0.985±0.003 bBB	0.988±0.007 bB	0.989±0.000 bB	0.985±0.006 aB	0.986±0.004 aB	0.983±0.004 aB	0.997±0.003 aC	0.994±0.006 aC	0.993±0.006 aC
	10	0.992±0.007 bC	0.986±0.004 bB	0.985±0.005 bB	0.985±0.007 bB	0.977±0.007 aA	0.986±0.000 bB	0.985±0.005 aB	0.986±0.004 aB	0.994±0.004 aB	0.987±0.003 aB	0.987±0.004 aB	0.985±0.005 aB
	15	0.975±0.006 aA	0.976±0.007 aA	0.985±0.006 bB	0.992±0.003 bC	0.975±0.007 aA	0.985±0.000 bB	0.988±0.007 aB	0.982±0.002 aB	0.985±0.006 aB	0.985±0.004 aB	0.984±0.005 aB	0.985±0.006 aB
	21	0.971±0.000 aA	0.977±0.003 aA	0.976±0.007aA	0.977±0.003 aA	0.971±0.001 aAA	0.977±0.002 a	0.986±0.007 aB	0.981±0.000 aB	0.984±0.006 aB	0.980±0.00 aB	0.986±0.004 aB	0.986±0.004 aB

* *L**, *a**, *b**, *C**, *h** – colour values; [a,b,c]means in the same column with different superscript are significantly different regarding the days of storage; [A,B,C]means in the same row with different superscript are significantly different regarding the treatment (BACs and HHP) (P<0.05). Colour values: L^*, lightness; a^*, redness; b^*, yellowness; h^*, $\mathrm{Tan}^{-1}\, b^*/a^*$; C*, $(a^{*2} + b^{*2})^{1/2}$; and WHC, water holding capacity.

4.5.2. Meat pigments (Metmyoglobin, deoxymyoglobin, and oxymyoglobin)

The result from the of αTPN and AITC with HHP on the profile of myoglobin pigments in chicken presented in Figure 31. The result shows that the percentage of MetMb and DeoMb in meat contain αTPN and AITC decreased especially in meat contain αTPN+AITC that declined from 65 to 63 % in MetMb 22 to 18 % in DeoMb on the end of storage, while OxyMb increased in meat contain BACs. Whereas the control and meat treated with BAC+HHP exhibited a decrease in MetMb and OxyMb and an increase in DeoMb for 21 days storage. This result is in agreement with the finding by Bak et al. (2019) who observed that a higher HHP level decreases the rate of autoxidation of OxyMband and increases MetMb. In beef, in accordance with our findings, it has been demonstrated by Jung et al. (2003) pressure values higher than 325 MPa cause discolouration of beef meat related to an increase in L^*, a decrease in a^*, and an increase in the content of MetMb form. The presence of natural antioxidants in some meat products seems to protect Mb against oxidation by HHP and prevents meat discolouration, as has been reported for cooked ham, salami, dry-cured ham, and ripened sausages (Toldrà et al., 2008). It has been reported that HHP treatments induced colour modifications in fresh meat due to conformational changes in Mb, such as globin denaturation, heme displacement or release, ferrous ion oxidation, and meat discolouration (Toldrà et al., 2008). The evolution of MetMb with HHP and storage time shows that up to around 300 to 600 MPa could disturb the enzymatic systems lead to progressive accumulation of MetMb content in meat (Jung et al., 2003).

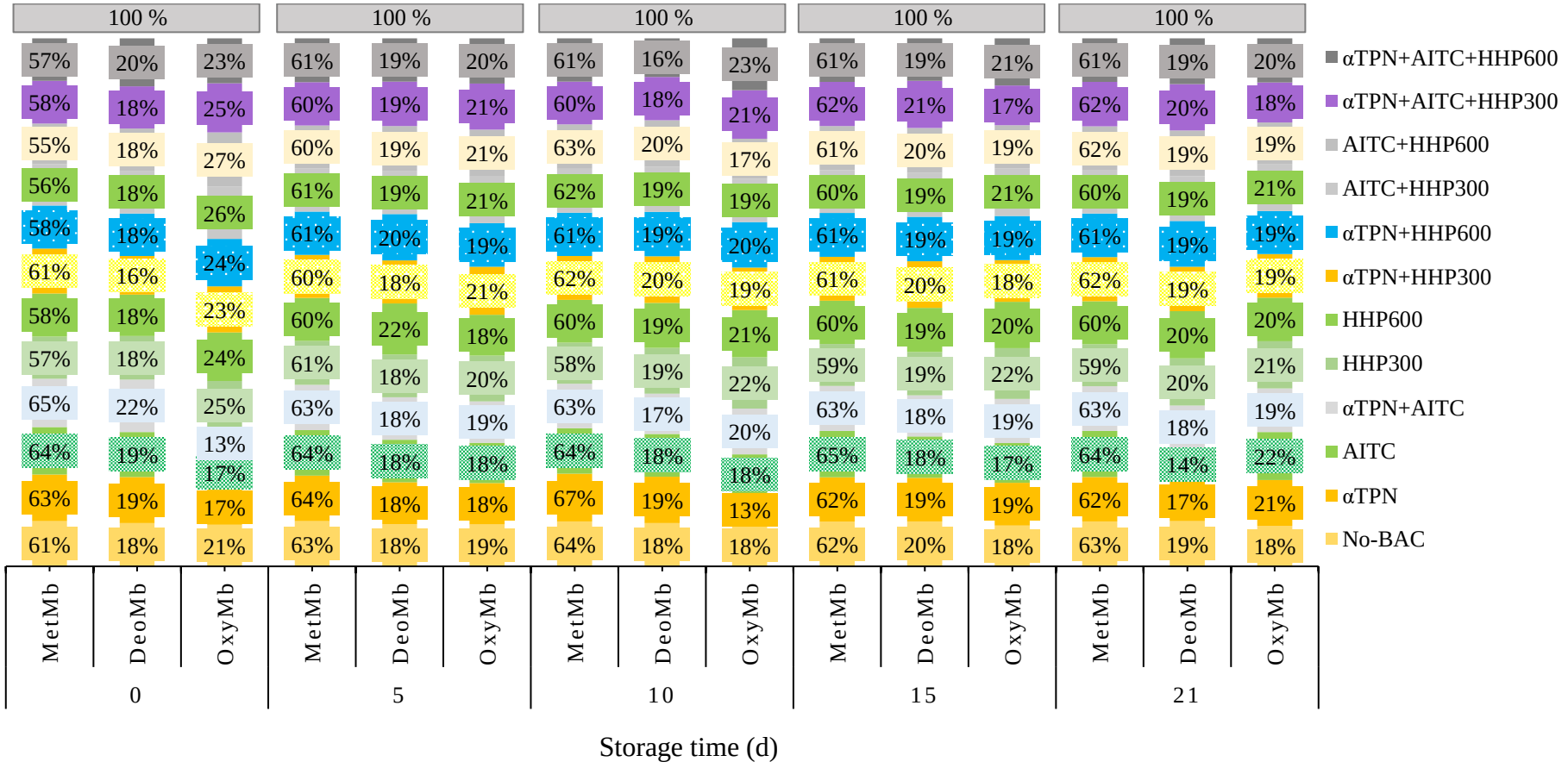


Figure 31: The influence of combined BACs (αTPN and AITC) with HHP on fresh chicken meat pigments {metmyoglobin (MetMb), deoxymyoglobin (DeoMb), and oxymyoglobin (OxyMb)} stored up to 21 days at 4 °C.

4.5.3. Thiobarbituric acid-reactive substances (TBARS)

In this study, at the beginning of storage, the differences in TBARS values between the meat groups were noticeable compared to control, while this variation was significantly increased on the last day of storage (Figure 32). At the end of storage, the highest TBARS values were observed in meat treated with HHP600 that was 0.207 mg MDA/kg, followed by αTPN+HHP600, AITC+HHP600 and control samples that showed TBARS of 0.201, 0.200 and 0.199 mg MDA/kg. The rest of the meat sample showed less than 0.193 mg MDA/kg. Overall, 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. This could be attributed to the strong potential antioxidant activity of combined αTPN+AITC in inhibiting the formation of secondary products of LO that may contribute to the off-flavour in stored meat products. It has been investigated that the exposure of chicken breast muscle to pressure treatment at 800 MPa for 10 min at subsequent storage at 5 °C was for 2 weeks leads to enhanced LO to the same extent as the heat treatment (80 °C for 10 min). While below 500 MPa showed no indication of rancidity (Orlien et al., 2000). It has been reported that HHP in meat and meat products can trigger LO reactions whereas the mechanisms by which HHP changes the thermodynamic equilibrium of chemical reactions and induces LO are not fully understood. However, the general suggestion for explaining this mechanism is could be: haemoproteins and membrane disruption that leads to increase in releasing and accessibility for iron, the release of iron can promote LO (Bajovic et al., 2012; Medina-Meza et al., 2014). Moreover, the enzymatic, hydrolytic and photooxidation mechanisms, autoxidation is recognized as a major oxidative fat degradation mechanism in foods (Bajovic et al., 2012)

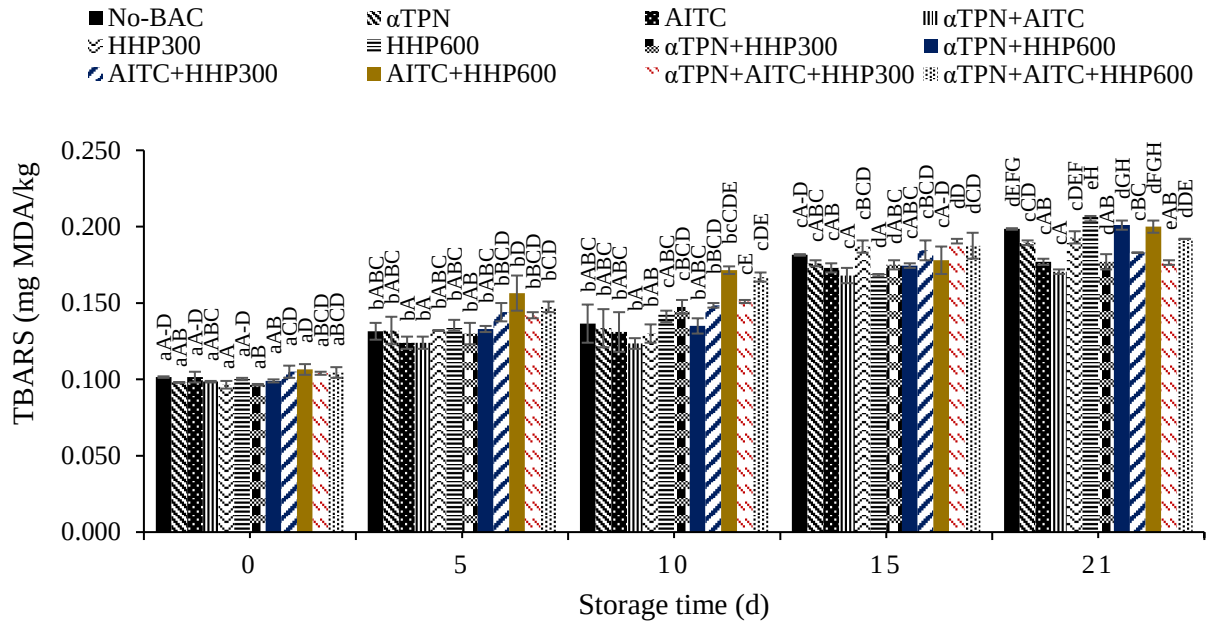


Figure 32: The influence of combined BACs (αTPN and AITC) with HHP on TBARS of fresh chicken meat stored up to 21 days at 4 °C. * [a,b,c]means with different superscript are significantly different regarding the storage days; [A,B,C]means with different superscript are significantly different regarding the treatment (P<0.05)

4.5.4. Microbiological characteristics

The result from the antimicrobial efficacy of BACs (αTPN and AITC) with HHP against aerobic mesophilic counts (AMCs), *L. monocytogenes*, *S.* Typhimurium, *P. lundensis* in chicken meat is presented in Figure 33. The initial AMCs population (day 0) in control and treated meat samples was less than 5 log CFU/g, which is acceptable quality characteristic of fresh chicken meat. The initial AMCs population were ranged between the 2.9 and 6.9 log CFU/g in meat treated with αTPN+AITC+HHP600 and in inoculated control, respectively. At the end of storage, the AMCs population in inoculated control remained higher than meat treated with BACs and/or HHP, while the highest cell count was observed in control with 8.2 log CFU/g followed by inoculated control with 7.9 log CFU/g. The meat treated with either with BACs and/or HHP exhibited less AMCs count, particularly the higher level of HHP600 was more effective in limiting the growth of AMCs. 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, followed by αTPN+HHP600, AITC+HHP600 and HHP600 that exhibited 5.8, 5.4. 5.1 log reduction in AMCs (Figure 33-A). These results indicate that the combination of BACs with a higher level of pressure

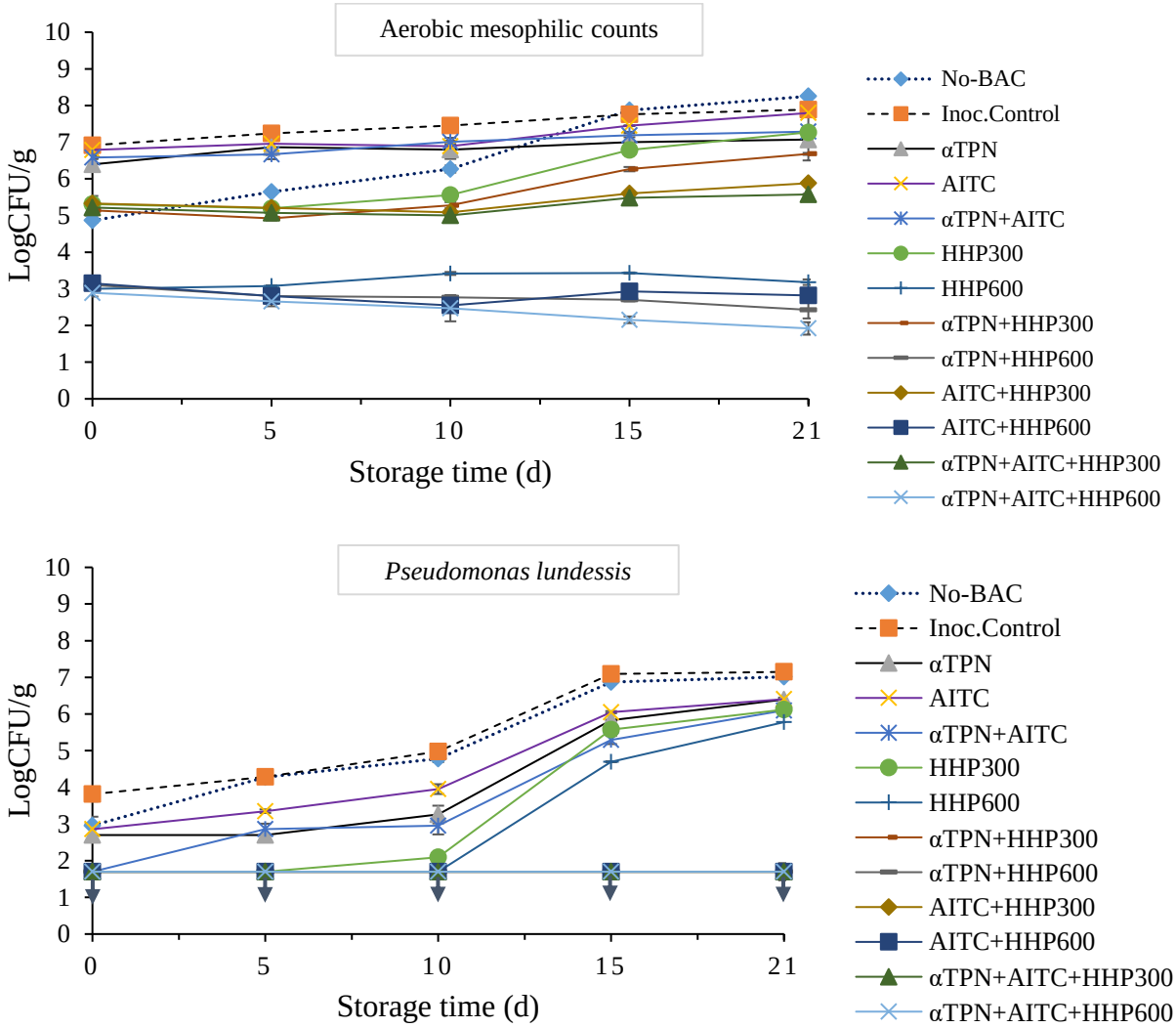
was more effective than BACs and/or HHP alone in preventing the meat from being affected by the growth of AMCs.

The microbiological statue of chicken meat was also monitored by investigating the effect of BACs and HHP on cell counts of *L. monocytogenes* and *S.* Typhimurium and *P. lundensis*. The growth of *P. lundensis* in chicken meat showed an overall less cell count in the current study. The highest initial cell counts were observed in inoculated control and control samples that showed 3.8 and 3 log CFU/g, respectively at the first day of storage, their count was increased significantly toward the end of storage to show 7.2 and 7.1 log CFU/g, respectively at day 21, and these values were higher than the rest of the treated meat samples. Additionally, the meat treated with BACs and HHP only showed the bacteria population ranged between 5.7 and 6.4 log CFU/g at day 21 that was found in the sample treated with HHP600 and AITC, respectively (Figure 33). However, the meat pressurised and treated with both BACs showed no growth of *P. lundensis.* This result indicates that the BACs only or HHP only did not show inhibitory effect against the growth of this microorganism while using BACs with pressure showed combined effect and was able to inhibit the growth of *P. lundensis.* On the other hand, the cell counts of *L. monocytogenes* did not seen in control at the beginning of storage indicating the meat was free from this bacterium. Whereas high counts were noticed at day 0 of storage in inoculated control, meat treated with AITC, αTPN, and αTPN+AITC that showed 4.7, 4.1, 3.9 and 3.6 log CFU/g respectively, and these counts were remained higher than other meat groups until day 21 and increased to 7.5, 6.9, 6.2 and 6.9 log CFU/g in the same meat group, respectively (Figure 33). 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 were also active enough to cause 4.9, 4.6, and 5.8 log reduction in the cell counts of *L. monocytogenes.* Regarding *S.* Typhimurium, the initial counts for all meat groups were less than 4.9 log CFU/g. Surprisingly the initial cell count was high in meat inculcated with microbes that treated with AITC, αTPN+AITC, and αTPN and showed 4.9, 4.9, and 4.8 log CFU/g, respectively. Throughout the storage period, no-growth or less than 2.7 log CFU/g was observed in control and meat treated with HHP600, αTPN+HHP600, AITC+HHP600, and αTPN+AITC+HHP600 (Figure 33). However at the end of storage, the cell count was the highest in inoculated control about 7.5 log CFU/g was detected, also the meat treated with BACs/HHP300-only/ combined-BACs and HHP300 showed cell count ranged between 5.4 and 7.1 log CFU/g that was found in meat treated with αTPN+HHP300 and αTPN+AITC or AITC, respectively. This finding indicates that the BACs only or low level of pressure such as 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 enhanced the antimicrobial effect against the growth of *S.* Typhimurium in chicken meat during 21 day storage period. Dias et al. (2013) applied antimicrobial packaging incorporated with AITC and carbon nanotube for cooked chicken meat inoculated with *Salmonella Choleraesuis*. They found a reduction in the microbial population, control of oxidation and reduction in the colour changes that lead to effectiveness of the packages for the 40 days of storage. Based on the effect of αTPN and AITC with HHP on AMCs and *P. lundessis,* less than 7 log CFU/g cell count was observed with BACs with HHP. Indicating that these BACs with HPP and particularly 300 MPs was sufficient to extend the shelf of chicken meat to 3 weeks storage at 4 °C. Additionally, regarding the pathogens *L. monocytogenes* and *S. Typhimurium* very strong effect of BACs was observed with HHP 300 MPs or higher, whereas for the safety of meat further accurate presence/absence detection test for *monocytogenes* and *S. Typhimurium* in ground chicken meat is needed.

In some studies, 7 logs CFU/g has been used to define the spoilage for the criterion of microbiological acceptability of meat (Höll et al., 2016; Rouger et al., 2017). In current study, above 7 logs were recorded only at day 10 in No-BAC, inoculated control, and day 15 αTPN, AITC, αTPN+AITC, and HHP300 meat. For *L. monocytogenes* more than 7 logs only noticed in inoculated control at day 21, while for *S.* Typhimurium 7.4 log was only observed in inoculated control on day 15 and in αTPN, AITC, and αTPN+AITC at day 21 of storage. Regarding *P. lundensis* above 7.1 log found in inoculated control at day 15 and 7.1 log control at day 21, whereas other meat groups exhibited less than 6.4 log of *P. lundensis.* Li and Gänzle (2016) studied the use of HHP (600 or 450 MPa) combined with 0.04, 0.025, 0.15, and 0.10 % for CARV, thymol, AITC, and cinnamaldehyde, respectively in beef steaks. They demonstrated that AITC and cinnamaldehyde exhibited synergistic activity with pressure on *E. coli* in the buffer; however, cinnamaldehyde did not affect the survival of *E. coli* and *L. monocytogenes* after pressure treatment of meat. Synergistic inactivation of AITC with pressure was observed only at concentrations that negatively affect meat quality. It has been reported by Porto-Fett et al. (2010) that the pressurization at 600 MPa or 483 MPa for 1 to 12 min in Genoa salami stored up to 28 d at 4 °C reduced numbers of *L. monocytogenes* 1.6 to ≥5.0, *E. coli* O157:H7 about 4.7 to ≥5.8, and *Salmonella* up to 1.9 to 2.4 log CFU/g. Huang et al. (2018) treated ground chicken meat with HHP *(*250-350) MPa with or without AITC (0.05-0.15 %, w/w) obtained from *Brassica nigra* (black mustard) to evaluated *E. coli* O157:H7 (STEC) as a common contaminant in meat and poultry at 4 and 10 °C for 10 days. HHP+AITC treatment indicated that AITC may continue depressing or killing the pressure-damaged cells. They found that combining HHP at 350 MPa, 20 min at 4 °C with 0.15 % AITC, a greater than 5 log reduction in *E. coli* O157:H7 was obtained. Li et al. (2015b)

suggested that αTPN has excellent antibacterial activity and could induce morphological changes of *E. coli*. It might have good potential to be used for medical purposes. It has been suggested that the treatment of meat with 600 MPa can control risks associated microorganisms in beef (Hugas et al., 2002), however, HHP does not effectively inhibit the growth of spores in the products and the resistant strains of *E. coli* or *L. monocytogenes* (Jofré et al., 2009; Li & Gänzle, 2016; Liu et al., 2012; Marcos et al., 2013). Chuang et al. (2020) treated fresh ground chicken meat HHP with CARV, their results are consistent with the generalization that G+veB bacteria are more resistant to HHP stress than are their G-veB counterparts. Yuste et al. (1998) investigated the microbiological quality of poultry meat using other physical decontamination processes such as HHP with the addition of nisin or glucono delta-lactone. They observed lesser extent activities in decreasing the AMCs compared to psychrotrophic bacteria.



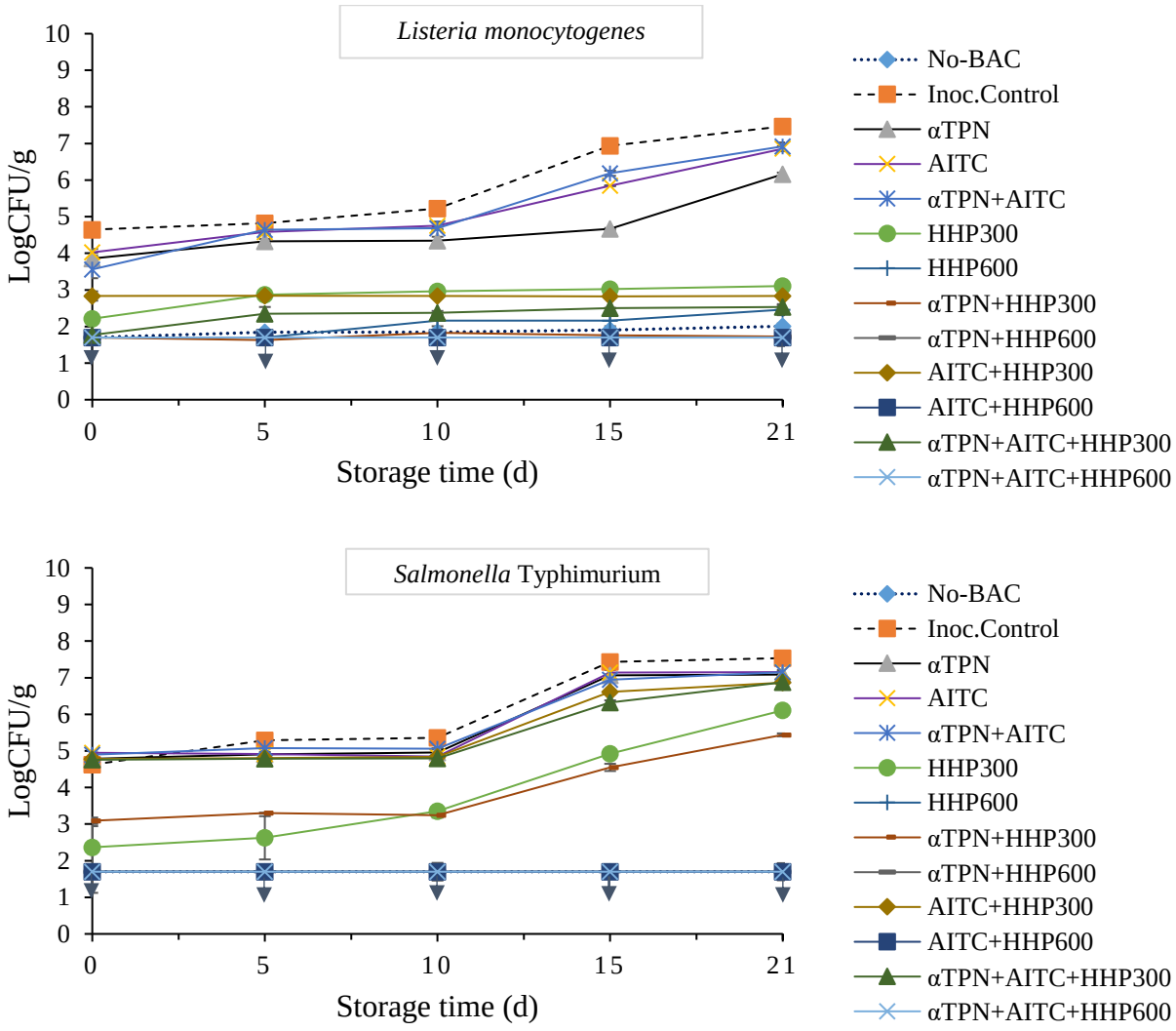
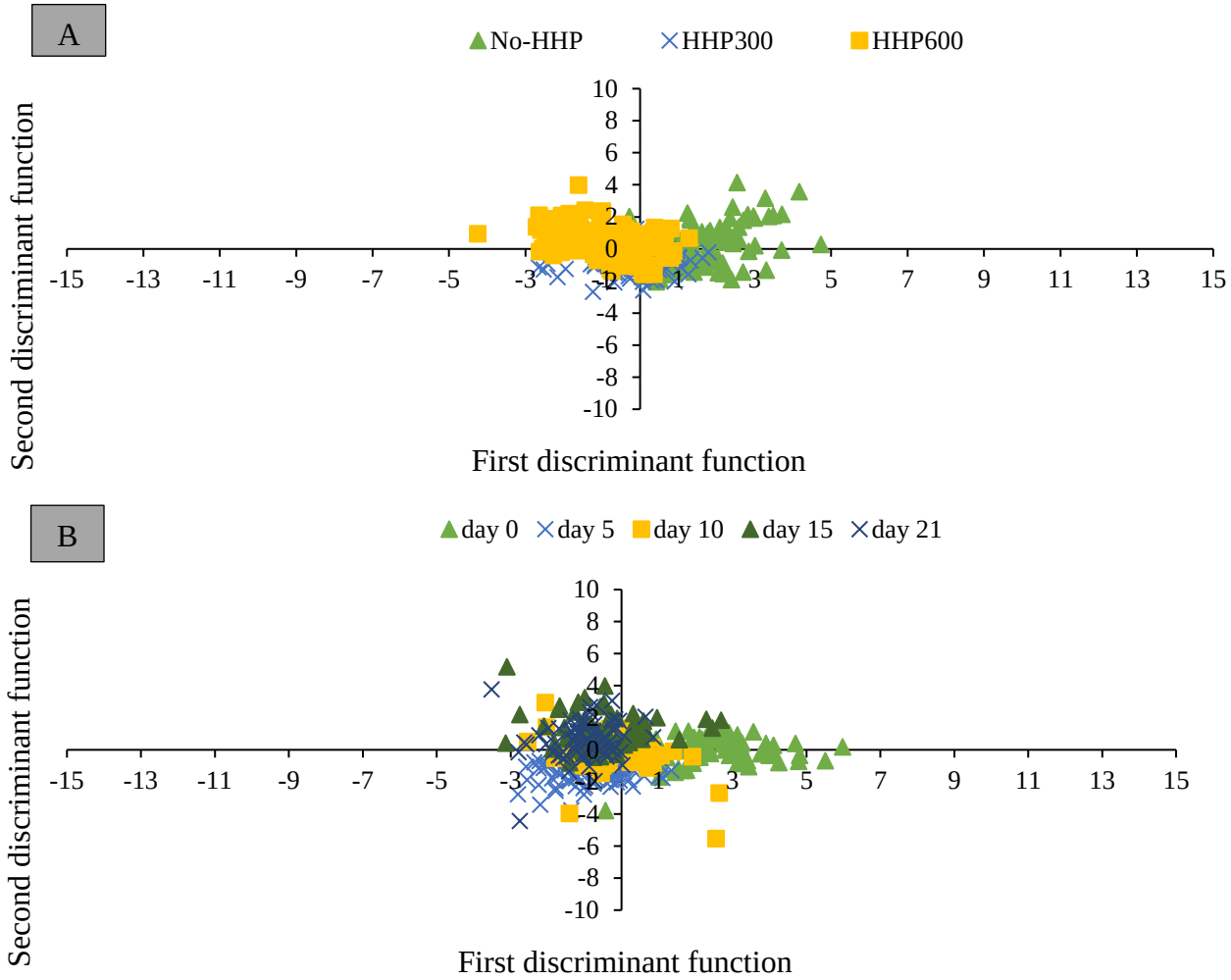


Figure 33: Effect of combined BACs (αTPN and AITC) with HHP on aerobic mesophilic counts-AMCs, *Pseudomonas lundessis*, *Listeria monocytogenes*, and *Salmonella* Typhimurium of chicken meat stored up to 21 days at 4°C. Arrow line (↓) represents the lower detection limit.

4.5.5. Electronic nose

The result from the effect of αTPN and AITC with HHP on meat and using E-nose to group the meat-based on smell detection are presented in Figure 34: A, B, C, and D. The E-nose separated the pressurized meat either that treated with or without αTPN and AITC toward 2nd discriminant function from unpressurized meat. Comparing the meat groups at different days of storage only a clear separation was observed on day 0, while overlapping noticed on the rest of the storage period. The E-nose also detected the odour and produced a clear separation of meat treated with αTPN, AITC, and No-BACs, whereas the meat contained αTPN+AITC had centered between the meat contain No-BACs, αTPN, and AITC, that can result from the reaction of meat content of these meat groups. The use of AITC has been identified as generally regarded as safe (GRAS) as a

flavouring substance “No safety concern at estimated levels of intake”, and evaluated by the Joint FAO/WHO Expert Committee on Food Additives and by the European Food Safety Authority (EFSA) Panel with an acceptable daily intake of AITC about 0.02 mg/kg BW/day allowed as a food additive and to be used in food-contact packaging materials (EFSA, 2010). Some factors can support the changes in the aroma profile of chicken meat during storage such as the progress of LO, fat content, the liberation of fatty acids, and increased microbial load during storage (Djenane et al., 2003; Mildner-Szkudlarz & Jeleń, 2008). Alongside meat treated with αTPN and HHP showed higher pH, *L**, *b**, and MetMb with low *a** values, DeoMb, and OxyMb. While meat treated with AITC and HHP exhibited lower pH, higher *L**, *b**, with low *a** values. As well as a reduction in TBARS compared to control, and reduction in *L. monocytogenes*, *S. Typhimurium*, *P. lundessis* count noticed particularly with BACs and HHP600. The current result indicates that the instrument can classify the chicken meat as either fresh or spoiled.



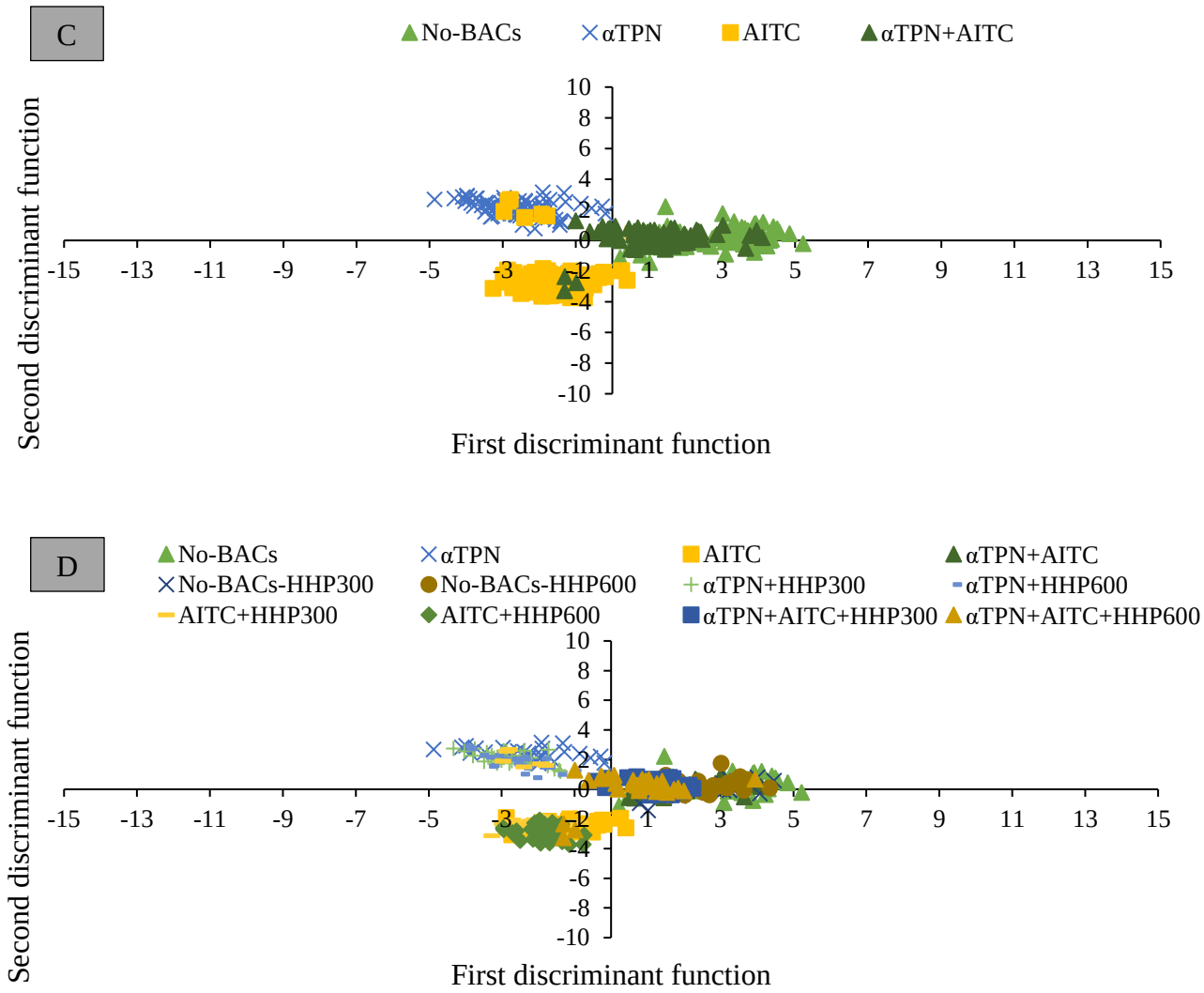


Figure 34: Efficacy of different concentrations of BACs (αTPN and AITC) combined with HHP on smell detection by E-nose in chicken meat stored up to 21 days at 4 °C, Canonical discriminant analysis score plot of A: The separation based the levels of HHP, B: The separation based on storage days, C: The separation based on the concentration of BACs, and D: The separation based the levels of HHP and the concentration of BACs.

4.5.6. Texture profile analysis (TPA) spreadability of meat

To examine the effect of BACs and HHP on the properties of the meat spread samples, the TPA of the meat were exploited. The results of the TPA test presented in Figure 35, indicates that were significant changes in the texture spreadable parameters between control (No-BACs, No-HHP), BACs, HHP300 and HHP600 MPa. The most spreadable samples were the softer ones which compressed more easily No-BACs-No-HHP, αTPN, AITC, and αTPN+AITC while the least spreadable samples were the firmer ones and the

more difficult to spread with HHP600 that does not contain BACs followed by AITC+HHP600, αTPN+HHP600 and αTPN+AITC+HHP600. At day 21 the penetration ability and spreadability of all meat groups significantly increased. The obvious softness enhances the ability of the specimen to spread mostly observed in samples treated with BACs combined with HHP. To the best of our knowledge, no work has been done that is associated with the effect of BACs and HHP on the spreadability of meat products thus far.

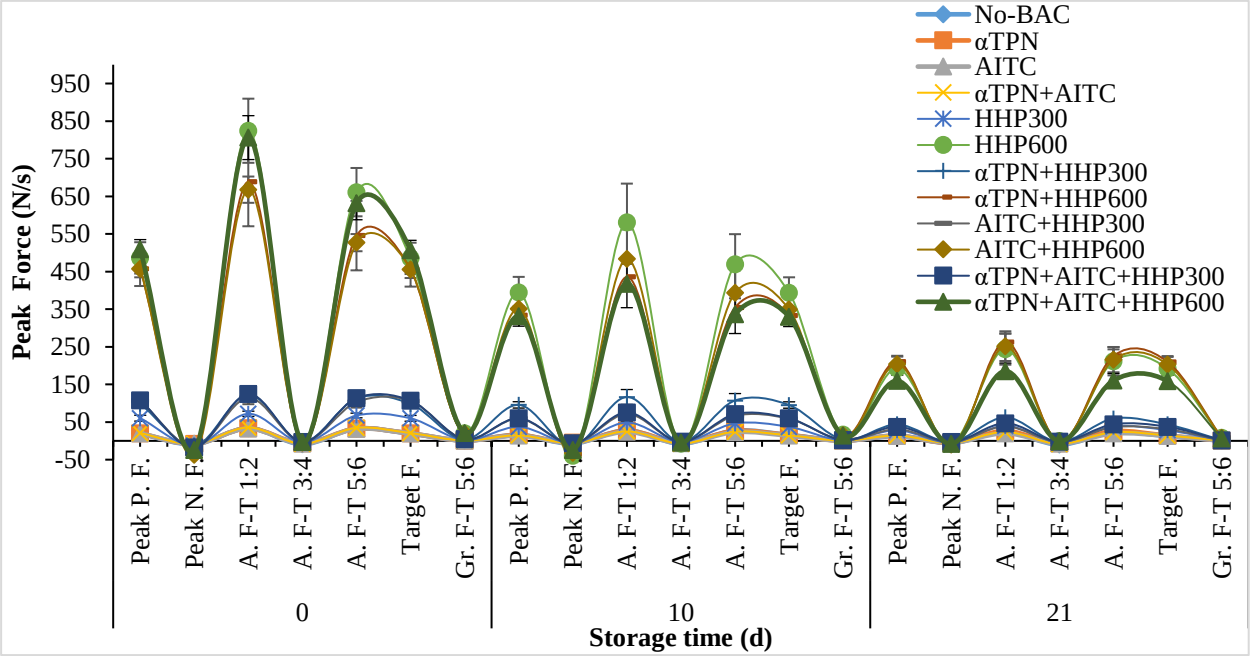


Figure 35: Spreadability of meat using TPA force versus displacement curves obtained during penetration of meat (20 °C) with crosshead rates (mm/s). For clarity, only seven points are presented by curve (Peak Positive Force, Peak Negative Force, Area F-T 1:2, Area F-T 3:4, Area F-T 5:6, Target Force, and Gradient F-T 5:6).

4.5.7. Sensory evaluation

The mean scores for the sensorial properties colour, odour, appearance, and acceptability to buy of raw chicken meat treated with combined BACs (αTPN and AITC) and pressurization stored up to 21 days at 4 °C were determined (Figure 36). The highest initial scores of sensory properties: colour, odour, appearance, and acceptability to buy was observed in control that was 8.0, 7.4, 8.1 and 7.7, followed by AITC with the scores of 8.0, 6.6, 7.5, and 7.0, αTPN with the scores of 7.9, 5.7, 7.0, and 6.3, and αTPN+AITC with the score of 7.8, 5.8, 7.6, and 6.5, respectively. At day 21 of storage, in control, the mean scores were 6.3, 2.9, 5.8, and 4.4, for AITC samples were 5.9, 4.5, 5.4, and 5.7, for αTPN were 6.2, 4.1, 5.8, and 6.6, and αTPN+AITC samples 7.0, 4.1, 6.1, and 5.1 for colour, odour, appearance, and acceptability to buy, respectively.

Additionally, it is observed that at the end of storage the highest scores of colour was seen in αTPN+AITC (score 7.0), odour in AITC+HHP300 (score 5.7), appearance in αTPN+AITC (score 6,1), and acceptability to buy in αTPN samples. 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.

In a study by Meira et al. (2017) they observed that the dry-fermented sausages treated with AITC and CARV in combination with other acids exhibited a lower sensory score in the texture, odour, flavour, appearance, and overall evaluation when compared to the control, but none of the parameters received a negative score during 21 d storage. Li and Gänzle (2016) noticed the synergistic inactivation of AITC with pressure (600 or 450 MPa) was only at concentrations that negatively affect sensory properties and meat quality. Huang et al. (2018) used HHP ≥450 MPa 20 min at 4 °C with 0.15 % AITC and noticed that the raw ground chicken meat's texture may start to deteriorate and became softer or mushy and eventually lose integrity. However, no visible colour change was noticed with the addition of 0.05-0.20 % AITC. The increase in the score of odour, appearance, and acceptability to buy in meat treated with BACs+HHP could attribute to the limited rancidity rate, and less growth of microbial spoilage. In accordance with our findings Chacon et al. (2006) found that during the sensory evaluation, sausages containing 500 ppm AITC were considered acceptable although slightly spicy by panelists. Nadarajah et al. (2005) also reported that panelists could distinguish untreated controls from mustard treatments (5 % mustard flour) but considered the mustard-treated meat to be acceptable. Wójciak et al. (2013) found that 1 % addition of ground mustard seed to the composition of cooked pork sausages did not significantly influence most of the sensory attributes. No study was found in the literature that pondering the effect of αTPN and HHP on the sensorial properties of meat.

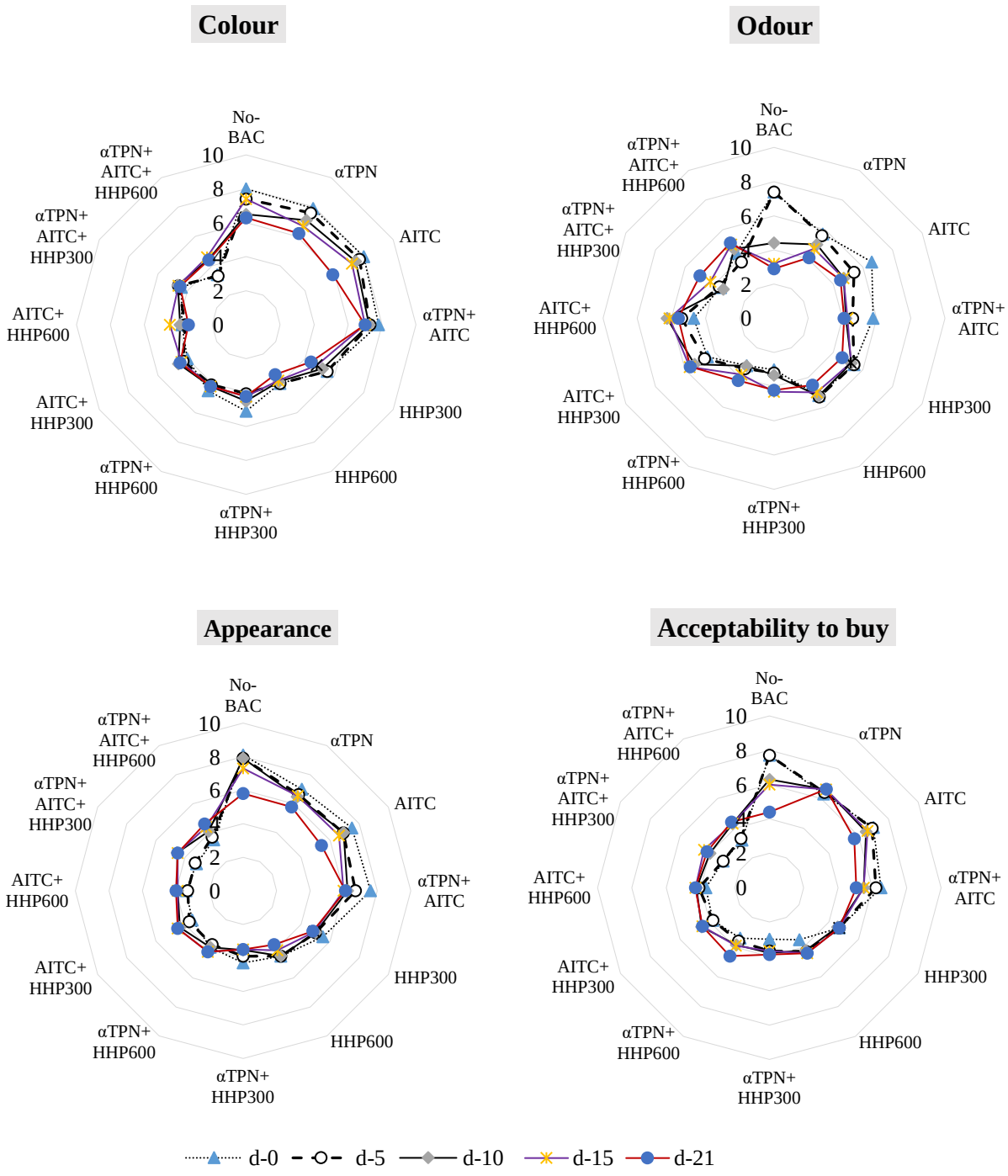


Figure 36: Spiderweb diagram of sensory evaluation showing the effect of BACs (αTPN and AITC) combined with HHP on colour, odour, appearance, and acceptability to buy properties of raw chicken meat stored for 0, 5, 10, 15 and 21 days at 4 °C.

4.6. 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, β-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.

5. CONCLUSIONS AND RECOMMENDATIONS

The use of bioactive compounds from plant materials as natural antioxidants/antimicrobials has a great potential to govern the organoleptic changes to preserve meat from deterioration. However, the high concentrations can be restricted to avoid unacceptable levels of flavours and odours. The current work was an attempt to obtain a clearer picture of the effects of BACs combined with HHP on the physicochemical, microbiological, and organoleptic characteristics of chicken meat during chilling storage (Figure 37-Appendix). In the first experiment, the use of allyl-isothiocyanate, carvacrol, linalool, and piperine in chicken meat had shown a protective effect against some colour parameters and a nonsignificant reduction in lipid oxidation compared to untreated meat. Allyl-isothiocyanate particularly 1000 ppm 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 aerobic mesophilic counts. Compared to untreated samples an increased *a** value was perceived in meat treated with carvacrol, linalool, and piperine and has a great contribution towards the final colour intensity of the meat. Based on the storage time only significant WHC was detected with linalool 1000 ppm compared to an increased drip loss in control, while piperine-500 exhibited almost stabilized efficiency in WHC. BACs mainly carvacrol and linalool had a clear protective effect against lipid oxidation by keeping TBARS scores lower than 2 mg MDA/kg with a smaller flavour impact. Prolongation of the lag phase of the growth of aerobic mesophilic counts was observed, except with piperine. Therefore *in vitro* antimicrobial effect of these BACs was studied. Allyl-isothiocyanate particularly 1000 ppm showed a considerably higher effect compared to carvacrol in reducing the growth of *P. lundensis*, *St. aureus,* and *B. cereus*. However, carvacrol was more active in reducing the growth of *E. coli*, *L. monocytogenes,* and *S.* Typhimurium. Linalool showed *in vitro* inhibitory effect against G+veB and G-veB bacteria except for *P. lundensis*. No inhibition activity was noticed for piperine. 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 (carvacrol, allyl-isothiocyanate, linalool, and piperine) to enhance the quality of meat and meat products.

In the second experiment, applying the disc method, the components with the widest spectrum of antibacterial activity against the studied bacteria were found to be carvacrol, followed by thymol, eugenol. While. using MIC method allyl-isothiocyanate showed the best activity among all the BACs followed by geraniol, β-citronellol, carvacrol, α-Terpineol, thymol, eugenol, linalool, and cuminaldehyde. The lowest MIC was found with allyl-isothiocyanate 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. Among the BACs allyl-isothiocyanate and α-Terpineol were chosen as the most effective BACs in liquid form against aerobic mesophilic counts, *L. monocytogenes, S.* Typhimurium, and *P. lundensis.*

In the third experiment, the different concentrations of α-Terpineol were able to alter the physicochemical attributes of chicken meat during 14-day storage. At the end of the storage period, α-Terpineol 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 α-Terpineol. Increasing trends of *b** value and *C** colour intensity were detected at day 14 in samples containing a higher rate of α-Terpineol compared to a slight decrease with no significant rate in MIC-1 and control. Moreover, different levels α-Terpineol particularly MIC-2 and MIC-4 were able to show a significant effect on decreasing WHC. Besides, α-Terpineol 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 α-Terpineol 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 α-Terpineol particularly higher level (MIC-4) showed antimicrobial activity against aerobic mesophilic counts, *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.

In experiment four, allyl-isothiocyanate 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 allyl-isothiocyanate (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. Allyl-isothiocyanate decreased MetMb and DeoMb and increased OxyMb in chicken meat. The meat containing allyl-isothiocyanate 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 aerobic mesophilic recorded in meat treated with allyl-isothiocyanate 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 allyl-isothiocyanate. Moreover, *P. lundensis* increased in all meat groups except in meat contained MIC-

4 of allyl-isothiocyanate did not exhibit the growth at day 10 onward. The E-nose showed that the concentration of allyl-isothiocyanate 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.

In experiment five, the less concentration of BACs (MIC-1) applied with HHP. At the end of storage the pH in control and meat treated with α-Terpineol were decreased, in contrast to HHP600 treated samples that showed a significant increase in pH values to reach 6.28, and the α-Terpineol 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 α-Terpineol and allyl-isothiocyanate 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 lipid oxidation 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 lipid oxidation was higher than either using BACs and/or HHP alone and even the combination of both BACs and HHP. At day 21 the lowest aerobic mesophilic counts were 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 aerobic mesophilic counts. 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 α-Terpineol and allyl-isothiocyanate 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 (α-Terpineol and allyl-isothiocyanate) with HHP decreased counts of aerobic mesophilic counts 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. Besides, very strong effect of BACs was observed with HHP 300 MPs or higher pressure on *L. monocytogenes* and *S. Typhimurium*, but for the safety of meat further accurate presence/absence detection test in ground chicken meat is needed.

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 α-Terpineol and allyl-isothiocyanate. 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.

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 aerobic mesophilic counts, *L. monocytogenes*, *S.* Typhimurium, and *P. lundessis*. 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, α-Terpineol alone was more effective in increasing *C** value of meat, while allyl-isothiocyanate was more effective with HHP in increasing *C** value of meat. The BACs α-Terpineol and allyl-isothiocyanate 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. Based on the results from current study it can be suggested that low level of α-Terpineol and allyl-isothiocyanate (MIC-1) with low level of pressure (300 MPa) are promising to preserve the quality attributes of fresh ground chicken meat during refrigeration conditions.

RECOMMENDATIONS

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.

6. Appendixes

6.1. References (Appendix-M1)

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6.2. Appendix (M2)

Appendix-Table 1: Composition of major plant BACs of potential application in meat and meat products.

Bioactive compounds	**Common and Latin name and sources of potential plants EOs with percentage composition of selected BACs (%, g/100gm)**																
	Balm 	**Oregano** 	**Thyme** 	**Clove – buds** 	**Rosemary** 	**Coriander - seeds** 	**Marjoram** 	**Sage** 	**Cumin** 	**Juniper** 	**Garlic** 	**Cinnmomum cassia** 	**Black pepper** 	**Lavender** 	**Basil** 	**Mustard** 	**Southern blue gum tree** 
	Melissa officinalis	Origanum vulgare	*Thymus vulgaris* L.	Myrtaceae *Syzygium aromaticum* (L.)	*Rosmarinus officinalis* L.	Coriandrum sativum	*Origanum majorana*	Salvia officinalis L	Cuminum cyminum	Juniperus communis	*Allium sativum*	Cinnamomum Verum	Piperaceae *Piper nigrum* L.	Lavandula officinalis	Ocimum basilicum L	Brassica nigra	*Eucalyptus globulus*
Carvacrol	14 (1)	6-80 (2,3,4,8,9,10,11, 21,24)	1.18-19.29 (2,4,5,11, 12,20)									0.84 (9)					
Thymol		16.22-64 (2,3,4,9,10, 11,21)	7.48(19), 10-64 (2,4,11,12,15,20)				10 (10)										
Eugenol				75-85 (2,10, 15), 49.85 (9)											3.76 (7)		
y-Terpinene		2.14-52 (2,3,4,9,10, 21,24)	2-31 (2,4,5, 19,20)		0.93 (19)		12-14 (10,11)		12.9-16.86	2.64 (15)							8.8 (22)

									(13,17)								
1,8-Cineole (eucalyptol)		0.09-0.6 (11,24)	0.12-2 (11,12,19), 4.35 (20)		3-89 (2,19), 43.99 (23)				26.75 (16)			4.15 (9)		8.6 (25)	10.5 (7)		45.4 (22),79.85 (23)
Geranyl acetate						5 (6)								0.98(7)			
Geraniol	6 (5)																
***p*-Cymene**		Trace-64 (2,3,4,5,8,9,11,21,24)	4·1-56 (2,4,8,11,12, 19, 20)		1.44 (23)	4 (6)			0.30-8.6 (13, 17)	1.55 (15)		3.92 (9)					9.5 (22), 5.14 (23)
Citronellal	40 (1)																
α-Terpineol		0.15 (11)	0.24 (11), 4.73 (23)											1.35 (7)			3.4 (22)
Bornyl acetate			1·0 (12)		0-17 (2)												
Camphor		0.15 (21)	1.55-2.33(19,23)		2-22 (2,8,19,23)	6 (6)		12.0 (8)						9.67(25)			
Linalool		1.05-2.73 (9,11, 21,24)	6.27-10.3 (11, 12) 0.66 (15), 18.18 (19), 4.69 (20)	0.01 (15)	3.37 (19)	70-75 (6,10)				0.10 (15)		7.39 (9) 2.51 (15)		34.44 (18) 36.97 - 47.95 (15,25)	65.95 (7)		
Iso-menthone	9 (1)																
Limonene		0.07-0.91 (9,21,24)	1.04 (20), 5.63 (15)						0.94 (16)	11.43 (15)		1.42 (15)		0.3 (15)			17.8 (22)
α-pinene		0.20-1.8 (11, 21,24)	25.2 (12),		2-25 (7,2,11,19)	6 (10)			1.41 (17)	27.49 (15)		2.17 (9)		0.54 (25)			4.2 (22),

			3.66 (15), 0.80 (19), 0.88 (20)		10.9 (23)												
Citronellol	6 (1)																
Cuminaldehyde									17.1 - 36.67 (16,17)			76.49 (15)					
Trans-cinnamaldehyde		65 (2)										65-66.32 (9,10)					
4-Thujanol							36.2 (8)										
Terpinene-4-ol							8.7-26 (8,10, 11)		2.44 (17)								
Eugenol acetate				8-22.59 (9,10)													
Caryophyllene		0.43-0.98 (9,11, 21)	0.82-2.03 (11,15)	14.39-14.81 (9,15)													
Humulene				3.58 (9)													
β-Caryophyllene		1.6 (24)	1·7 (12)									3.61 (9)					
Allyl isothiocyanate															56.52 (9)		
Piperine													33.5 (11)				

Diallyltrisulfide+ diallyl disulfide											33.8+ 18.86 (14)						
β-Pinene		0.54-0.6 (21,24)	0.65 (16), 3.56 (19)		3.14 (19)					6.8 - 18.76 (15,17)							
a-terpinene		0.43-1.7 (11,21,24)	0.86 (11), 1.32 (20)		0.50 (19)												0.15 (23)
4-terpineol		0.23 (11)	0.41-5.66 (11,19), 0.74 (20)		3.50 (19)					0.09 (17)	2.5 (14)						

19. (Gouveia et al., 2017)
20. (Boskovic et al., 2017)
21. (dos Santos Rodrigues et al., 2017)
22. (Tyagi & Malik, 2011)
23. (Ait-Ouazzou et al., 2013)
24. (Luz et al., 2015)

13. (Pajohi et al., 2011)
14. (García-Díez et al., 2017)
15. (Herman et al., 2016)
16. (Chaleshtori et al., 2016)
17. (Patil et al., 2016)
18. (Marín et al., 2016)

7. (Predoi et al., 2018)
8. (Gutierrez et al., 2008)
9. (Radha krishnan et al., 2014)
10. (Oussalah et al., 2007)
11. (Calo et al., 2015)
12. (Cosentino et al., 1999)

1. (Fratianni et al., 2010)
2. (Burt, 2004)
3. (Fasseas et al., 2008)
4. (Karabagias et al., 2011)
5. (Marino et al., 1999)
6. (Samojlik et al., 2010)

Appendix-Table 2: Applications of BACs alone/or in combinations in meat and meat products

Bioactive compounds	**The concentration of BACs or method used**	**Meat product tested**	**Storage conditions**	**Results**	**References**
				Antimicrobial, antioxidant, and sensory	
CARV and thymol	5 % for thymol and 1.5 % for CARV in pure ethanol.	Coating of microcapsules (*in vitro*)	The release of 10 % of thymol and 10 % of CARV was carried out at 4 °C for up 28 days	Thymol and CARV showed significant antimicrobial activity against the *E. coli* O157:H7, *St. aureus*, *Listeria innocua*, Saccharomyces cerevisiae, and Aspergillus Niger with MIC of 125–250 ppm and 75–375 ppm for thymol and CARV respectively. Besides a range of zones of inhibition of 4.3 ± 1.3, 8.8 ± 0.9, 9.0 ± 0.8, and 11.3 ± 1.3 mm for the S. cerevisiae, *Listeria innocua*, *E. coli*, and *St. aureus*, respectively. The synergistic effect of combinations of thymol and CARV was achieved at a concentration of 50 % and 50 %.	(Guarda et al., 2011)
CARV with HHP	CARV (0.75 % and ≤30 %) at 250, 300, 350, 400, and 450 MPa	Fresh ground chicken meat	Stored at 4 °C and 10 °C for up to 8 d	The 10-min HHP treatments at 250, 300, 350, 400, and 450 MPa reduced *Salmonella* by 1.08, 1.71, 2.46, 4.65, and 6.01 log CFU/g, respectively, and reduced *L. monocytogenes* by 0.21, 0.54, 1.39, 4.40, and 5.25 log CFU/g, respectively.	(Chuang et al., 2020)
Thymol	250, 500, 750 mg/Kg	Fresh minced beef patties	Samples were packed using a high barrier film and stored under normal conditions, and MAP for up to 16 days at 4 °C	Thymol alone was effective on coliforms and Enterobacteriaceae, whereas it does not show to inhibit to a great extent the growth of the other microbial populations. Moreover, an increased amount of thymol, under MAP conditions, had better effects on the product quality, with a prolongation of the shelf life about 7 days.	(Del Nobile et al., 2009)
Thymol	500, 1000, 1500 ppm	Peeled shrimps	Coating and packaged under MAP	Active coating (1000 ppm) under MAP extended the shelf life to about 14 days, compared to the same samples in the air for about 5 days.	(Mastromatteo et al., 2010)
Eugenol	0.1g /100g	Chicken noodles	Stored at 35 ± 2 °C	Protect against an increased colour L* and decrease in a* and b* values during storage. Eugenol treatment also showed the lowest TBARS and FFA (Free fatty acids) contents, also showed a positive impact on microbiological quality and sensory attributes.	(Khare et al., 2014)
Eugenol	0.1 ml	Cooked beef sirloin, and	Spread over surface	Controlling the growth of *L. monocytogenes* at 30 °C and 7 °C and 1 % level had a greater inhibitory activity	(Hao et al., 1998)

		sliced	of 25g slice then refrigerated		
AITC	AITC (0.6 and 1.2 μg/h) with MAP	Fresh chicken breast	samples were inoculated with pathogens at 10^4 CFU/g, and the packages (with and without AITC-with ambient air or 30 % CO2/70 % N2) stored at 4 ◦C for up to 21 days	The maximum reduction in MAP plus AITC (compared to MAP alone) was 0.77 log CFU/g for *L. monocytogenes* and 1.3 log CFU/g for *S.* Typhimurium. A release rate of 0.6 μg/h of AITC was found to not affect the colour, whereas at 1.2 μg/h of AITC the surface of the chicken was discoloured.	(Shin et al., 2010)
AITC	Volatile horseradish distillate contained approximately 90% AITC	Roasted beef slices and bacteria inoculated on agar	Stored at abusive temperature (12 °C), AITC contained on a filter paper disk which was packaged with a ground beef patty for 7 days	AITC could be effective as a vapor to kill *E. coli* O157:H7, bactericidal activity was observed for *St. aureus*, *E. coli*, and *Serratia grimesii*	(Ward et al., 1998)
AITC and coumaric acid	AITC and CARV in combination with phenolic acids (PA) ferulic acid (FA), o-coumaric acid (CA) and p-hydroxybenzoic acid (AHB)	Dry-fermented sausages	Stored for 35 days	The MIC values of AITC, CAR, FA, CA, and AHB for 5-strain mixture of *E. coli* O157:H7 were 0.25; 1.3; 5.12; 18.27; and 37 mM respectively. AITC showed synergism with all phenolic acids (FA, CA, and AHB, while CARV only showed synergism with CA, and the combination of AITC and CA had the strongest synergistic effect which applied in dry-fermented sausages at 10- and 20-fold using the FIC. *E. coli* O157 was reduced ≥5 log CFU/g after 21 d accompanied by lower sensory scores in the texture, odour, flavour, appearance, and overall evaluation when compared to the control, but none of the parameters received a negative score, whereas the pH of 10× FIC and 20× FIC were higher than the control.	(Meira et al., 2017)
AITC with HHP	AITC (0.05–0.15%, w/w), and HHP *(*250–350) MPa	Ground chicken meat	Stored at 4 and 10 °C for 10 days	HHP with AITC treatment indicated that AITC may continue depressing or killing the pressure-damaged cells of *E. coli* O157:H7 (STEC). HHP alone (350 MPa, 4 °C) in 15 min can only attain a 2.1 log CFU/g reduction of *E. coli* O157:H7. Combining HHP at 350 MPa, 20 min at 4 °C with 0.15 % AITC concentration, a greater than 5 log reduction was obtained. The raw ground chicken meat's texture may start to deteriorate and became softer or mushy and eventually lose	(Huang et al., 2018)

				integrity at pressure ≥450 MPa. However, no visible colour change was noticed with the addition of 0.05-0.20 % AITC	
AITC with HHP	CARV, thymol, AITC, and cinnamaldehyde with 0.04, 0.025, 0.15, and 0.10 %, respectively, and HHP (600 MPa or 450 MPa).	Beef steaks	Stored at 4 °C for 16 days	HHP with AITC had a synergistic effect against *E. coli* and *L. monocytogenes*. However, the levels applied negatively affected the sensory properties of the product. AITC and cinnamaldehyde exhibited synergistic activity with pressure on *E. coli* in buffer; however, cinnamaldehyde did not affect the survival of *E. coli* after pressure treatment of meat. Synergistic inactivation of AITC with HHP was observed only at concentrations that negatively affect meat quality.	(Li & Gänzle, 2016)
AITC	Microencapsulated AITC was added to three batters at 500, 750, or 1,000 ppm	Four sausage batters (17.59 % beef, 60.67 % pork, and 17.59 % pork fat)	Dry-cured sausages tainted with *E. coli* O157:H7 (6.0 log UFC/mL), stored at 75 % RH and 13 °C for 25 days	AITC showed no effect on meat pH reduction. While AITC with 750 and 1,000 ppm reduced *E. coli* O157:H7 by 6.5 $\log^{10}$ CFU/g after 21 and 16 days of processing. *E. coli* O157:H7 numbers were reduced by 4.75 $\log^{10}$ CFU/g after 28 days by 500 ppm AITC. The pathogen population was reduced to undetectable levels after 16 and 21 d using 1000 and 750 ppm of AITC. During the sensory evaluation, sausages containing 500 ppm AITC were considered acceptable although slightly spicy by panelists.	(Chacon et al., 2006)
CARV and nisin	250 to 500 µg/ml	Exposed bacterial cells of *L. monocytogenes* sliced bologna sausages *to* CARV	Stored at 4 °C storage	They noticed significant growth rate reductions compared to those of controls (p<0.05) with a MIC of 250 µg/ml and MBC ranging from 250 to 500 µg/ml. They also found synergistic interaction of CARV and nisin against *L. monocytogenes* and three food isolates (CM2, CM8, and CM11) by using *in vitro* checkerboard assay. Their findings showed degenerative changes of cell wall and cytoplasmic membrane leading to cell lysis, structural disruption, increased membrane permeability and depolarization, and changes in respiratory activity	(Churklam et al., 2020)
Thymol and CARV	Thymol and CARV at 0-300 ppm	Poultry patties	Conventionally packaged in air and MAP: 40 % CO_2; 30%O_2; 30 % N_2) at 0-18 °C	Higher log reduction for *Pseudomonas* spp. during all the storage time was observed in both packaging atmospheres. Reduction about 1–1.5 log CFU/g in the final cell load of lactic acid bacteria and Enterobacteriaceae. the combination of the BACs and	(Mastromatteo et al., 2009)

				low temperature determined no modification for off-odour during the first 4 days of storage.	
Thymol and CARV	50 mg/kg of each BHT (positive control), thymol, and CARV.	Supplemented to the feed to broiler chickens.	Broiler breast and thigh samples were stored for 10 d at 4 °C	Feed supplementation did not significantly affect breast sample oxidation. However, after 10 d of storage, increasingly higher values of TBARS were detected in thigh samples of the control compared to other groups and the lower TBARS were detected between those feed-supplemented groups. The authors suggested the application of thymol or CARV could be useful to improve poultry meat quality.	(Luna et al., 2010)
CARV and thymol	0.4 and 0.8 % v/w	Marinated fresh chicken	Stored up to 21 days at 4 °C in air and under vacuum packaging (VP).	They found that 0.8 % v/w combination with VP had a significant decrease in the population of spoilage microbiota (e.g *Pseudomonas* spp) about 2.9–3.1 log CFU/g and based on the TVC extended the shelf-life of the meat microbiologically by >6 days whereas a combination of BACs at the 0.4 % v/w with packaging (air or vacuum) showed significant extension of meat shelf-life sensorially up to 15 and >21 days, as compared to 9 days in controls	(Karam et al., 2019)
Geraniol, and CARV	Ratios of 1:0, 2:1, 1:1, 1:2, and 0:1 (2.5 % v/v) in oil-in-water emulsions	Raw goat meat surface	during extended storage at 4° C.	The geraniol and CARV emulsion-entrapped formulations could extend antimicrobial efficacy on the goat meat model until 9 days as compared to samples with oil only, non-emulsion formulations.	(Syed et al., 2020)
The combination of thymol, CARV and grapefruit seed extract (GFSE)	50, 100, 150, 200 and 300 mg/L[1]	Poultry meat patties	Packaged in air or (MAP: 5 % O_2; 30 % CO_2; 65 % N_2)	Thymol and CARV, as individual antioxidants were more effective than GFSE and retarded the LO by maintaining MDA values below 2 mg kg^{-1} meat, and the colour a* decreased, an increase in b* values. The effect of GFSE was less than thymol and CARV. Although colour acceptability decreased with time, all meat preparations packaged in air maintained desirable appearance better than samples in MAP.	(Lucera et al., 2009)
CARV and Cinnamaldehyde	0, 0.5, and 0.75 %)	Baked chicken	Chicken wrapped with apple and tomato films containing BACs	The taste panel indicated a higher preference for (0.5 %) CARV -containing tomato coated chicken over corresponding apple coating There was also a higher preference for cinnamaldehyde-containing apple films over corresponding CARV-containing wrapping. films can be used to protect raw chicken pieces against bacterial contamination without adversely affecting the sensory qualities of the wrapped chicken pieces.	Du et al. (2012)

Limonene	limonene (0, 4, 6 and 8 %, w/w) with polylactic acid (PLA) in composite-based packaging	Fresh beef meat	12 day at 4 ºC	They monitored total viable count (TVB), total coliform, *Pseudomonas spp*. and *St. aureus*, and found Prolonging the shelf life using PLA-limonene at the concentration 8 % assured up to 12 days compared to 6 days for sample packed with PLA-limonene (4 %)	(Sangkasanya et al., 2018)
Thyme EO and BACs	Thyme EO (0.3 %, 0.6% and 0.9) and thymol, CARV and *p*-Cymene, cinnamaldehyde, and eugenol	Minced pork	Packaged under vacuum or MAP at 3 ± 1 °C for up to 15 days	They found MIC was greatest for thymol and CARV followed by thyme EO against four serovars of *Salmonella* (S. Enteritidis, *S. Typhimurium*, *S. Montevideo*, and *S. Infantis*). Thyme EO exhibited greater antimicrobial activity than *p*-Cymene, cinnamaldehyde, and eugenol and lower than thymol and CARV. Based on sensory data pork packaged under MAP with 0.3 % thyme EO added was most acceptable.	(Boskovic et al., 2017)
Thymol and CARV)	Commercial bacteriophage and emulsions of 1.6 % (w/v)	Boneless and skinless chicken breasts	Stored at 4 °C for 5 d, through dipping of inoculated chicken meat	They observed that 1.6 % (w/v) thymol and CARV for 3 min dipping resulted in 1.3 and 1.6 log CFU/g reduction, respectively, While, the dipping in both bacteriophages (1.1×10^8 PFU/ml) and 1.6 % (w/v) thymol or CARV for 3 min resulted in reductions of 1.9–2.0 log CFU/g of a cocktail of *Salmonella* strains (S. Typhimurium, *Salmonella* Enteritidis, and *Salmonella* Dublin SP.). Moreover, the i*n vitro* MIC against *S.* Typhimurium for BACs; CARV, eugenol, geraniol, *p*-Cymene, and thymol were 0.5–1, 1, 2, >2 and 0.5–1 mg/ml, respectively.	(Moon et al., 2020)
Oregano oil	0.1 %	chicken breast meat	Stored at 4 °C. Aerobically or under MAP + 30 % CO_2–70 % N_2, and under MAP + 70 % CO_2–30 % N_2.	They revealed that exhibited reduction in TVC, *Pseudomonas* spp., Enterobacteriaceae, and lactic acid bacteria populations and an additive preservation effect that extended the product shelf life by ca. 3-4 days for samples containing 0.1% oregano oil, and 5-6 days for samples under MAP containing 0.1 % of oregano oil.	(Chouliara et al., 2007)
Thyme oil and balm oil	0.5 %	Fresh chicken breast meat	Chicken breast meat stored at 4 °C for 21 days	Lower TMCs compared to control. Significant inhibition of the growth of E. coli and LAB. and balm oil effectively inhibited the growth of Salmonella spp.	(Fratianni et al., 2010)

* MAP (modified atmosphere packaging), AITC (Ally isothiocyanate), TVC (total viable counts), LAB (lactic acid bacteria), TMC (total microbial counts), EO (essential oil), CARV (carvacrol).

Appendix-Table 3: Concentration and dilution of αTPN applied in meat

Total weight	100	g						
MIC1:	2.5	g αTPN in	1000	g final mixture		let 1 ml αTPN is 1g αTPN		
if	2.5	g αTPN in	1000	g final mixture				
then	0.25	g αTPN in	100	g final mixture				
				αTPN	0.25	g		
			+	Ethanol	1.25	g		= 5x weight of αTPN
				αTPN+ethanol	1.5	g		
			+	DW	3.45	g		
				αTPN+DW+ethanol	5	g	5	% of meat
			+	meat	95	g	95	% of total weight
					100	g		total weight
The concentration of αTPN in MIC-2 were x2, and in MIC-4 were x4								

Appendix-Table 4: concentration and dilution of AITC applied in meat

Total weight	100	g						
MIC1:	0.0088	g AITC in	1000	g final mixture		let 1 ml AITC is 1g AITC		
if	0.0088	g AITC in	1000	g final mixture				
then	0.00088	g AITC in	100	g final mixture				
				AITC	0.00088	g		
			+	ethanol	0.0044	g		= 5x weight of AITC
				AITC+ethanol	0.00528	g		
			+	DW	4.99384	g		
				AITC+DW+ethanol	5	g	5	% of meat
			+	meat	95	g	95	% of total weight
					100	g		total weight
The concentration of AITC in MIC-2 were x2, and in MIC-4 were x4								

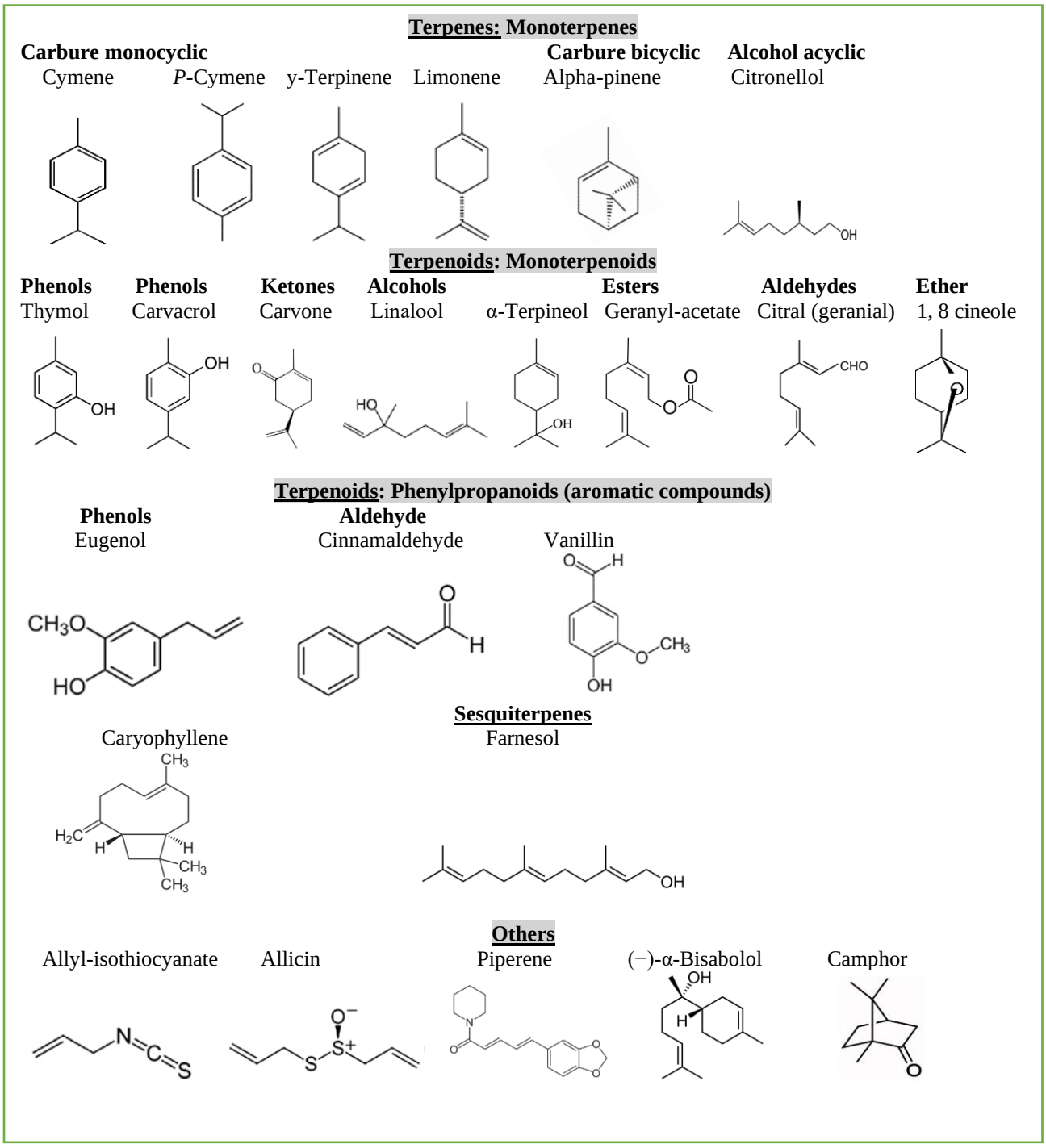

Figure 10- Appendix: Chemical structures of selected bioactive constituents of EOs (Bakkali et al., 2008; Burt, 2004; Hyldgaard et al., 2012).

					
No-BAC	**αTPN**	**AITC**	**αTPN+AITC**	**HHP300**	**HHP600**
					
αTPN+HHP300	**αTPN+HHP600**	**AITC+HHP300**	**AITC+HHP600**	**αTPN+AITC+HHP300**	**αTPN+AITC+HHP600**

Figure 14-Appendix: Examples of meat in a sealed bag used in the experiment

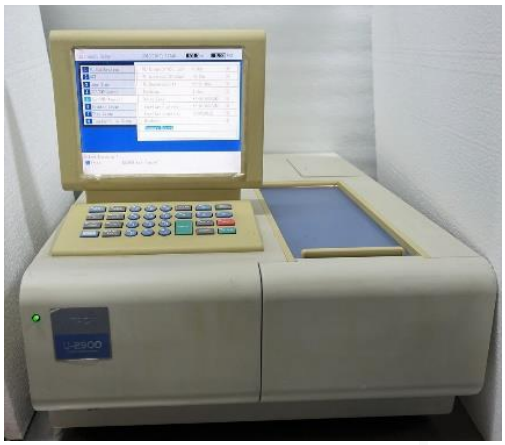

Figure 16-Appendix: Spectrophotometer Hitachi U-2900.

Figure 17-Appendix: Electronic nose NST 3320 instruments (Applied Sensor Technologies)

Figure 18-Appendix: An example of a meat sample used for sensory evaluation

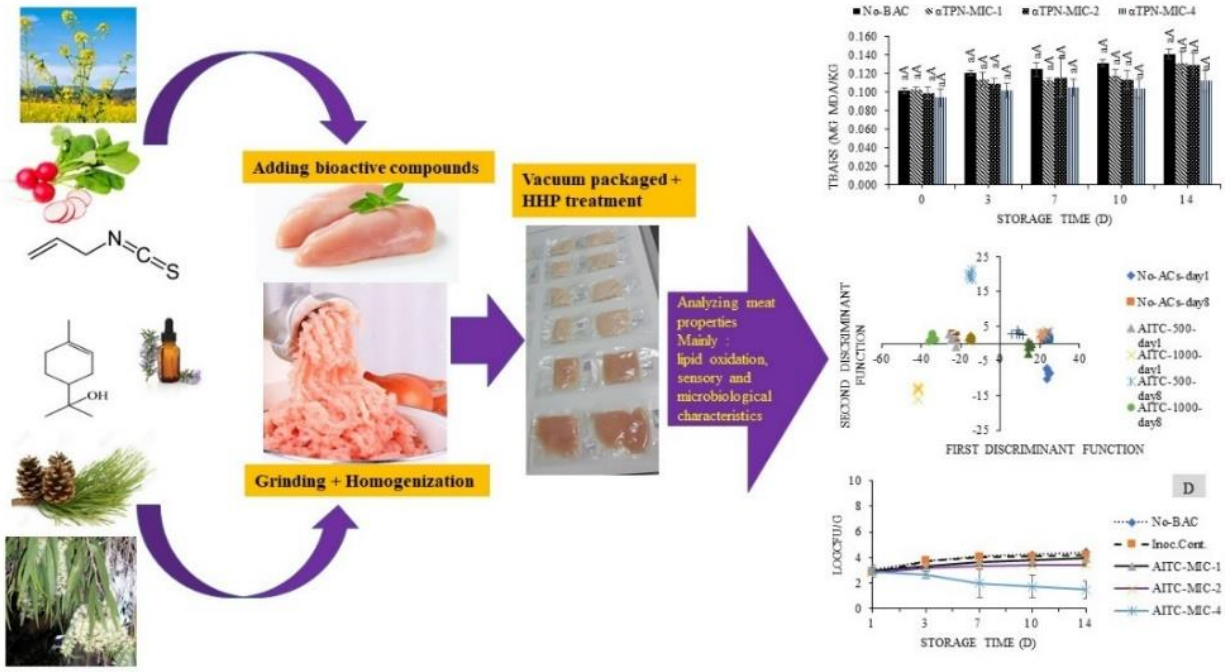

Figure 37: Graphical summary

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Khabat Noori Hussein

DEDICATION

IT IS AN HONOUR TO DEDICATE THIS THESIS TO:

MY LOVELY PARENTS

MY BROTHERS AND SISTERS

TO TEMPOUS PUBLIC FOUNDATION AND MY COLLEAGUES AT SZIU- HUNGARY

MY BELOVED HOMELAND “KURDISTAN”