Total volatile basic nitrogen in meat products: occurrence, method of determination and use as a freshness indicator

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1.0 EXECUTIVE SUMMARY

Meat and muscle food freshness is paramount to its acceptance by end-consumers. Total volatile basic nitrogen (TVB-N) is often applied as an objective biomarker for meat freshness, safety and suitability for consumption.

Major sources for TVB-N generation are the degradation of proteins and amines – specifically, trimethylamine-N-oxide (TMAO) to trimethylamine (TMA), dimethylamine (DMA) and formaldehyde, as well as the deamination of adenine nucleotides. The formation pathways are related to the activity of endogenous enzymes and spoilage bacteria on methylated amines.

TVB-N increases with meat storage and is aligned with other biomarkers of spoilage (i.e. function of duration, temperature, packaging, etc.). But these increases are not always consistent.

The relationship between TVB-N production and enzymatic activities in red meat have not be confirmed by experimental evidence (unlike with fish). It is worth noting that all the studies investigating the contribution of proteases and proteolytic activities (both endogenous and exogenous) from the bacteria have been conducted on seafood and similar studies on red meat are urgently needed to understand the phenomena in red meat context. Since protein degradation infers increased tenderness in red meat products, it is reasonable to suggest that more tender products would have higher TVB-N levels and therefore subject to associated penalties.

The rapid increase in TVB-N is observed at the point of glucose depletion in meat. High pH facilitates the gradual transition from glycogen dependent microbes to the protein degrading types of bacteria and its impact on TVB-N should be investigated.

Similar studies (to those conducted with fish, chicken and pork) using red meat are needed to clearly identify the main TVB-N producing bacteria and devise strategies to control their activities. This observation merits consideration before interpreting or using TVB-N as a proxy measurement for total viable microbial counts.

Many studies offer limited interpretation of the TVB-N results and its association with other spoilage biomarkers. Instead reference to TVB-N guidelines or recommendations are made – an observation that suggests it is policy not evidence that underpins many conclusions of meat product freshness.

There are different recommendations of TVB-N limits for meat freshness. These are primarily for fish and seafood products and therefore inappropriate for other meat types, species specific, arbitrary, or

have an element of ambiguity (e.g. an intermediary 'stale' classification between fresh and spoilt states). The selection of TVB-N limits can therefore introduce inaccuracy and subjectivity into the appraisal of meat freshness.

Research of TVB-N often uses inappropriate experimental designs and analysis. It is important to address this paucity to provide confidence in the mechanics between TVB-N and production, processing and preservation strategies.

Insights from animal models have shown a positive correlation between TMAO levels and development of cardio vascular disease (CVD). Among these, TMA and TMAO have been suggested as potential predictors of an individual's risk of developing CVD, coronary and kidney complications, insulin resistance, and diabetes. TMA-related compounds can act as markers for determining the risk of acquiring metabolic-related diseases in the ensuing one to three years, which emphasises the importance of understanding and quantifying TVB-N content in meat.

Apart from the impact of TVB-N on meat quality/freshness, the level and type of biogenic amines present in meat is considered a health concern, owing to their known toxic effects. A recent growing health concern has also emerged for TMA and its derivatives TMAO-N and formaldehyde (FAD), owing to their association with an array of dietary-related illnesses including cardiovascular diseases, diabetes, cancer and renal complications.

The primary method for TVB-N determination is destructive. Spectroscopic, polymerase chain reaction probes, odour sensors and electronic nose techniques have been investigated as non-destructive alternatives. Initial results are encouraging, however their capacity is limited to the differential between spoilt and non-spoilt samples through chemometric classification systems based on predefined TVB-N guidelines. Consequently, further reference populations and investigations of red meat types are required.

A frequent assumption made with non-destructive methods is the uniform distribution of the measured parameter within the food sample which is not the case for fresh meat products where a defined sampling region is planned. This is especially true for surface colour, surface microbial counts, and texture changes. Despite the lack of mechanistic relationships that can explain spectra and TVB-N, spectroscopy is a beneficial technology in generating models that could predict meat freshness with very high accuracy.

2.0 PROJECT OBJECTIVES

The aims of this project were to

- Review the scientific literature on the use and application of TVB-N and outline its applicability for red meat.
- Develop recommendations for a research plan to underpin Australian exports of beef meat with respect to robust indicators of freshness.

3.0 INTRODUCTION

Muscle foods (e.g. fish, seafood, poultry, and red meats) are known for their tendency to undergo fast degradation. This is because of their wide range of endogenous hydrolytic enzymes and their capacity to support a wide range of microorganisms (Mills et al., 2014). The decision to purchase raw muscle foods is similar to other foodstuffs, that is – motivated by its appearance (e.g. colour, size, shape, etc.) as well as other physiochemical properties such as smell and texture (Banovic et al., 2009, Reicks et al., 2011, Mao et al., 2016, Holman et al., 2020). On the contrary to the majority of foods, where a characteristic and pleasant odour is desirable (e.g. strong characteristic fruity smell), the absence of malodours in raw muscle foods is preferred. With this in mind, the sensory evaluation of meat and fish is often the most efficient and appropriate method to evaluate their freshness. Indeed, this practise is commonplace to customers around the globe. Its premise is supported by the rapid processing of information by humans and the direct relevance to each consumer's experience and constraints for muscle food acceptance.

Several chemical methods have been proposed to examine the freshness of muscle foods. These include assessments of lipid oxidation, nucleotide degradation, methylamine and related compounds, and total volatile basic-nitrogen (TVB-N) levels (Botta, 1995). These methods have been regularly used to evaluate the freshness of the seafood. For other muscle food types, lipid oxidation and, to a lesser extent, nucleotide degradation are more widely used. Nevertheless, recent studies of pork have used TVB-N to determine its quality and freshness (Cai et al., 2011, Huang et al., 2014, Xu et al., 2018, Zequan et al., 2019) and the same is true for beef (Akkose and Aktas, 2008, Lu et al., 2019). The term TVB-N describes a wide range of chemical compounds that commonly have nitrogen in their structure (trimethylamine, dimethylamine, ammonia, and other nitrogenous compounds). These compounds are formed mainly post-slaughter and accumulate during the interim prior to consumption. Past research has suggested that the generation of TVB-N is due to the decomposition of protein by the actions of enzymes and microorganisms. This is supported by several studies that observed a gradual increase in TVB-N content as chilled or frozen storage periods continued (Frank et al., 2019, Chen et al., 2020). TVB-N levels are often quantified using a standard method, with results interpreted using established guidelines, standards and freshness limits.

While the methods used in TVB-N determination, the formation of the compounds and their usefulness as quality measure have been extensively reviewed in seafood (Howgate, 2010b, Howgate, 2010a), such information are not readily available for red meat. The mechanisms of TVB-N formation

in red meat are not established nor understood. Further, the effects of the TVB-N compounds on nutritional and health aspects have not been critically examined, and the topic requires urgent attention.

4.0 AETIOLOGY OF MEAT SPOILAGE

Meat spoilage occurs when metabolic processes or microbial activity alter its physicochemical properties to a level or *limit* that is unacceptable. These limits can be established by consumers and be based on their opinions of hedonic and aesthetic meat properties. Alternatively, limits can be defined by regulatory agencies and be based on safety and health-hazard considerations. From consumers' point of view, meat is considered spoilt when undesirable changes to its sensory characteristics can be detected (Fletcher et al., 2018). This point of rejection will vary among consumers and depend on their culture/background, experience, and affordability (McCullough et al., 2012, Font-i-Furnols and Guerrero, 2014). Irrespective, to better meet the specifications of these different consumers, we should first understand the four main spoilage mechanisms. These include: 1. Microbiological; 2. Enzymatic autolysis; 3. Lipid oxidation; and 4. Protein oxidation (Dave and Ghaly, 2011).

4.1 Microbiological spoilage

Microorganisms are a major cause of quality deterioration and food spoilage. Microbial spoilage occurs when visible changes (slime and colonies formation), the development of malodours and off-flavours, and the extent of textural degradation or loss (decay) of structure exceed acceptable consumer expectations. Characteristic microbial spoilage of meats depends on the availability of glucose, lactic acid, amino acids, and other nitrogenous compounds that can be utilised as an energy source for growth. Glucose is preferentially catabolised during the first phase of microbial growth under aerobic and anaerobic conditions (Nychas and Drosinos, 2014). Lactic acid is the second major source for carbon and energy and is efficiently utilised, often after glucose substrates are exhausted.

For meat, the depletion of glycogen reservoirs in the muscles due to malnutrition or stress before slaughter will impact the ultimate pH of the meat, and this, in turn impacts on the microbial spoilage of fresh meat. For example, pre-slaughter stress causes glycogen depletion, which reduces the production of lactic acid after slaughter, and results in the production of meat with high pH (6.0-6.8) (Dave and Ghaly, 2011, Ponnampalam et al., 2017a) that is normally termed dark, firm and dry (DFD) meat. Apart from having undesirable sensory attributes, DFD meat has a reduced shelf-life

because of its high pH that supports microbial growth.

The microbial spoilage of beef and pork were investigated by Mayr et al. (2003) under refrigerated (4 °C) aerobic and vacuum packed storage conditions over an eleven day period. The authors determined the mesophilic total aerobic bacteria, *Pseudomonas* spp., *Enterobacteriaceae,* Lactic acid bacteria (LAB), and *Enterococcus* spp. counts were increased in parallel with increased volatile organic compounds (VOCs) emissions. This study used proton transfer reaction mass spectrometry (PTR-MS) to identify gradual increases in the VOCs, especially sulphur compounds, and related the VOCs to the microbial population, especially *Pseudomonas* spp. and *Enterobacteriaceae*, over the storage time. Further, trends of the determined parameters were higher in aerobic packaged samples when compared to their anaerobic vacuum-packed counterparts, regardless of the meat type. Total aerobic bacteria, *Pseudomonas* spp., and *Enterobacteriaceae* populations of aerobically stored samples were found to be correlated with several VOCs (r = 0.43-0.93). Less strong correlations were found with vacuum packaged samples. Significant correlations (0.37-0.70) were observed between *Enterococcus* spp. and VOCs in both packing systems. Strong correlations were found between LAB and the VOCs in vacuum-packed samples only.

Balamatsia et al. (2007) investigated the spoilage of fresh chicken breast under four packaging systems (aerobic, vacuum, and two modified atmosphere packaging [M1, 30%/65%/5% (CO_2/N_2/O_2) and M2, 65%/30%/5% (CO_2/N_2/O_2)]. The authors determined TVB-N, trimethylamine (TMA-N), Total viable counts (TVC), *Pseudomonads*, *Brochothrix thermosphacta*, *Enterobacteriaceae*, LAB, and yeasts populations over a 15 day storage period at 4 °C. It was found that TMA-N and TVB-N concentrations increased with the storage time, but concentrations were lower for samples held in modified atmosphere packaging.

Carnobacterium spp. was isolated from beef loin samples (a total of 103 isolates), before and after the samples had been stored under aerobic or vacuum packaging for 20 days (Ercolini et al., 2009, Casaburi et al., 2011). The samples were characterised using random amplification of polymorphic DNA (RAPD) and PCR and were identified by 16S rRNA gene sequencing. The authors carried out characterisation on 45 strains of *C. maltaromaticum* that were investigated for their growth conditions using temperature, NaCl concentration, and pH as growth variables. In addition, these authors investigated the *in vitro* lipolytic and proteolytic activities of the *C. maltaromaticum* strains. The production of VOCs in beef inoculated samples with *C. maltaromaticum* was investigated using

headspace solid-phase micro-extraction (HS-SPME)-gas chromatography-mass spectrometry (GC-MS) analysis and the samples were subjected to sensory analysis. Meat samples stored under aerobic conditions had a higher number of VOCs than the vacuum-packed samples. The authors found *C. maltaromaticum* was able to grow at low storage temperatures. A wide range of VOCs were identified (esters, aldehydes, ketones, alcohols, carboxylic acids, and sulphur compounds) where a higher number of VOCs were found for aerobically packed than the vacuum-packed beef samples (33 and 24 VOCs, respectively) with acetoin, 1-octen-3-ol, and butanoic acid found to be the dominant VOC in both packaging systems. These volatiles had a low sensory impact on the meat and had a trivial effect on meat spoilage.

Mesophilic and psychotropic spoilage bacteria in beef were identified by random amplified polymorphic DNA-PCR (Ercolini et al., 2009), and selected bacteria (*Serratia proteamaculans*, *Pseudomonas fragi*, and *C. maltaromaticum)* were further used to investigate their production of VOCs. In the beef samples, *Acinetobacter baumannii*, *Buttiauxella* spp. and *Serratia* spp. were found in the mesophilic isolates, whereas *Pseudomonas* spp. were the most common psychotropic isolate. Both *C. maltaromaticum* and *C. divergens* were found to be common isolates. *C. maltaromaticum* produced a number of VOCs, including aldehydes, lactones, sulphur compounds and other specific compounds (e.g. 2-ethyl-1-hexanol, 2-buten-1-ol, 2-hexyl-1- octanol, 2-nonanone, and 2-ethylhexanal). *P. fragi* produced the highest number of alcohols and ketones. Specific VOCs found with *S. proteamaculans* bacteria were 1-octen-3-ol, and isoamyl acetate. The major classes of spoilage bacteria associated with meat products are shown in Table 1. Extensive discussion of these bacteria and their contribution to spoilage is outside the scope of this review. Nonetheless, general observations relevant to this review are:

- In addition to their ability to grow and being active under aerobic conditions, many microorganisms can survive limited oxygen or vacuum packaged environments where the substrate may also vary. Thus, many microorganisms can be found under various packaging systems commonly used in the meat industry (Table 1).
- Microbes such as *Pseudomonas*, *Shewanella putrefaciens*, *Enterobacteriaceae* and *Lactobacillus spp.* utilise glucose as the primary source of energy under reduced oxygen or modified atmosphere (Nychas and Drosinos, 2014), and their preference toward amino acids as a source of energy occurs under limited oxygen environments. The dynamics of microbial growth and the dominant species will vary depending on the availability of preferred substrate,

oxygen, moisture, and the pH of the meat product.

- Several microorganisms (including *Pseudomonas, Photobacterium,* and *Vibrionaceae*) have the ability to contribute to the TVB-N levels as a result of the production of ammonia and methylamines. Indeed, the total aerobic bacteria count was somewhat (but not exactly) paralleled with the TVB-N level of super-chilled and frozen beef during 24 weeks of storage (Lu et al., 2019).
- Compared to studies conducted on seafood (Kobatake et al., 1988, Goulas et al., 2005, Lalitha et al., 2005, Erkan, 2007, Parlapani et al., 2019), very little research has examined the impact of packaging systems, storage conditions and the dynamics of microbiota on the generation of TVB-N in other meat types. Recently, Saenz-Garcia et al. (2020) demonstrated that TVB-N formation in chicken inoculated with *Pseudomonas* sp., *Brochothrix* sp., *Hafnia* sp., *Acinetobacter* sp., or sterile saline solution (negative control) during storage at 4 °C for eight days was in the following order; *Pseudomonas sp*. > *Brochothrix sp*. = *Hafnia sp.* > *Acinetobacter sp.* > sterile saline solution from day four onward (Figure 1). Similar studies on red meat are needed to clearly identify the main TVB-N producing bacteria and devise strategies to control their activities.

Table 1

Reported spoilage bacteria by type of meat product, substrate and spoilage compound. Abbreviations include colony-forming units (CFU), nitrogenous (N), fresh meat (FM), processed meat (PM), meat stored under a vacuum atmosphere (VP), meat stored under modified atmosphere packaging (MAP), cured meat (CM), facultative anaerobes (FA), and aerotolerant anaerobes (AT). Biogenic amines includes (phenyl-ethylamine, tyramine, methylamine, ethylamine and butylamine. [a]Perceptible changes in the sensory properties of meat occur at $>10^8$ CFU. Asterisk (*) indicate most frequently observed.

Microorganism Genus	Meat packaging	Substrate utilized by bacteria	Spoilage product	Spoilage attribute[a]	Growth conditions	Reference
Achromobacter	VP-FM, MAP	TMAO, choline	TMA	Colourless rots	Psychrotrophic	Osman and Bozoglu (2016) Clark and Burki (1972) Campbell and Williams (1951)
Acinetobacter*	FM, PM, VP, MAP	Amino acids and N-compounds, lactic acid	Methyl sulphides, esters, and acids.	Fresh beef – surface slime and off odour	Psychrotrophic, FA,	Osman and Bozoglu (2016)
Aeromonas*	FM, VP, MAP	Amino acids and N-compounds.	Methyl sulphides, esters, and acids.		FA	Iulietto et al. (2016) Osman and Bozoglu (2016)
Alcaligenes	FM, VP, MAP	Amino acids and N-compounds.	Methyl sulphides, esters, and acids.	Souring, putrefaction, gas	Psychrotrophic, prevalent in frozen meat	Osman and Bozoglu (2016), Dave and Ghaly (2011)
Alteromonas	FM, PM, VP, MAP	Amino acids, myoglobin, TMAO	Hydrogen sulphide (HS), TMA	Off odour, green discolouration	Aerobic, FA	Nychas and Drosinos (2014) Smolander et al. (2002)
Arthrobacter	FM, PM, VP, MAP	Nitrate, glucose, choline amine oxidation biogenic amines	Nitrite, TMA, acids		Aerobic storage, psychrotrophic (frozen meat)	Nychas and Drosinos (2014) Hacisalihoglu et al. (1997)
Bacillus	FM, PM, MAP	Amino acids, TMA, glucose	Acids	Souring, flat sour spoilage	FA, psychrotrophic, Present in cured bacon. Produce amino acid decarboxylases	Wang et al. (2015) Nychas and Drosinos (2014) (Fung, 2009)
Bacteroides	FM	Peptides, hemicellulose, and pectin	Short-chain fatty acids, including acetate, succinate,	Off odour	Anaerobic	Li and Guan (2017) Nychas and Drosinos (2014)

Microorganism Genus	Meat packaging	Substrate utilized by bacteria	Spoilage product	Spoilage attribute[a]	Growth conditions	Reference
		hydrogen, methylamines (TMA), L-carnitine	propionate, indole, γ-butyrobetaine			Fung (2009)
Brochothrix	FM, VP, PM, MAP (50% CO_2 – 100% CO_2)	Glucose, amino acids, ribose, glycerol, N-compounds.	Acetoiniacetyl, 3-methylbutanol, acetoin, acetic acid, methyl sulphides, esters.	Slime production, souring, greening	Psychrotrophic, FA, meat at 4 °C or frozen	Iulietto et al. (2016) Osman and Bozoglu (2016) Nychas and Drosinos (2014)
Carnobacterium	FM, PM, VP, MAP	Ribose and gluconic acid, citrate	Lactic acid, acetoin, acetate, 1-octen-3-ol, and butanoic acid	Negligible contribution to overall meat spoilage	AT, meat storage 1.5 °C	Casaburi et al. (2011)
Citrobacter	FM	Amino acids, Choline, L-carnitine	TMA, histamine, putrescine, cadaverine, and histamine	Off odour	FA, psychrotrophic, frozen meat	Jameson et al. (2018)
Clostridium	FM, VP, ham	Choline, L-carnitine	Sulphide, TMA oxygen, carbon dioxide	Methyl Sulphide odour, blown pack, bone taint	Anaerobic	Iulietto et al. (2016) Dave and Ghaly (2011)
Corynebacterium	FM, PM, VP, MAP	Amino substrates, TMAO	TMA	Off odour	AT, psychrotrophic, frozen meat	Falony et al. (2015) Nychas and Drosinos (2014)
Cronobacter (Enterobacter)*	FM, PM, VP, MAP	Glucose, lactic acid, amino acids and N-compounds.	Methyl sulphides, esters, and acids. >10^8 CFU	Off odour	FA	Osman and Bozoglu (2016) Nychas and Drosinos (2014)
Enterobacteriaceae	MAP <50% CO_2 with O_2, CM, ham	Lysine, TMAO	Hydrogen sulphide, methyl sulphide, dimethyl sulphide, hypoxanthine, cadaverine, putrescine, histamine, TMA	Putrefaction	Mainly decarboxylation of lysine Present in cured bacon (smoked, salted, ripened)	Iulietto et al. (2016) Kumudavally et al. (2001) Nychas and Drosinos (2014) Dave and Ghaly (2011)
Enterococcus	VP, MAP	Amino acids	H_2O_2	H_2O_2 greening, slime production, bone taint, souring	Produce amino acid decarboxylases	Iulietto et al. (2016) Dave and Ghaly (2011)
Escherichia	FM	Mixed acid fermentation, TMAO	Succinate, propionate, lactic acid, succinic acid, acetic acid, formic acid, ethanol, TMA	Souring	FA	Li and Guan (2017) Nychas and Drosinos (2014)
Erwinia	FM	TMAO,	TMA, mixed-acid fermentation products	Off odour	Psychrotrophic, FA, frozen meat	Nychas and Drosinos (2014)

Microorganism Genus	Meat packaging	Substrate utilized by bacteria	Spoilage product	Spoilage attribute[a]	Growth conditions	Reference
Flavobacterium	FM	TMAO	TMA	Off odour, yellow discoloration	Psychrotrophic, obligate aerobe, frozen meat, dried meat	Osman and Bozoglu (2016) Fennema et al. (2016) Nychas and Drosinos (2014) Gutierrez et al. (1998)
Hafnia	FM, VP, MAP	Amino acids	Amines, ammonia, methyl sulphides, and mercaptans	Sulphide odour, putrefaction, pinkish-red meat	FA, changes pH to alkaline	Iulietto et al. (2016) Osman and Bozoglu (2016)
Janthinobacterium	VP, MAP	Amino acids	-	-	Aerobic	Nychas and Drosinos (2014) Rosenberg et al. (2014)
Klebsiella	VP, MAP	Choline, methylated amines, amino acids	TMA, histamine	Off odour	FA, psychrotrophic, frozen meat	Nychas and Drosinos (2014) Dave and Ghaly (2011)
Lactobacillus	VP, VP-Pork, FM, PM, VP, MAP	Glucose	H_2O_2	H_2O_2 greening, slime, souring	FA, AT, 4 – 7 °C (fresh beef), cooked meats	Nychas and Drosinos (2014) Osman and Bozoglu (2016)
Leuconostoc	FM, PM, VP, MAP	Amino acids, glucose	Formic, acetic, butyric, propionic, and lactic acids, H_2O_2, gas	Greening, slime, off odour souring, swelling packages	AT, also occurs in ham, and sausage at 4-7 °C	Iulietto et al. (2016)
Micrococcus*	FM, PM, VP, MAP	Amino acids, TMAO	Methyl sulphides, TMA	Off odour	AT, present in cured bacon	Nychas and Drosinos (2014)
Moraxella*	FM	Amino acids and N-compounds, lactic acid, TMAO	Methyl sulphides, esters, and acids, TMA.	Off odour	Psychrotrophic	Osman and Bozoglu (2016)
Photobacterium		TMAO	TMA, hypoxanthine	Off odour	FA	Dave and Ghaly (2011)
Pseudomonas*	VP, VP-pork, MAP (>50% CO_2 with O_2), FM, PM, ham	Glucose, amino acids, glucose 6-phosphate, lactic acid, pyruvate, gluconate, gluconate 6-p, creatine, creatinine, citrate, aspartate, glutamate	Cysteine, cysteine, methionine. *P. fluorescens* (Methylamine, dimethylamine, trimethylamine, ethyl esters)	Fresh beef – blue spot, green rots, slime, foul odours. Ham- souring,	Frozen meat, psychrotrophic, fresh beef at 4 °C, FA, ham, dried meat	Braun et al. (1999) Dave and Ghaly (2011) Nychas and Drosinos (2014) Osman and Bozoglu (2016)
Psychrobacter*	FM, VP, MAP	variable	variable	variable	Psychrotrophic	Nychas and Drosinos (2014)

Microorganism Genus	Meat packaging	Substrate utilized by bacteria	Spoilage product	Spoilage attribute[a]	Growth conditions	Reference
Serratia	FM, PM, VP, MAP	Amino acids and N-compounds.	Methyl sulphides, esters, and acids.	Red rots, slime	FA, fresh beef at 4 °C	Osman and Bozoglu (2016) Carrizosa et al. (2017)
Shewanella	FM, VP, MAP	Amino acids and N-compounds, glucose, lactic acid, pyruvate, gluconate, propionic acid, ethanol, acetate,	Hydrogen sulphide, methyl sulphides, esters, acids, and TMA	H_2S greening	FA	Osman and Bozoglu (2016) EFSA Panel on Biological Hazards (2016) Nychas and Drosinos (2014)
Staphylococcus	FM, PM	Glucose, amino acids, TMAO	Acetate, pyruvate, and succinate, TMA	Souring, off odour	FA, cured meats, psychrotrophic	Onyango and Alreshidi (2018) Nychas and Drosinos (2014) Dave and Ghaly (2011)
Streptococcus	FM, PM, VP, MAP	Amino acids, glucose	Biogenic amines, formic, acetic, butyric, propionic, and lactic acids	Slime, greening, souring	Common in fresh meat, hamburger	Nychas and Drosinos (2014) Osman and Bozoglu (2016)
Streptomyces	FM	Amino acids		Earthy or musty odour	Aerobic	Nychas and Drosinos (2014)
Vibrio	PM, CM	Amino acids – (cysteine and methionine), TMAO	H_2S, methyl mercaptan and dimethyl sulphide, TMA	'Sulphury' Off odour	FA, salt tolerant, common at 4-10 °C	Iulietto et al. (2016) Ufnal et al. (2015) Nychas and Drosinos (2014)
Weisella	PM, VP, MAP	Glucose, amino acids	H_2O_2	Slime production, H_2O_2 greening, gas production and bulging of vacuum-packed meat	AT	Iulietto et al. (2016) Nychas and Drosinos (2014)

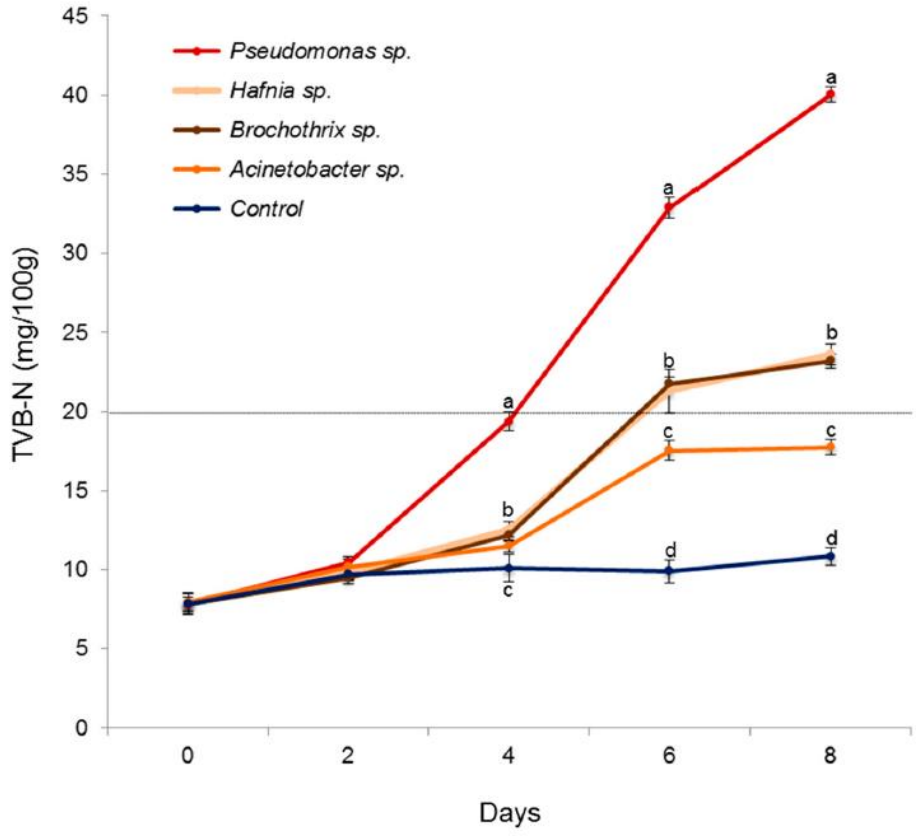

Figure 1

Fresh decontaminated meat samples were inoculated (~4.0 log CFU/g) with either Pseudomonas sp., Brochothrix sp., Hafnia sp., Acinetobacter sp., or sterile saline solution (negative control) and stored at 4 ˚C across 8 days. Total volatile basic nitrogen (TVB-N) >20 mg/100 g (pointed grey line) was used as an indicator of meat spoilage. The mean ± standard error (bars) from three independent experiments are shown. Different letters indicate the means are significantly different ($P < 0.05$). Statistical analysis was performed using ANOVA and means were separated using Fisher's protected least significant difference test. Sourced from Saenz-Garcia et al. (2020) under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

4.2. Enzymatic spoilage

Following the death of an animal, many endogenous enzymes remain active and participate in biochemical changes in the muscles pre- and post-rigor. The actions of proteases during *post-mortem* storage play an important role in the overall quality of the meat. For example, the role of the muscle's endogenous enzymes such as calpains in meat tenderisation (Bhat et al., 2018) and aminopeptidases in meat flavour (Nishimura, 1998) have been established. Similar roles have been reported for exogenous proteases produced by bacteria in a wide range of meat products (Berdague et al., 1993, Toldra, 1998, Jurado et al., 2007). While it is widely reported that TVB-N production is associated with increased enzymatic activities, especially proteases due to their decomposition/degradation of meat protein structures (Howgate, 2010b, Howgate, 2010a, Huang et al., 2014), the relationship has not been confirmed clearly by experimental evidence. For example, the addition of a protease extracted from *Pseudoalteromonas* sp. NJ276 obtained from Antarctic sea ice to the meat of bigeye tuna produced lower TVB-N and TMA-N concentrations compared to non-treated control samples (Quan-fu et al., 2012). Conversely, the production of TVB-N and TMA were decreased in irradiated fish myofibrillar protein compared to unirradiated samples upon treatment with protease-producing bacteria (which included *Aeromonas hydrophila, Bacillus megaterium, Pseudomonas marinoglutinosa , and Salmonella typhimurium*), where lower bacterial protease activity was found in irradiated fish myofibrillar protein compared to the unirradiated samples (Alur et al., 1993). The authors suggested that irradiation of the bacteria reduced its ability to produce proteases. Rodríguez et al. (2003) reported a linear increase in TVB-N and TMA-N in Turbot stored on ice kept in a chiller at 2 °C between 19-40 days of storage only. The authors determined the count of four microbial groups (aerobic, anaerobic, coliforms, and proteolytic bacteria) and an increasing trend in the 19-40 day storage period was only observed in the anaerobic and coliform bacteria, suggesting their potential involvement in the production of volatile nitrogenous bases.

When the above information and those presented in Section 4.1 (Microbiological spoilage) are considered together, it is suggested that specific proteases produced by certain microorganisms (e.g. anaerobic and coliform) or these microbial groups themselves produce other complementary compounds that facilitate the decomposition of protein. This contention is aligned with the suggestion that physical alterations in the muscle structure facilitate proliferation of the bacteria, which produce ammonia and sulphur compounds, volatiles and non-volatile bases (Berdague et al., 1993, Toldra, 1998, Jurado et al., 2007, Dave and Ghaly, 2011). It is worth noting that all the studies investigating the

contribution of proteases and proteolytic activities (both endogenous and exogenous) from bacteria have been conducted on seafood and similar studies on red meat are urgently needed to understand the phenomena in red meat context. There are several significant compositional and biochemical differences between red meat and seafood, and it is expected that these differences could lead to different outcomes than those reported for seafood.

4.3 Lipid oxidation

Lipid oxidation is the reaction of unsaturated (containing double bonds) fatty acids (UFA) with molecular oxygen resulting in rancidity and deterioration of fats. Recent comprehensive reviews on lipid oxidation are already available (Papuc et al., 2017, Domínguez et al., 2019). Three main UFA oxidation pathways have been documented, including autoxidation, photo-oxidation, and enzymatic hydrolysis, with the activity of the latter being of little significance in meats and meat products (Mariutti and Bragagnolo, 2017). Autoxidation of fatty acids (FA) occurs in three main steps (initiation, propagation, and termination) and results in malodours. The generation of hydroperoxides, carbonyls, and hydrocarbons causes off odours that lead to rejection of the products. Lipid oxidation may be mediated by the actions of lipases (EC 3.1.1), lipoxygenases (EC 1.13.11), and cyclooxygenase (EC 1.14.99.1), as well as several enzymatic systems involved in cholesterol oxidation (Papuc et al., 2017). It is generally believed non-enzymatic lipid oxidation plays a major role in lipid oxidation in meat and that lipoxygenases contribute to the reaction to a lesser extent (Papuc et al., 2017). Several studies have reported lipoxygenase and lipoxygenase-like activities in pork (Min and Ahn, 2005, Jin et al., 2011), chicken (Grossman et al., 1988), beef (Min et al., 2008) and fish (Mohri et al., 1990, Hsu and Pan, 1996). Lipoxygenase in chicken was found to be stable during storage at -20 °C for one year, and it was suggested it could contribute to lipid oxidation during frozen storage (Grossman et al., 1988). Min et al. (2008) reported that raw beef loin had higher lipoxygenase-like activity than chicken and pork. The term “lipoxygenase-like activity” was used since the activity was determined using crude meat extracts that catalysed the formation of conjugated dienes from the substrate linoleic acid, where the oxidation could also be affected with other components (reducing activity and myoglobin derivatives). Interestingly, in the study of Min et al. (2008) the lipoxygenase-like activity in raw beef loin was increased during 7 days of storage at 4 °C, whereas the activity was decreased in chicken (breast and leg muscles) and pork loin samples. Lipoxygenase obtained from fresh pig belly was found to have maximum activity at 39 °C and pH $\geq$ 9.0, and the activity was reduced by the use of NaCl above 3% (Jin et al., 2011), but this does not explain the suggested species effect. The pH of normal meat cut

(≅ 5.5) and normal storage conditions of fresh meat (≅ 5°C) suggest unfavourable conditions for this activity.

Many of the above-mentioned enzymes (lipases, lipoxygenases, and cyclooxygenase) are secreted by bacteria (Brenda, 2020), and they may be involved in lipid oxidation during the storage of meat. However, this has not been widely investigated in fresh meat. Increased lipolysis and generation of volatiles have been well documented in fermented meat products (Selgas et al., 1993, Toldrá, 2008). A recent study on Chinese-style sausage (Bian et al., 2019) demonstrated the changes in 13-hydroxyoctadecadienoic acid (13-HODE) and 9-hydroxyoctadecadienoic acid (9-HODE) during the curing and early drying stages were catalysed by lipoxygenase, but the source of the lipoxygenase (the meat or microbial) was not distinguished in that study. The total phospholipase, acid lipase, and neutral lipase activities in Cantonese-style sausage were investigated (Shang et al., 2019). The lipase activity was in the following order; neutral lipase > acid lipase > total phospholipase, and the activities were decreased over three days of processing. Carbonyl value was correlated with neutral lipase and total phospholipase activities. Again, the reported activities of the samples were not differentiated by its source (microbial or meat).

4.4. Protein oxidation

Protein oxidation has attracted much interest in recent years as a hot new topic, especially in meat science. Various reviews on the topic that examined protein oxidation (Davies and Gardner, 1996), protein oxidation in muscle foods and its effects on meat quality (Lund et al., 2011, Bekhit et al., 2013, Zhang et al., 2013b), the chemistry and mechanisms of protein carbonyls formation in meat systems (Estévez, 2011), general mechanisms of protein oxidation in muscle foods and their effects on human health (Soladoye et al., 2015, Papuc et al., 2017). Further, the impact of redox status and sulphur amino acids on protein oxidation (Estévez et al., 2020), and lipid-protein oxidation interactions (Guyon et al., 2016, Papuc et al., 2017) have been reviewed. It is well established that protein oxidation induces significant modifications in meat, affecting its nutritional (e.g., reduced protein digestibility and loss of amino acids), functional (e.g. solubility, water holding capacity, emulsifying, gelling, binding, hydrophobicity, susceptibility to enzyme hydrolysis, and inactivation of enzymes), and sensory (e.g. colour, flavour, texture, and juiciness) properties. Whether the oxidation occurs on the side chains of the amino acids or the protein's backbone, modification affects the ability of the protein to undergo biochemical reactions and subsequently, its physiological properties. Only off-flavour and discoloration as consequences of protein oxidation may affect the marketability of meat products. However,

defining spoilage as an “unfit for use” concept, protein oxidation could have significant commercial effects due to lower yield of processed products, poor utility, and discrimination against the product due to poor quality.

The health effects of oxidised proteins have been the subject of consideration for two reasons:

1) The inability of the digestive system to have effective access to breakdown aggregated proteins; and
2) The formation of protein hydroperoxides.

The reduction in protein digestibility decreases amino acid residue bioavailability and leads to undigested protein becoming available for fermentative actions of colonic microbiota, which will lead to several outcomes. While some published reviews (Rowland and Hughes, 2000, Papuc et al., 2017) have focussed on negative effects and suggested potential intestinal tumorigenesis and reported the occurrence of small intestinal neoplasms and adenocarcinoma upon digestion of digestion-resistant potato, information from these reviews suggests increased production of short-chain fatty acids (SCFA), branched-chain fatty acids (BCFA) such as isobutyrate, isovalerate, and 2-methylbutyrate, and organic acids that might be beneficial to health. For example, SCFAs are known to be neuroactive compounds that play an important role in the gut-brain axis and consequently, human behaviour (Cryan et al., 2019, Cryan et al., 2020, Roubalová et al., 2020). In addition, BCFAs have been suggested to be health-benefiting compounds (Ran-Ressler et al., 2011). It should be mentioned that SCFA production during protein fermentation is dependent on the availability of carbohydrates, where reduced total SCFA concentration was reported in the absence of carbohydrates (Yao et al., 2016). Furthermore, small peptides generated from the breakdown of proteins show high antioxidant capacity and have the ability to scavenge ROS and to activate antioxidant enzymes (Wang et al., 2016, Yu et al., 2016, Zhao et al., 2017) leading to improved antioxidant-prooxidant balance in the gut.

On the other hand, the production of ammonia, amines, phenols, indoles, cresols, N-nitroso compounds, and sulphides from the fermentation and series of modifications (oxidation, deamination, and decarboxylation) of sulphur-containing amino acids (cysteine and methionine) and aromatic amino acids (tyrosine and phenylalanine) can create significant health problems such as irritable bowel syndrome, ulcerative colitis and colorectal cancer (Rowland and Hughes, 2000, Yao et al., 2016). These metabolites can exert toxic effects such as changing the microbiota communal structure supporting the growth of pathogenic bacteria, causing mucosal inflammation and modulate the intestinal motility

(Yao et al., 2016). The formation of protein hydroperoxides is facilitated by several radicals and some two-electron oxidants such as singlet molecular oxygen (Davies, 2016). In the absence of metal ions and reducing agents, the lifetime of protein hydroperoxides is in the order of days at room temperature. This lifetime is increased substantially in the order of months to years with a reduction in temperature to < -20 ˚C (Davies, 2016). The presence of transition metals, such as in red meat, can accelerate the decay of the hydroperoxides. However, free iron can catalyse the production of peroxide radicals as well as degradation of lipid peroxides to produce peroxyl and alkoxyl radicals.

Considering the above pathways, the roles of microbial proteases and endogenous meat proteases in degrading protein (Figure 2) to yield free amino acids that undergo metabolism by microorganisms yields a wide range of volatile compounds that are associated with the loss of freshness (Table 2).

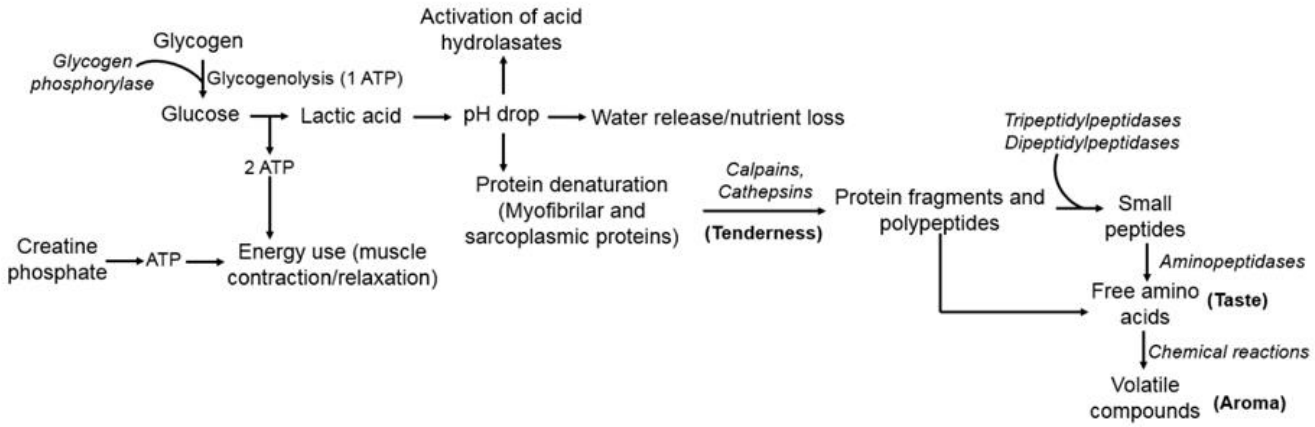

Figure 2

Scheme of anaerobic glycolysis and proteolysis in *post-mortem* muscle. Modified from Nip et al. (2006)

Table 2

Metabolic products of amino acid degradation. Contents modified from Paczkowski and Schütz (2011).

Amino acid	Pathway, Enzyme Source, and Conditions of Degradation	Metabolic products
Leucine	Ehrlich pathway, anabolism	propan-1-ol, 2-methyl-propan-1-ol, 2-methyl-butan-1-ol, 3-methyl-butan-1-ol
	M. phenylpyruvica, *S. xylosus*, *S. starnosus*	3-methyl-butan-1-ol, 3-methyl-butanal, 3-methyl-butanoic acid
Isoleucine	Ehrlich pathway, anabolism	1-propanol, 2-methyl-propan-1-ol, 2-methyl-butan-1-ol, 3-methyl-butan-1-ol
	Yeast	propan-1-ol, 2-methyl-butan-1-ol, 3-methyl-butan-1-ol, pentanol
Threonine	Yeast	1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, pentan-1-ol
Arginine > Ornithine	Ornithine decarboxylase	putrescine
Lysine	Lysine decarboxylase	cadaverine
Tyrosine	*S. albus*, *B. fragilis*, *Fusobacterium* sp., *Bifidobacterium* spp., *C. paraputrificum*, *C. butyricum*, *C. sporogenes*, *C. septicum*	4-methylphenol (anaerobe)
Phenylalanine	*E. coli*, *Proteus* sp., *E. faecalis*, *S. albus*	2-phenylethanol, phenylacetaldehyde, phenylacetic acid
		phenol (facultative anaerobe)
	Phenylalanine decarboxylase + Fe^{3+}	green complex
	Pseudomonaceae (aerobe)	2-phenylethanol, phenylacetaldehyde, phenylacetic acid
		phenylpropanoic acid
	M. phenylpyruvica, *S. xylosus*	ethylbenzene, benzaldehyde, benzonitrile, 2-hydroxybenzaldehyde
	P. putida, *E. coli*, *K. pneumoniae*, *B. halodurans*	ethenylbenzene, ethylbenzene
	Denitrifying bacteria	1-phenylethanol, phenylethanone, benzoyl-acetate
Tryptophan	Bacteroides, *Lactobacillus*, *Clostridium*, *Bifidodobacterium*, *Peptostreptococcus*	indole, indoyl acetic acid and indoyl propanoic acid
Cysteine	Anaerobe	Elemental sulphur, hydrogen sulphide
		Hydrogen sulphide, dimethyl sulphide, dimethyl disulphide, dimethyl trisulfide, dimethyl tetra sulphide
Methionine	Aerobe	Methanethiol, dimethyl disulphide, dimethyl trisulphide
		Dimethyl sulphide
	H. alvei, *E. agglomeran*, *S. liquefaciens*, *A. putrefaciens*, *A. hydrophila*	Methanethiol, dimethyl sulphide

5.0 DETERMINATION OF MEAT FRESHNESS OR SPOILAGE

Freshness of muscle food is paramount since it is associated with good eating quality (presence of inosine monophosphate, texture, and residual glucose) and lack of any off-flavours due to microbiological activities or enzymatic reactions. As mentioned above, oxidative processes and hydrolytic reactions caused by bacteria and enzymes are the leading causes of meat spoilage. More generally, methods used for the determination of spoilage/freshness of meat products can be broadly classified as either destructive or non-destructive methods. Destructive methods require preparation, extraction, and manipulation of the sample and this may cause undesirable changes in the samples and have the characteristics of being laborious and expensive. Destructive methods include, but are not limited to sensory evaluation (subjective and objective); microbiological analysis (total viable count and specialized tests); pH to determine DFD meat; ATP and its degradation products; biogenic amines; lipid oxidation methods (thiobarbituric acid reactive substances (TBARS), conjugated dienes, peroxide value, HPLC, GC analysis of free FAs, FA profile, and cholesterol oxidation co-products); protein oxidation (colorimetric, fluorescence, SDS-PAGE, turbidity, column chromatography, proton-transfer-reaction mass spectrometry (PTR-MS), LC-MS MALDI or ESI MS/MS, and HPLC/UV); gaseous and volatile spoilage indicators (gas chromatography-mass spectroscopy (GC-MS), and gas chromatography olfactory (GCO), TMA, DMA, TVB-N, NH_3, CO, H_2O, NO, and SO_2).

Non-destructive methods use a large number of techniques that do not require too much manipulation of the samples, and thus provide data that reflects actual sample conditions, without modifications caused by the various preparation steps (Chen et al., 2014b, Huang et al., 2015a, Kucha and Ngadi, 2020). Furthermore, they are generally fast and in many cases, are inexpensive and safe alternatives to traditional chemistry methods (Cozzolino and Murray, 2002, Cozzolino et al., 2003, Barbin et al., 2013). Examples of technologies used in non-destructive methods are colour measurements; imaging (computer vision, hyperspectral imaging, and near-infrared hyperspectral); spectroscopy (Fluorescence, Near-Infrared Reflectance, Raman, and Fourier Transform Infrared spectroscopy); electronic nose; electron spin resonance; and conductivity meters, metal oxide composites and probes (Table 3a). Given the diversity of the techniques and assays, these have been categorically reviewed.

Table 3a

Spectroscopic method and technique used to determine meat freshness or extent of spoilage

Method/technique	Spoilage measurement	Approach	Sensitivity/response time	Comments	References
FT-NIRS	TVB-N	Prediction model	Prediction coefficient of determination R = 0.81	A rapid and non-destructive method	Cai et al. (2011)
NIR hyperspectral imaging	Total viable count TVC and psychotropic plate count (PPC), surface colour attributes	Prediction of TVC and PPC using near-infrared (NIR) range (900–1700 nm) coupled with computer	Coefficient of determination (R2) > 0.82 between NIR-spectral data and TVC/ PPC. A 95% accuracy of NIR-linear discriminant analysis.	Relation of chemical changes during microbial spoilage to NIR spectral data.	Barbin et al. (2013)
Fourier transform infrared spectroscopy (FTIR)	Microorganisms	Detection of aerobic spoilage using FTIR and adaptive learning - Fuzzy Inference Neural Network system (AFINN) for monitoring microbial spoilage/biochemical activity	Two minutes	Rapid detection of spoilage from the surface.	Alshejari et al. (2015)
GC-MS	Volatile organic compounds (VOCs)	Quantitative identification of VOCs	-Correctly measured 39 VOCs -Sensitive to potential indicators of meat spoilage - nonanal, benzaldehyde, 1 1-octen-3-ol, acetic acid, and 1-hexanol	High correlation between spoilage progression and level of VOCs and microbial composition	Li et al. (2020)
Mass Spectroscopy	Volatiles organic compounds	Proton transfer reaction mass spectroscopy for continuous detection of volatiles organic compounds	-PTR-MS has low sensitivity when meat headspace contain compounds with the same nominal mass, i.e. isomers and isobars.	Real-time measurements	Frank et al. (2020) Franke and Beauchamp (2017)
Raman spectroscopy	Overall quality	Prediction of sensory tenderness, chewiness and juiciness based on Raman spectroscopic data	<10 s for acquisition of Raman spectral 83 % correlation with sensory data	Potential for rapid, on-line screening High correlation with sensory panel for tenderness and chewiness	Wang et al. (2012)
Portable non-invasive optical sensor using fluorescence	Oxygen and carbon dioxide	Detection from the headspace volatiles	CO_2 increase 700 – 3800 ppm -O_2 decrease – 7.5-7ppm	Rapid non-destructive method	Anusankari et al. (2019)

Table 3b

Advances in the evaluation of meat freshness using chemo-sensors. Abbreviations include intelligent packaging (IP), and metal-organic semiconductor sensor (MOS).

Method	Method/technique	Spoilage measurement	Approach	Sensitivity/response time	Comments	References
Chemical responsive films	Colorimetric sensor – (optoelectronic nose)	Microbial and sensory analysis	Combined pH sensors and metabolites selective chromogenic reagents. Sensory evaluation.	Colour differences compared using PLS model to predict mesophilic aerobic counts, sensory scores, and storage time (R^2 = 0.9381, 0.9300, and 0.9472).	Able to differentiate samples at different storage times. Provide characteristic colorimetric fingerprints. Samples classification, according to freshness (85% of correct classification.	Salinas et al. (2014)
	Indicator dyes immobilised onto cellulose micro-particles covalently embedded in food-grade silicone	Ammonia and biogenic amines (TVB-N), meat spoilage	Natural dyes responsive to amine changes colour from green to dark red	Response time 1.5 h and the reverse response of 20 h	Detection of colour change based on the CIE system	Schaude et al. (2017)
	Colorimetric NH_3 indicator films	NH_3 measurement	Packaging film using Tara gum/polyvinyl alcohol incorporating curcumin	1-3 minutes	Rapid system that could be used for TVB-N	Ma et al. (2017)
	Colorimetric array indicator for	Protein decomposition products - NH_3 and CO_2 detection	Colour patterns produced by the gaseous compounds are developed by the multicolour array. Obtained images are used in multivariate analysis to discriminate the concentrations of NH_3 and CO_2.	Not stated	Potential use during storage and in intelligent packaging	Huang et al. (2011) Zhang and Lim (2018)
	Polyaniline film-based chemical sensor	Microorganisms and TVB-N	Colour development by the sensor correlated to microbiological analysis and TVB-N	2 hrs	Good correlation between colour developed by the sensor and TVB-N concentrations, total viable count and Pseudomonas spp.	Matindoust et al. (2017) Kuswandi et al. (2012)

Method	Method/technique	Spoilage measurement	Approach	Sensitivity/response time	Comments	References
	Poly(ortho-phenylenediamine-co-aniline) copolymer as a sensor for freshness	TVB-N and microbial (Total and *Pseudomonas spp*.)	Colour development by the sensor was correlated to microbiological analysis and TVB-N	Variable depending on TVB-N concentration	Colour changes in the copolymer was correlated with the TVB-N concentration and microbial growth patterns.	Domínguez-Aragón et al. (2018)
	Titanium dioxide-Polyaniline/Silk fibroin fibre (TiO_2-PANI/SFF) composite	NH_3 and TVB-N	TVB-N measurements in pork.	Response time of 10 s to NH_3 (100 μg/L). Correlation of R2= 0.990 for TVB-N levels in pork and 6 mg/100 g minimum response.	Linear discriminant analysis (LDA) predicted the freshness of pork at 86.38%,	Shi et al. (2018b)
	Artificial vision technique and pattern recognition algorithms.	Colour and texture	Determination of freshness using surface texture and colour	93.83% success in identification of surface colour and texture associated with spoilage	Non-destructive real-time prediction of spoilage	Arsalane et al. (2019)
	Amine-responsive bilayer films - agar (AG), anthocyanins (AN), gellan gum (GG) and TiO_2 nanoparticles	Gas sensor - TMA	AG-AN layer – volatile amine sensor GG-TiO_2 layer – protective conducting layer/light barrier	0.018 mM TMA.	Electrochemical writing. Detection limit of 7.54 mg/100 g Colour change indicator – red-to-green	Zhai et al. (2020)
	Au patch electrode Ag-SnO_2/SiO_2/Si Metal-insulator-semiconductor capacitive sensor	Gas Sensor (NH_3, TMA, ethanol, and H_2S)	Detection of gas in meat samples and measurement of TVB-N, TVC, pH and sensory evaluation	Concentrations in the 25 ppb–10 ppm. Times for response and recovery are 55 and 90 sec, respectively.	A high correlation coefficient was found for TVB-N, TVC, pH, and sensory evaluation results	Senapati and Sahu (2020)
	L-cysteine-modified gold electrode - polyglutamate–glucose oxidase (GOx) complex	Glucose	Detection of glucose depletion/onset of nitrogen degradation	Not provided	Fast and reliable for detection of glucose in meat and onset of off odours development	Choi et al. (2017)
	pH indicator film (IP)	Meat spoilage sensor	Based on natural dyes, anthocyanins on agar/potato starch films base	Visible with pH changes of ~ 0.1	Colour change correlated with pH increase	Pereira et al. (2015)
	pH-based time temperature indicator	Freshness monitor	Anthocyanins on poly-vinyl alcohol /chitosan polymeric base	Visible with pH changes of ~ 0.4	Colour change correlated with pH decrease	Kumudavally et al. (2001)

Method	Method/technique	Spoilage measurement	Approach	Sensitivity/response time	Comments	References
High-performance thin-layer chromatography		Cadaverine	Fractionation separation of cadaverine from other amines	1-5 ppm cadaverine in 30-40 min (5-6 samples). Detection of 3 h post-mortem quality	Rapid detection of bacterial quality. 1-5 ppm cadaverine related to 10^7 CFU/mL	Papadopoulou et al. (2011)
		Volatiles	Identification of volatile fingerprints and use of chemometrics to determine meat quality	>76 % correct classification of meat freshness	Freshness of meat was partially discriminated by Principal component analysis (PCA). The use of stochastic resonance (SR) signal-to-noise ratio (SNR) spectrum was successful in discriminating meat samples and various experimental conditions.	Li et al. (2014)
Electronic nose	Portable electronic nose (E-nose) based on a colorimetric sensor array of pork	Spoilage monitoring using volatile organic compounds	12 chemically responsive dyes on a silica-gel base giving specific fingerprint for volatile compounds	Discrimination rate of 97.5 % on prediction assay using back propagation artificial neural network (BP-ANN) model.	Real time monitoring of meat freshness Correlated with TVBN and TVC	Xiao et al. (2014b)
	Real-time responses of e-nose sensors to Beef strip loins	Headspace volatile measurements	Based on MOS sensors	Distinguish fresh from not fresh at day 9 of storage	High accuracy prediction in daily spoilage differences of beef High correlation with TVBN response increase with storage time	Salinas et al. (2014)
	incorporation of pH indicators and chromogenic reagents		Colour modulations of the chromogenic array processed using principal component analysis (PCA) and partial least squares (PLS)	Discrimination of sample freshness from day-to-day	High correlation between the sensory score and conventional freshness tests (TVC).	Gil et al. (2011)
Redox Potential (Eh)	Redox electrode (Platinum rod and oxidized iron rod moulded in resin) measures electrical signals and connected to a computer. Parallel colour measurement of meat juice using image analysis	Oxidation of myoglobin	Colour changes and Eh measurements under various conditions	unknown	The system is not well defined, and more research is required with robust experimental design.	Cucci et al. (2020)

5.1 Multivariate exploratory techniques

Multivariate exploratory techniques have been widely used to detect meat spoilage and monitor its quality. Interrelationships among the various observed parameters (e.g. sensory, TVC, TVB-N, TBARS, and pH) are typically observed under controlled experimental work to define the freshness of meat under refrigeration and frozen storage (Custodio et al., 2018). In many studies, the measurements have been used at room temperature to demonstrate the effect of temperature abuse, accelerated shelf life testing or effectiveness of the developed sensors (Kuswandi et al., 2012, Domínguez-Aragón et al., 2018, Senapati and Sahu, 2020, Zhai et al., 2020). Studies conducted in this category have used mostly destructive methods and involve cumbersome sample preparation steps and time-consuming procedures (Kodogiannis, 2016). Recent development in metal oxide-based gas sensors (Galstyan et al., 2016, Galstyan et al., 2018) have provided an opportunity to develop probes that are sensitive to a wide range of gaseous and volatiles produced by food during storage and sales (Table 3a and Table 3b). The main advantages of this new generation of sensors are their ability to assess, provide rapid real-time monitoring of the product quality, and be integrated into existing quality management systems (Galstyan et al., 2018).

5.2 Non-destructive methods

The use of non-destructive methods in the assessment of meat quality and detection of meat spoilage is gaining popularity due to their obvious advantages of being less laborious, rapid, less sample manipulation and interference as well as their ability to be adapted in inline/online systems (Kamruzzaman et al., 2012, Feng and Sun, 2013, Chen et al., 2014b, Xiong et al., 2015). A frequent assumption made in non-destructive methods is the uniform distribution of the measured parameter within the food sample (Barbin et al., 2013), which is not the case for fresh meat products where a defined sampling region is planned. This is especially true for surface colour, surface microbial counts, and texture changes (Alshejari et al., 2015).

5.2.1 Rapid methods

The changing demands in the food supply chain have accelerated the development of rapid quality and safety assessment methods. These methods involve real-time measurement of organoleptic properties associated with bacterial growth and biochemical activities (Barbin et al., 2013, Alshejari et al., 2015, Dong et al., 2019). Rapid detection techniques such as mid- and near-infrared spectroscopy, electronic nose (chemo-sensors), hyperspectral imaging technology, conductivity meter,

and colour chemo-sensors (Table 3a and Table 3b) can determine multiple physicochemical properties simultaneously. The level of accumulation or loss of certain chemical compounds is then related to the level of meat spoilage using predetermined reference-quality attributes and compared with standard methods. Rapid quality detection methods involving single or combined techniques are discussed in the following sections.

5.2.2 Polymerase chain reaction

Polymerase chain reaction (PCR) and deoxyribonucleic acid probes are designed to provide rapid detection and identification of microbial species, which is specifically useful for the identification of pathogenic strains of microbes (Fletcher et al., 2018). These methods allow specific and rapid detection of microbes as an alternative to the conventional microbial enumeration techniques (Gutierrez et al., 1998, Yost and Nattress, 2000), which reduce products holding time, cost and allow prediction of the products' safety through the production chain. The drawbacks of PCR methods is they may not be able to differentiate between viable and dead cells (Ma et al., 2017), and they have low reproducibility especially when used in the identification of closely related bacterial strains (Doulgeraki et al., 2012). PCR tests or PCR combined with other techniques such as microarray, ELISA, bioluminescence, and flow cytometry, have been applied to monitor changes in meat quality. Ercolini et al. (2010) demonstrated the use of PCR-denaturing gradient gel electrophoresis to monitor changes in the microbial profile of meat microbiota by analysis of 16S rRNA gene of DNA extracted directly from meat. Recently, Martins et al. (2020) combined the high specificity and selectivity of quantitative PCR (qPCR) and traditional plate count techniques to develop mathematical models for prediction of microbial growth of isolated species. Although molecular techniques can correctly identify and quantify specific strains of bacteria in many meat studies, little is known regarding specific strain-specific volatile compounds relationships. Odeyemi et al. (2019) studied the use of 16S rRNA amplicon sequencing to characterize the microbes involved in spoilage of fresh mussels under modified atmosphere packaging. The authors were able to identify *Shewanella baltica* as a producer of H_2S and other spoilage volatile metabolites. The bacteria can accept electrons from trimethylamine-N-oxide (TMAO) in the absence of oxygen and are capable of surviving under aerobic and anaerobic conditions.

Nieminen et al. (2011) used the 16S rRNA gene-targeted terminal restriction fragment length polymorphism (T-RFLP) method and traditional specific enumeration techniques to examine microbial LAB in minced (80% pork and 20% beef) meat under modified atmosphere packaging (MAP) (65% O_2 + 25% CO_2 + 10% residual air). Both analyses techniques found that *Leuconostoc spp*. and

Carnobacterium spp. were the dominating LAB in the samples during cold storage for eight days, followed by *Lactobacillus algidus*, *Lactococcus spp*. and *Weissella spp.* (Chen et al., 2020).

PCR technology was used to investigate microbial diversity in goat meat stored in MAP under refrigerated conditions (Carrizosa et al., 2017). The technique showed that *Hafnia alvei* dominated high CO_2-low O_2 (45% CO_2 + 20% O_2 + 35% N_2) samples whereas *Serratia proteamaculans* dominated the low CO_2-high O_2 samples (20% CO_2 + 55% O_2 + 25% N_2). The study highlighted the potential use of PCR in monitoring bacterial species in meat during storage and documented the critical role of modified atmosphere packaging and its composition in controlling the microbial population. Li et al. (2019a) used 16S rDNA data to predict microbial metabolic pathways associated with spoilage of pork during refrigerated storage with *Pseudomonas, Acinetobacter* and *Photobacterium,* the dominating species during the aerobic storage of pork at 4 ˚C. The concentration of TVB-N and the changes in pH were correlated with TVC. The studies, as mentioned above, support the use of PCR and other molecular techniques as tools for rapid and high-throughput identification of microorganisms and their potential use as predictive tools to identify microorganisms involved in the generation of volatile detection. The potential use of 16S rRNA sequencing as an operational tool to predict VOCs have also been proposed (De Vrieze et al., 2018), but the concept has not been widely investigated in red meat studies.

5.2.3 Spectroscopy

The ability to measure reflected, absorbed and emitted light over a broad wavelength spectrum has been utilized with the aid of chemometric analysis to investigate the quality and freshness and determine the authenticity of foods using a wide range of techniques (Fletcher et al., 2018). These include laser-induced breakdown spectroscopy, Raman spectroscopy, fluorescence spectroscopy, Mid-infrared, Near-infrared, attenuated total reflectance infrared spectroscopy, nuclear magnetic resonance [low field, ^{1}H and ^{13}C, ^{31}P, low-resolution, high-resolution, magnetic resonance imaging] (Table 3a). Others spectroscopy techniques are NMR-mobile universal surface explorer (NMR-MOUSE), impedance spectroscopy, acoustic spectroscopy, and dielectric spectroscopy (Fletcher et al., 2018).

Infrared spectroscopy (IRs) involves the characterisation of samples based on their interaction with infrared light. The light that is emitted, absorbed, or scattered by a material in the spectral region between 4000 and 700 cm^{-1} can be quantified as a basis of information on fundamental vibration and

stretching of the dipole moment of specific functional groups of molecules. The technique can be used to study and identify materials or substances that vary biologically (Reddy Gangidi and Proctor, 2009, Fletcher et al., 2018). Modification of the functional groups C-H, O-H, N-H, and S-H in the products due to microbial activity or chemical/ biochemical activity (e.g. lipid and protein oxidation) can be easily visualized in the FT-IR spectra. Spectral techniques have attempted to translate the chemical changes during spoilage to IR spectral data (Barbin et al., 2013). The efficiency of the techniques is measured by correlation measurements between the spectral method and a standard determination procedure.

The near-infrared spectroscopy (NIR) fibre-optic reflectance probe radiation wavelength ranges from 12500 to 4000 cm^{-1} on the electromagnetic spectrum (Atanassova et al., 2018). NIR hyperspectral images are obtained in reflectance mode using *push broom* hyperspectral imaging system mounted with a camera (Barbin et al., 2013). Recorded hyperspectral images are three dimensional with a spatial dimension of x $\times$ y pixels (for example, 320 $\times$ 450 pixels in the x and y-axis respectively). Barbin et al. (2013) obtained hyperspectral images in the NIR range of 897 – 1753 nm with a spectral increment of 3.34 nm. The authors reported a coefficient of determination (R^2) of > 0.82 for the prediction of TVC and psychotropic plate count using NIR-hyperspectral imaging. NIR and MIR to determine the beef product quality covers other aspects, including adulteration, tenderness, chemical composition.

Fourier transform Near-infrared spectroscopy (FT-NIR) is a simple, fast, and non-destructive method for monitoring changes in meat freshness during storage (Sinelli et al., 2005, Huang et al., 2012). Cai et al. (2011) suggested the use of FT-NIR to predict meat tenderness and TVB-N. The authors used Warner–Bratzler shear force (WBSF) and TVB-N methods to evaluate the predictive capability of FT-NIR and to develop the models using a synergy interval partial least square (SI-PLS) algorithm to predict textural properties and TVB-N of pork meat. Although the prediction model presented a moderate coefficient of determination (R^2 = 0.70), the technique could present a rapid and non-destructive method of texture/spoilage prediction. The correlation coefficients in the calibration and prediction sets were Rc = 0.84 and Rp = 0.81 for TVB-N; and Rc = 0.75 and Rp = 0.70 for WBSF, respectively. Fluorescence spectroscopy was used to quantify adenosine triphosphate (ATP) content and plate count in pork aerobically stored at 15 °C for three days (Oto et al., 2013). Excitation (Ex) and emission (Em) matrices of fluorescence intensity for tryptophan (Ex = 295 nm and Em = 335 nm) and nicotinamide adenine dinucleotide phosphate (NADPH) (Ex = 335 nm and Em = 450 nm) were obtained and related to microbial growth using partial least squares (PLS) regression analysis. The generated

model predicted the ATP content and TVC with a high R^2 of 0.94–0.97 for the calibration set and 0.84–0.88 for the validation set. A similar approach was applied by Sahar and Dufour (2014), who used FT-IR (4000–1000 cm^{-1} range) coupled with attenuated total reflectance (ATR) accessory to investigate the microbial population in chicken breast meat stored aerobically at 5 °C for eight days, and at 15 °C for five days. The method was successful in determining total viable count (TVC), *Pseudomonas*, *Enterobacteriaceae*, and *B. thermosphacta* in the samples. PLS regression was successful in classifying the samples across the various storage times using four PLS factors. The freshness of pork (90 samples) was also evaluated by determining the TVB-N content using NIR, computer vision, and electronic nose techniques (Huang et al., 2014) after treatment with several pure cultures *Bacillus fusiformis J4*, *Acinetobacter guillouiae P3*, *Pseudomonas koreensis PS1* and *Brochothrix thermosphacta S5* isolated from chilled stale pork in addition to a control fresh group. Several mathematical systems (principal component analysis and back-propagation artificial neural network (BP-ANN)) were used to develop a model to predict the concentration of TVB-N in pork. The model that had the three methods had a high accuracy with an R^2 of 0.95 but is not a practical approach.

Visible and near-infrared (Vis/NIR) reflectance spectroscopy were used to investigate their ability to determine the concentrations of TVB-N content in duck breast meat (Qiao et al., 2017). The models were constructed using partial least square regression (PLSR) between data obtained from the spectra of the samples and results from the standard TVB-N method. The analysis of the spectral data was carried out using synergy interval partial least squares and principal component analysis methods to obtain the appropriate set of wavelengths required for discrimination among the samples. The derived model had a correlation coefficient (Rp) of 0.86 (RMSEP of 1.060 mg/100 g). The study suggested the potential use of Vis/NIR spectroscopy to quantify the TVB-N content in duck meat. Follow on research was reported by (Qiao et al., 2020) using model updating (MU), direct standardization (DS), and slope/bias correction (SBC) mathematical approaches with a root mean square errors of prediction as 1.35, 1.91, and 0.93, respectively. The prediction model performance was affected by the number of samples with the correlation coefficient of calibration set (Rc) decreased from 0.91 to 0.86 by increasing the addition of extra 25 samples while a moderate correlation coefficient of predication (Rp) of 0.54-0.58 was reported.

Liu et al. (2020) investigated the use of front-face synchronous fluorescence spectroscopy ($\triangle\lambda$ = 75 nm) to evaluate the TVB-N, TBARS, and TVC of beef during refrigerated storage at 4˚C for 25 days packed in MAP (60% O_2 + 40% CO_2). Fluorescence peaks for the amino acids and conjugated Schiff base

compounds were strongly correlated with the TVB-N and TBARS values, respectively, and the peaks for the amino acids and collagen were highly correlated with the TVC values (correlation coefficient = 0.87-0.91). The use of partial least squares discriminant analysis (PLS-DA) algorithm enabled the classification of beef samples as fresh, acceptable, or spoiled at 92.5% and 87.0% for the calibration and validation sets, respectively. A similar approach was used by Ouyang et al. (2020) to develop a model to predict TVB-N content in frozen pork with a R^2 of 0.97.

The use of viscoelasticity to develop a mathematical model that predicts the concentrations of TVB-N in chilled beef was reported by Li et al. (2019b). The viscoelasticity was based on airflow and laser measurements. The model was created using TVB-N content prediction models generated by partial least squares regression (PLSR) and support vector machine regression (SVR). The model had a correlation coefficient of 0.89. The use of viscoelasticity to predict TVB-N was proposed for chicken breast meat (Tong et al., 2010), chicken and pork (Myhan et al., 2015) and chilled beef (Li et al., 2019b) using various mechanical testing systems resulted in models with R^2 in the range of 0.75-0.89.

Despite the lack of mechanistic relationships that can explain spectra and TVB-N, spectroscopy is a beneficial technology in generating models that could predict meat freshness with very high accuracy. The technology offers excellent advantages compared to the traditional methods (rapid, high throughput, cheaper, and the ability to be automated and integrated into online systems).

5.2.4 Odour sensors and electronic nose technology

Lipid oxidation, degradation of protein and metabolism of S-containing compounds (such as sulphides and thioesters sulphur) result in the production of odorous volatiles (Feng and Ahn, 2016) that reduce food freshness and limit shelf life. Odour sensing technologies involve optical, chemical, and electrical sensors that can identify individual chemical compounds or multiple components in the gases and volatiles phases emitted by the food (Boothe and Arnold, 2002). The technologies have been used to establish associations among the volatile compounds, acceptability of products, and changes in microbiological quality in relation to storage time and temperature. The volatiles detected in the early stages of meat spoilage include aldehydes, ketones, esters, and amine compounds. Headspace volatiles produced during the spoilage of meat are considered significant in characterizing the spoilage process in a non-invasive sampling and characterization manner. Several sensors with varying degrees of technical complexity have been developed for sampling, quantification, and identification of headspace volatiles, including a metal oxide semiconductor and conducting organic polymers (Schaude

et al., 2017). Chemical-based sensors including pH chromogenic materials (Schaude et al., 2017) and gas sensors for volatiles (Chen et al., 2014b, Xiao et al., 2014b, Zhu et al., 2017) that can be used for real-time monitoring the freshness and estimation of shelf life throughout the production chain (harvesting, processing, storage, and display). These systems can be developed as small-size sensing systems and printed on the package (Zaragozá et al., 2013), allowing versatile detection systems applied directly to products to the individual package scale.

Met and Yeşilçubuk (2017) demonstrated the use of solid-phase micro-extraction (SPME) and gas flushing on Tenax (fibres with different chemical compositions that are used for the collection of volatiles). The results of the study showed that interactions and competition among the volatile compounds to be bound on the fibre, the loading efficiency of volatiles was dependent on the type of volatile and detection method applied. Odour sensors rely on the polarity and acid/base properties of the volatiles. Therefore, they are prone to interferences caused by the presence of moisture in the headspace or adsorption onto the equipment surface (Met and Yeşilçubuk, 2017). The combined use of an odour sensor and hyperspectral imaging (for the colorimetric spectral and textural data sensing) was shown to increase the efficacy of freshness evaluation of chicken and provided a non-destructive means for the evaluation of TVB-N and shelf life (Khulal et al., 2017).

The development of electronic nose (E-nose) sensor-based technology for the rapid detection of off-flavours and classification of meat spoilage has recently attracted much interest. A wide range of sensors are available that have different detection principles (e.g. acoustic, quartz crystal microbalance, surface acoustic wave, metal oxide semiconductors, conducting polymers, calorimetric, electrochemical, fluorescence, infrared, and optical). The conducting sensors are characterised by having low response time at room temperature, high sensitivity, and are compatible with portable devices and suited to hand-held applications. Emerging E-nose methods are based on conducting polymers (CP) and metal oxide semiconductors (MOS) (Ajaykumar and Mandal, 2020). A study by Zhu et al. (2017) investigated the use of an E-nose system based on MOS sensors for automatic detection of crab meat freshness. The authors observed high discrimination accuracy on the third day of storage, which was highly correlated to the TVB-N levels. However, a link between specific detected volatiles and specific microbial species was not made. This may be partly due to the interactions of the volatile compounds in the headspace. This area is worthy of further investigation since little is known about the reactivity and interactions among various VOCs and whether more degradation or condensation reactions take place in the headspace.

The E-nose has been frequently used for the evaluation of cooked (Siegmund and Pfannhauser, 1999) and raw chicken meat (Boothe and Arnold, 2002). Rajamäki et al. (2006) used an E-nose equipped with MOS field-effect transistor to study chicken meat packed in MAP (80% CO_2 + 20%N_2) at different storage durations and conditions (frozen, chilled, and temperature abused). A gradual increase in dimethyl sulphide and dimethyl disulphide over the storage period was evident in all treatment groups, but higher amounts were found in the temperature-abused samples. A similar trend was found for Pentane. The prediction of *Enterobacteriaceae* content and of hydrogen sulphide-producing bacteria based on PLS regression models was high ($r > 0.90$). Lower prediction coefficients ($R = 0.71$-0.75) were found with aerobic mesophilic bacteria and anaerobic and facultative anaerobic bacteria. Chen et al. (2014a) fabricated a low-cost colorimetric sensor array with a specific calorific fingerprint for VOCs that was used to investigate the freshness of chicken breast meat over nine days of storage at 4 ˚C. The authors used classification algorithms (a nonlinear learning algorithm "BP-ANN", Linear algorithm "linear discriminant analysis (LDA)," a new AdaBoost–OLDA algorithm "orthogonal linear discriminant analysis (OLDA) and adaptive boosting (AdaBoost) algorithm" to model the response of the E-nose data and the measured the TVB-N concentration in the samples. The freshness classification obtained from the models had a success rate of 87–100%, and the AdaBoost–OLDA algorithm generated the best model. Estelles-Lopez et al. (2017) developed a web-based system "MeatReg" that is capable of identifying the best machine learning method to compare analytical data obtained from different techniques and predict the microbial species responsible for meat spoilage. The authors verified the platform by using a commercial E-nose system consisting of eight quartz crystal microbalance (QMB) sensors coated with various poly-pyrrole derivatives and examined mincemeat stored under various aerobic and modified atmosphere conditions. The authors used a number of destructive (HPLC and GC-MS) and non-destructive (FTIR and multispectral imaging) techniques and several regression methods (ordinary least squares regression, stepwise linear regression, partial least square regression, principal component regression, support vector regression, random forest and k-nearest neighbours) to achieve successful prediction of TVC, LAB, *pseudomonads*, *Enterobacteriaceae* and *B. thermosphacta.*

5.2.5 Fingerprinting

Fingerprinting techniques have been made possible by the developments in volatile sensing, classification, and quantification. The techniques are based on the unique functional groups and fragmentation patterns of biological materials. Kodogiannis (2016) studied the use of an E-nose

through a multi-input-multi-output clustering-based fuzzy wavelet neural network system. The author reported that volatile fingerprints of odour profiles could be used in the classification and prediction of the meat freshness. The potential of using PCR-denaturing gradient gel electrophoresis (DGGE) analysis in monitoring, isolation, and classification of microbes has also been reported (Ercolini et al., 2006, Lin et al., 2013, Zhang et al., 2018). Ercolini et al. (2006) used PCR-DGGE to monitor the profiles of *Pseudomonas, Enterobacteriaceae, Brochothrix thermosphacta* and LAB in beef during storage for up to 14 days at 5 °C under varied storage conditions (aerobic, MAP1(60% O_2 + 40% CO_2) and MAP2 (20% O_2 + 40% CO_2)]. The authors identified thirteen different genera and 17 different species during the storage period with different spoilage microbial populations found under different packaging systems and at different time intervals (2, 4, and 7 days) during the storage. Under aerobic packaging, the spoilage bacteria were *Rahnella aquatilis*, *Rahnella spp*., *Pseudomonas spp*., and *Carnobacterium divergens*; under MAP1, *Pseudomonas* spp. and *Lactobacillus sakei* were the dominant bacteria; and under MAP2, *Rahnella* spp. and *L. sakei* were the main bacteria (Lin et al., 2013). In another study Zhang et al. (2018) accompanied PCR-DGGE with selective culturing of *Enterobacteriaceae*, *Pseudomonas* spp. and *B. thermosphacta* to track the changes in the complexity of the microbial diversity in beef samples. Aside from monitoring the changes in microbial diversity, the previous studies managed to yield consistent level data linking the yield of TVB-N compounds and the freshness of the meat products (Lin et al., 2013, Zhang et al., 2018).

5.2.6 Intelligent packaging

Intelligent packaging is a component of smart packaging techniques aimed at providing information on quality and safety of a product by monitoring and reporting specific attributes of the food or the internal environment in a package (O'Grady and Kerry, 2008). Intelligent packaging techniques use built-in sensors and indicators that aid in detecting and reporting changes in the headspace and monitoring spoilage progression (Park et al., 2015). The four commonly used intelligent systems included barcodes, sensors, radiofrequency identification (RFID) tag, and indicators. The indicators systems include freshness (e.g. microbial growth based on pH or volatile measurement), time-temperature (TTI) (for storage monitoring) indicators and pathogen indicators for detection of metabolites from specific microbes e.g. *Escherichia coli*. (Park et al., 2015, Fang et al., 2017). Some examples of commercially available intelligent packaging systems with potential for application in meat packaging are summarised in Table 4 and Holman et al. (2018).

Table 4

Some commercial intelligent packing systems for potential meat packaging applications. Modified from Biji et al. (2015) and Ahmed et al. (2018). Abbreviations include time-temperature indicator (TTI), radio frequency identifier (RFID)

Trade Name	Manufacturer	Type
Freshtag®	COX Technologies	Freshness indicator
Sensorq®	DSM NV And Food Quality Sensor International	Freshness indicator
SensorQ.	DSM NV, Heerlen, Netherland. Food Quality Sensor International Inc., Lexington, USA.	Freshness indicator
Fresh Tag.	COX Technologies, Louisville, Kentucky	Freshness indicator
Raflatac	VIT Technical Research Centre, Finland. UPM Raflatac, Scarborough, UK.	Freshness indicator
O_2 Sense ™	Freshpoint Lab., Florida, USA.	Integrity indicator
Novas®	Insignia Technologies Ltd.	Integrity indicator
Ageless Eye®	Mitsubishi Gas Chemical Inc.	Integrity indicator
Timestrip®	Timestrip Ltd.	Integrity indicators
Novas®	Insignia Technologies Ltd.	Integrity indicators
Easy2log®	CAEN RFID Srl	RFID
Intelligent Box	Mondi Plc	RFID
CS8304	Convergence Systems Ltd.	RFID
Temptrip	Temptrip LLC	RFID
Timestrip®PLUS Duo	Timestrip UK Ltd.	Temperature indicator
Timestrip Complete®	Timestrip UK Ltd.	TTI
Monitormark™	3M™, Minnesota	TTI
Fresh-Check®	Temptime Corp	TTI
Onvu™	Ciba Specialty Chemicals and Freshpoint	TTI
Checkpoint®	Vitsab	TTI
Cook-Chex	Pymah Corp.	TTI
Colour-Therm	Colour Therm	TTI
Thermax	Thermographic Measurements Ltd.	TTI
WarmMark.	DeltaTrak, Pleasanton, CA, USA.	TTI
Onvu	Bizerba SE & Co., KG, Balingen, Germany.	TTI

While indicators communicate the status of a product through a simple observable response, sensors encompass receptors and transducers that enable sensing, identification and quantification of a physical or chemical property, and provides continuous output of the signal. TTIs are developed on the basis that meat storage temperature has significant consequences on the shelf life of a product. While meat handling is maintained under the cold chain throughout its processing and distribution, additional use of TTIs provides extra safety precaution to enable quick action to be taken on the product. Hsiao and Chang (2017) showed that a microbial-based TTI (Man Rogosa Sharpe agar (MRS) broth enriched with 2% (w/v) glucose and 0.5% (w/v) yeast extract and incorporated with a pH indicator and appropriate concentration of the *Lactobacillus sakei* strain) could be applied for monitoring of vacuum-packed fish fillets. The changes in the colour of the indicator correlated with the *L. sakei* fermentation activities and pH alteration. Under constant conditions, *L. sakei* maintains a constant fermentation regime, which can be related to the freshness of fish fillets or TVB-N production levels.

Freshness indicators in meat packaging have been developed to register changes in pH, gas/volatiles changes and the formation of biogenic amines (Schumann and Schmid, 2018). Studies have shown that the concentration of biogenic amines including putrescine, cadaverine, histamine, spermidine, tyramine and spermine could be used as an indicator for accumulation of TVB-N and consequently the progress of meat spoilage (Zhang et al., 2019b). In fact, as observed later in this review, detection of TVB-N can be used as a freshness indicator in muscle-based foods (see Section 6 Total Volatile Base Nitrogen (TVB-N)). Rapid detection and quantification of TVB-N using colorimetric sensors and dye-based systems for gas/volatiles detection present potential for use in intelligent packaging (Kim et al., 2017, Zhai et al., 2017, Chi et al., 2020, Kang et al., 2020). For instance, Zhai et al. (2017) developed a colorimetric film based on starch/polyvinyl alcohol (SPVA) incorporated with 30-120 mg of roselle (*Hibiseus sabdariffa* L.) anthocyanins (RACNs) for real-time freshness monitoring in meat-based products, using a case study on fish (silver carp). The authors targeted the changes in TVB-N production in the fish for 165 days at 4 °C and reported high efficiency in matching the colour changes on the films with measured TVB-N and the spoilage of fish, suggesting the use of the film in intelligent packaging. Kim et al. (2017) developed a pH-based sensor using colorimetric bromocresol purple dye-based pH-responsive indicator, which demonstrated high response to changes in meat quality as validated using microbiological and colour measurements.

Another class of intelligent detection in packaging systems are gas sensors. These are able to

detect and process signals from the changing headspace volatiles produced during the spoilage of meat including CO_2 or H_2S, and other volatile compounds (Wells et al., 2019, Zhai et al., 2019, Zhai et al., 2020). Recently, Zhai et al. (2020) proposed an amine responsive bilayer film as a potential meat spoilage monitor, which was prepared from agar (AG), anthocyanins (AN), gellan gum (GG) and TiO_2 nanoparticles. The system which was based on the electrochemical writing ability of the AG-AN layer on GG/TIO_2 as the light barrier material presented a limit of detection of 0.018 mM to trimethylamine (TMA), which was a significant observation in the current search for cheaper and friendly techniques to monitor meat quality and safety.

6.0 TOTAL VOLATILE BASIC NITROGEN (TVB-N)

The sum primary, secondary, and tertiary amines in the form of volatile amines and toxic nitrogen compounds are classified as TVB-N compounds (Li et al., 2015b). In living animals, gut microbiota and endogenous enzymes continually produce a wide range of volatile and non-volatile compounds including biogenic amines; methylamines (MA), dimethyl-amine, N-nitroso dimethylamine, putrescine, and cadaverine (Saleem et al., 2012). These are assimilated for storage and utilisation for normal body function (Chen et al., 2011). Biogenic amines are produced from decarboxylation of amino acids (arginine, lysine, and arginine/ornithine) by rumen microbes and endogenous enzymes. Some of these metabolites are directly deposited in muscle tissues (such as TMA and TMAO) and other naturally muscle compounds, choline, betaine and carnitine give rise to TVB-N generation during post-mortem storage. *Post-mortem* biochemical and chemical activities due to exogenous and microbial enzymes in meat result in the generation of ammonia, biogenic amines, organic acids, and sulphur compounds from amino acids, hypoxanthine from ATP degradation products, and generation of acetate from lactate (Chen et al., 2014b, Ajaykumar and Mandal, 2020).

The formation of ammonia, which is produced from the deamination of amino acids, and other biogenic amines such as methylamines, putrescine, cadaverine, tyramine, tryptamine, 2-phenylethylamine, and histamine (Custodio et al., 2018) have drastic effects on the sensory properties of meat products and lead to consumers' discrimination against the products. Post-mortem TVB-N levels are dependent on the level of microbial and enzymatic activities that lead to spoilage; therefore, they are used as indices of meat freshness and food safety (Saccani et al., 2005, Ruan et al., 2019).

Apart from the impact of TVB-N on meat quality/freshness, the level and type of biogenic

amines present meat is considered of health concern, owing to their known toxic effects. For example, tyramine, putrescine, and cadaverine are known to be precursors for the formation of carcinogenic N-nitrosamines (De Mey et al., 2014, Zhang et al., 2019b). A recent growing health concern has also emerged for TMA and its derivatives TMAO-N and formaldehyde (FAD), owing to their association with an array of dietary-related illnesses including cardiovascular diseases, diabetes, cancer and renal complications (Zeisel et al., 1983, Koeth et al., 2013, Nowshad et al., 2018). Therefore, there is a growing number of studies reporting determination of TVB-N from a food safety and freshness perspective (Huang et al., 2017a, Zhang et al., 2018, Ezati et al., 2019, Fan et al., 2019, Lu et al., 2019, Zhang et al., 2019b, Zhao et al., 2019a, Zhao et al., 2019b). This appears to be very important due to the fact that biogenic amines such as polyamines (spermidine, spermine, and agmatine) are stable during storage (Saccani et al., 2005).

Biogenic amines are commonly determined using chromatography (Galgano et al., 2009), colorimetric (Khulal et al., 2016a), or combined methods such as GC-MS (Wojnowski et al., 2019), all which may involve derivatisation during sample preparation. Underivatized methods for biogenic amine determination that is based on separation by cation-exchange chromatography and suppressed conductivity coupled with mass spectrometry for overall detection of biogenic amines in fresh and frozen meat was proposed by (Saccani et al., 2005).

6.1 Factors affecting the occurrence of TVB-N in meat

Pre-slaughter formation and accumulation of volatile and non-volatile nitrogenous compounds cannot be ignored in the discourse of factors determining the quality of muscle foods. This fact has been extensively documented in fish and shellfish (Haard and Simpson, 2000, Howgate, 2010b) and to lesser extent in meat (Tables 3a and b), where freshness, sensory properties and acceptability were shown to be correlated with these metabolites.

Ruminants have been reported to produce volatile reduced alkaline N-compounds including ammonia, and aliphatic amines such as trimethylamine (TMA) methylamine (MMA) and dimethylamine (DMA), during the degradation of plant-based materials by rumen microorganisms (Sintermann et al., 2014, Kelly et al., 2019). TVB-N compounds also accumulate following microbial and enzymatic degradation of non-protein-nitrogen compounds (Zhao et al., 2019b). Although the majority of ‘gut produced TVB compounds’ can be excreted (e.g., aliphatic amine emissions), significant amounts were reported to be assimilated in the body or stored in the hepatic system and other tissues

(Sintermann et al., 2014). For instance, assimilated TMAO was shown to be further converted by trimethylamine oxide aldolase (TMAOase) to formaldehyde (assimilated as carbon source) and ammonium (assimilated as nitrogen source) via intermediate monomethylamine (Chen et al., 2011). Similarly, TMA is oxidised to TMAO by hepatic trimethylamine monooxygenase (flavin-containing monooxygenase 3 [FMO3]) (Niizeki et al., 2002, Chen et al., 2011, Sintermann et al., 2014).

The major dietary precursors of TMA and its products (DMA, FAD and TMAO) in ruminants include L-Carnitine (Carlson et al., 2007, Servillo et al., 2018b), choline (phosphatidylcholine and associated phospholipids) (Neill et al., 1978, Morgavi et al., 2015) and betaine (Mitchell et al., 1979, Eklund et al., 2005). Therefore, the dietary intake of these compounds will influence the generated amounts of methylated amines in the animal's tissues. Pre-slaughter practices that could influence the meat pH (Ponnampalam et al., 2017a) will play an important role in determining the pH (this will directly affect the activities of microbial populations and enzymes in the meat) and the amount of carbohydrates available for bacteria before being forced to metabolise protein compounds for energy (Ponnampalam et al., 2017b). Rapid increase in the TVB-N concentration from 6 mg N/100 g to 13.5 mg N/100 g between the second and third day of storage at 4 °C was found upon the depletion of glucose in meat (Umuhumuza and Sun, 2011). The rapid increase was attributed to the microbial degradation related deamination of adenine nucleotide and conversion of TMAO in the muscle to TMA, DMA, and formaldehyde. The concentration of TVB-N compounds are normally accompanied by a lag phase before reaching an exponential increase phase (Colby and Zatman, 1973), which is probably due to the delay in the adaptation and growth of bacteria that produces the enzymes responsible for various metabolic processes e.g. the conversion of TMA. The spoilage microflora is initially dominated by aerobic bacteria, which are gradually replaced by anaerobic microbes that can breakdown the proteins and amino acids to generate amine compounds and release of CO_2. Tan et al. (2019) showed a lag phase in the detection of amine compounds in chicken meat stored at 20 °C in accelerated shelf life experiments. Xu et al. (2019) reported strong association between the lag phase in the microbial growth and changes in pH and TVB-N values (Figure 3). The authors attributed the delayed production of TVB-N values to the initial slow establishment of *Aeromonas*, a common bacterium involved in meat spoilage and protein degradation. The authors demonstrated that the suppression of the *Aeromonas* growth resulted in lower TVB-N levels. These findings indicate the significance *post-mortem* storage conditions on meat quality and TVB-N production.

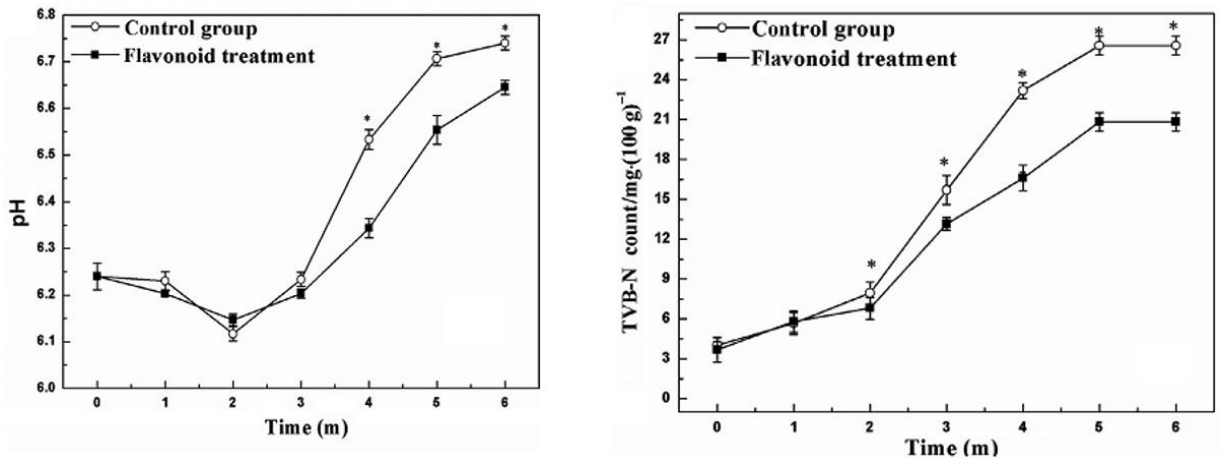

Figure 3

Effect of flavonoids from *Sedum aizoon L.* on pH and TVB-N levels in pork meat stored at -18 °C (a) pH, (b) TVB-N. Each data point is the mean ± standard deviation (bars) of three replicate samples, $P < 0.05$. Sourced from Xu et al. (2019)

6.2 Effect of animal species and body component

To date, there has not been a comprehensive account for the levels of TVB-N in meat from various ruminant species. The extent of spoilage in different meats is unlikely to be conceived by the assignment of a single TVB-N acceptable threshold of ≤ 15 mg/100 g for beef, pork and lamb meats. Clear differences are expected in the composition of the TVB-N generated in these meats due to differences in the concentrations of TVB-N precursors and microbiota composition. The precursors for TVB-N formation through degradation by microbial and endogenous enzymes are summarised in Table 1. The liver and liver-containing products have high levels of choline, which may explain the high levels of TVB-N compounds reported in liver products. Generally, beef, pork and lamb have similar contents of betaine and choline. Therefore, differences in the TVB-N levels among the species may perhaps result from the *ante mortem* depletion of glycogen influencing the ultimate pH as well as handling conditions that influence microbial proliferation (Medic et al., 2018). The composition of skeletal muscle as influenced by its physiological activity has a great impact on the ultimate pH and amount of exudates and therefore the oxidative capacity of the muscle fibres and enzymatic activities (Karlsson et al., 1993). This can clearly influence the contents of TVB-N precursors such as L-carnitine in different meat cuts.

Custodio et al. (2018) investigated the quality of pork loin and leg obtained at 24 h *post-mortem* and storage temperature of 5 °C. Despite the relatively similar initial levels of biogenic amines, storage for 16 days at 5 °C or frozen storage (-18 ± 1 °C) for 180 days resulted in significant differences between the two meat cuts concentrations of spermidine (leg and loin had 0.77 mg/kg and 6.26 mg/kg, respectively after 16 days storage). Other biogenic amines (spermine, agmatine, putrescine, histamine and cadaverine) where higher in the leg compared to the loin samples. The authors attributed these variations to differences in the proliferation of mesophilic and psychotropic bacteria in both meat cuts. The TVB-N values in the loins decreased from 24.2 mg /100 g (day 0) to 3.6 mg N/100 g after the frozen storage for 180 days, high fluctuation was found in the leg portion with the final level remaining high (11.1 mg/100 g) after the 180 days of frozen storage, from an initial value of 22.0 mg/100 g. There was no clear explanation for the reduction in TVB-N during frozen storage.

6.3 pH

The ultimate pH reached during rigor is about ~ 5.5 in normal meat obtained from unstressed animals (Devine et al., 1995). The degradative processes during storage that result in the production

of ammonia, amines and organic sulphides increases the pH (Muela et al., 2012). The increase in pH influences the rate of biochemical processes and provides better growth conditions for the microbial population in the meat, hence pH control interventions have been proposed as strategies for management of TMA production (Howgate, 2010b, Howgate, 2010a). The increase in pH during *post-mortem* storage has been frequently found to have a strong positive correlation with the concentration of TVB-N (Cao et al., 2013, Sun et al., 2018, Yang et al., 2018, Zequan et al., 2019, Chi et al., 2020, Senapati and Sahu, 2020). This positive correlation between pH and TVB-N is related to the favourable environment for proliferation of specific spoilage microbes at the higher pH (Sun et al., 2018). High pH facilitates the gradual transition from glycogen dependent microbes to the protein degrading types of bacteria (Lyu et al., 2016). Microbial and endogenous enzymes acting on low molecular weight compounds such as amino acids flourish at a high pH and decompose alkaline ammonia compounds, which in turn result in an increase in the pH of the meat (Cao et al., 2013).

Addition of preservatives stabilising the pH of pork meat stored under vacuum packaging attained low TVB-N levels of < 12.5 mg/100 g after 21-42 days of storage (Yang et al., 2018). Pork with an initial pH value of ~ 5.4 at day 1 of storage at 4 °C was treated with *Portulaca oleracea* L. extract (POE) at a concentration of 0.25-1.0% (Fan et al., 2019). At day 9 of storage, the treated samples had pH values in the 5.7-5.9 range, whereas the control samples had a pH of 6.35. The control of pH by POE resulted in lower microbial growth and lower TVB-N values, and the results from these analyses paralleled the pH trends over the storage time (Fan et al., 2019). The POE has a strong antimicrobial activity against *Pseudomonas aeruginosa, Bacillus subtilis and Bacillus cereus,* thus it is not clear if the inhibition of the microorganisms reduced the increase in pH or the inhibition of the pH change resulted in lower microbial growth. What is clear from this study and those reported in section 10, is the close relationship between pH, TVC and TVB-N. Similarly, the use of dense phase CO_2 and rosemary extract as a preservation method resulted in decreased the microbial growth and lower pH and TVB-N during 7 days of chilled storage (Huang et al., 2017a).

6.4 Preservation methods

Balamatsia et al. (2006) investigated the changes in microbiological, chemical and sensory attributes of chicken fillet meat treated with γ-radiation (0.5, 1, and 2 kGy) and stored aerobically for 21 days at 4 °C. The TVC, LAB and *B. thermosphacta* were decreased by the irradiation treatment with the highest reduction found with the highest treatment dose. Complete elimination of Pseudomonads, yeasts and moulds, and *Enterobacteriaceae* was observed in all treated samples. Irradiation

significantly reduced the concentrations of TMA and TVB-N (ranged from 2.2 to 3.6 and 30.5 to 37.1 mg/100 g of sample, respectively) during the 21 days of storage compared to untreated control (20.3 and 58.5 mg/100 g of sample). It is worth noting that the high concentrations in the control group are due to the long unrealistic storage time that led to excessive putrification. Histamine was found in control samples only, whereas putrescine and cadaverine respectively were < 27% and < 18% than their concentration in the control samples after 21 days of storage. It was clear that the reduction in microbiological counts resulted in the lower biogenic amines. The irradiation treatment increased the shelf life of the chicken by 4-5 days at 0.5 and 1.0 kGy and by 15 days at 2 kGy. Similarly, the authors in a following study demonstrated that the manipulation of microbial activities through modified atmosphere packaging resulted in reduced TMA and TVB-N concentrations in chicken breast meat (Balamatsia et al., 2007). In that study, chicken breast meat was stored under four different conditions [aerobic, vacuum-packed, MAP 30%/65%/5% (CO_2/N_2/O_2) and 65%/30%/5% (CO_2/N_2/O_2)] for 15 days at 4 °C. MAP treatments and vacuum packaging decreased the aerobic bacteria whereas vacuum packaging and 65%/30%/5% (CO_2/N_2/O_2) reduced Pseudomonas spp. in the samples. LAB and *B. thermosphacta* were lower in vacuum-packed chicken samples compared to other treatments. At the end of storage time (15 days at 4 °C), the concentrations of TMA were the lowest (~ 5 mg/100 g sample) in 65%/30%/5% (CO_2/N_2/O_2) treatment group and the highest TMA concentrations were found in aerobically and vacuum-packed samples (23.2 and 21.5 mg/100 g sample, respectively). Chicken samples in modified atmosphere 30%/65%/5% (CO_2/N_2/O_2) had an intermediate concentration (~ 12 mg/100 g sample). At the end of the storage period, the TVB-N concentrations in the samples were 54.5, 45.8, 43.1 and 29.6 mg/100 g respectively, for aerobically stored, vacuum-packed, 30%/65%/5% (CO_2/N_2/O_2) and 65%/30%/5% (CO_2/N_2/O_2) treatment groups, having started at 20.5 mg/100 g at 0 storage time. Based on sensory analysis results, the authors suggested TMA concentration of 10.0 mg/100 g and TVB-N concentration of 40 mg/100 g as limit values of acceptability for chilled chicken breasts. The same research group reported the combination of nisin and EDTA at various concentrations as an antimicrobial intervention was successful against mesophilic bacteria, *Pseudomonas* sp., *B. thermosphacta*, LAB, and *Enterobacteriaceae* in chicken breast fillets stored in modified atmosphere (65%/30%/5% (CO_2/N_2/O_2) packaging for 24 days at 4 °C (Economou et al., 2009). The most effective treatment for inhibition of TMA and TVB-N was 1500 IU nisin + 50 mM EDTA per g sample, which was the most effective antimicrobial combination.

7.0 TMA/TMA-N-O BIOMARKERS

7.1 Biosynthesis pathways of TMA/TMA-N-O

To enable better understanding of the role of TVB-N in meat quality and health, it is important to examine the nature of TVB-N and associated compounds. Major sources for TVB-N generation were shown to be the degradation of TMAO to TMA, DMA and formaldehyde as well as deamination of adenine nucleotides (Chen et al., 2019a). TMA and TMA-O are endogenously synthesised, or they are obtained from consumed food/feed directly (Figure 4). There are three pathways involved in the formation of TMA and TMAO, choline, L-carnitine and glycine betaine metabolisms. The formation pathway is related to the activity of endogenous enzymes and spoilage bacteria on methylated amines. These mechanisms are discussed in detail hereafter:

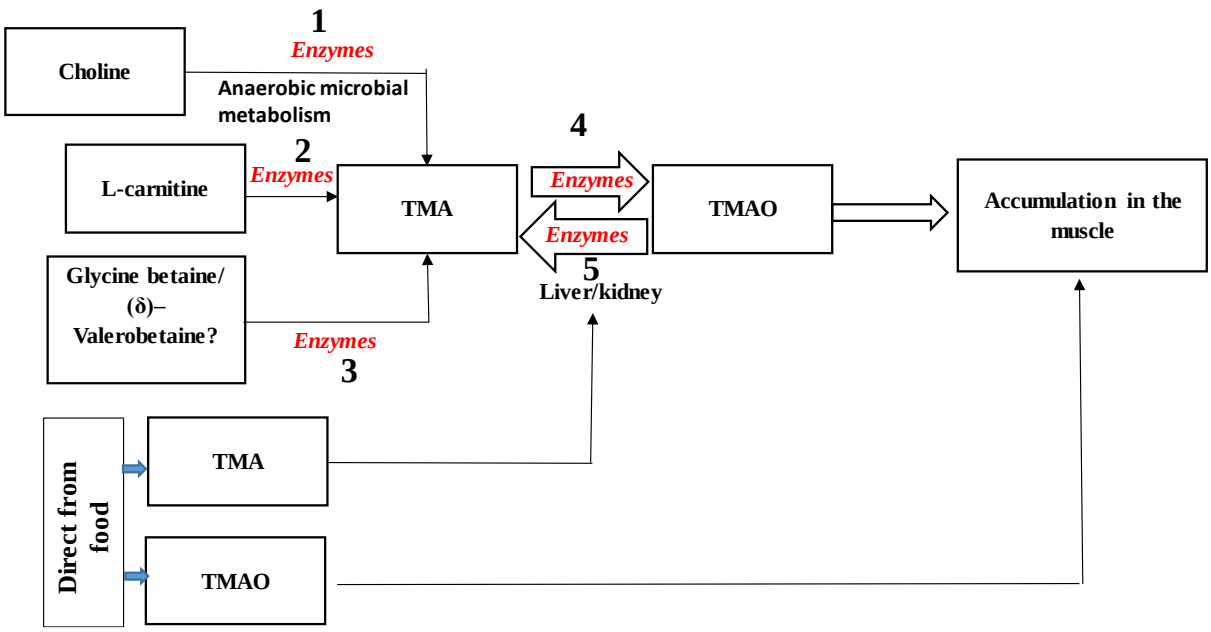

Figure S1

Trimethylamine and trimethylamine-oxide biosynthesis pathways.

7.1.1 Mechanisms of TMA and TMA-N-O formation

TMAO is derived from quaternary ammonium compounds containing the N-trimethylamine moiety, such as choline derivatives, carnitine, and betaine (γ-butyrobetaine) (Servillo et al., 2018a). Formation of trimethylamine (TMA) occurs through the metabolism of dietary L-carnitine, choline (available in glycolipids and phospholipids such glycerophosphocholine, phosphocholine, phosphatidylcholine and sphingomyelin) and betaine. Animal diets provide these compounds to gut microbiota and production of TMA takes place in the large intestine (Niizeki et al., 2002, Fava, 2015), which is then converted to TMA-O and distributed to various organs by serum (e.g. choline metabolism is shown in Figures 5 and Figure 6). These compounds are present in red meat (Koeth et al., 2013). The production of TVB-N and TMA in meat is facilitated by the metabolism of TMA-O and its precursors by spoilage microorganisms in meat. This is evident by the generation of TMA and TVB-N in chicken and pork that parallel microbial activity during *post-mortem* storage. Attempts have been made to identify microorganisms responsible for the formation of TMA in human gut (Jameson et al., 2018), and several animals (Rath et al., 2020). Attempts to identify microorganisms involved in TMA during *post-mortem* storage has been limited to chicken mostly. Such information for beef and lamb are limited/ unavailable.

The production of TMA occurs by microbial degradation (usually anaerobic) of carnitine, lecithin (phosphatidylcholine) and other choline derivatives (Anthony, 1982, Schugar et al., 2017). Following production, TMA is absorbed into the blood and transformed to trimethylamine oxide by Flavin monooxygenase enzymes (FMOs) in the liver.

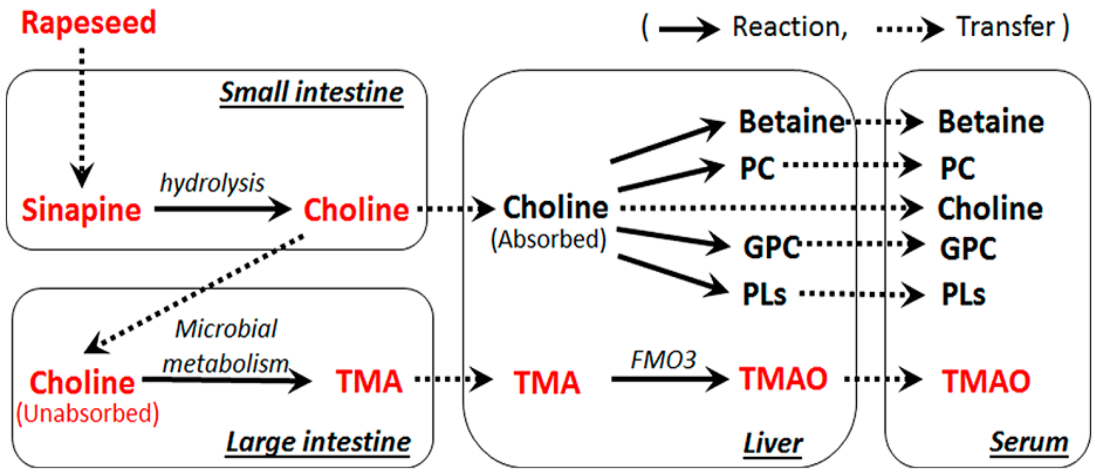

Figure 5

Major metabolic routes of sinapine-derived choline in the pigs after RSF feeding. Sinapine from rapeseed meal undergoes extensive hydrolysis in the small intestine to generate additional choline. Because of low bioavailability, sinapine-derived choline does not increase the choline pools in the liver and serum. Instead, it is mainly degraded by microbial metabolism to form TMA. After absorption, TMA is completely oxidized by flavin monooxygenase (FMO) in the liver to form TMAO. The metabolites that increased by rapeseed feeding are marked in red. Adapted from (Chen et al., 2019a)

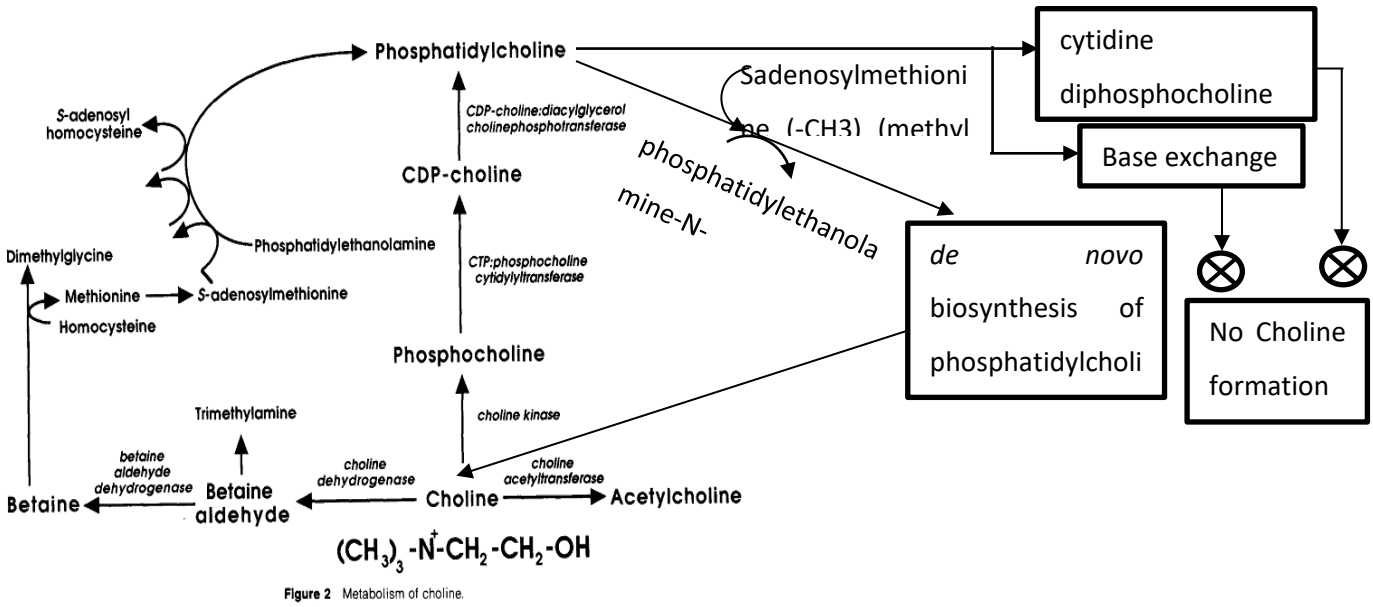

Figure 2 Metabolism of choline.

Figure 6

Biosynthesis and metabolism of choline – adapted and edited from (Zeisel, 1990)

7.1.2 Formation of TMA via L-carnitine pathway

Carnitine (β-hydroxy-c-trimethyl aminobutyrate) is a constituent of mammalian plasma and is found in skeletal and cardiac muscles (Shimada et al., 2004). Its presence in animal tissues has been attributed to biosynthesis from lysine and methionine (Shimada et al., 2004). L-Ca is a cofactor of acetyl-CoA carnitine transferase and its primary role in animals is channelling of activated long-chain fatty acids (LCFA) across inner mitochondrial membranes to the sites of enzymatic processes of oxidative degradation (β-oxidation) (Shimada et al., 2004, Szymeczko et al., 2007, Knüttel-Gustavsen and Harmeyer, 2011). In addition, it participates indirectly in lipid transformations as well as the metabolism of carbohydrates and nitrogen compounds. L-CA also controls excess acetyl-CoA in the mitochondria by facilitating the formation of acetyl carnitine, which liberates free acetyl-CoA (Knüttel-Gustavsen and Harmeyer, 2011). Deficiency in dietary L-CA in animal feeds can cause muscle weakness, and dysfunctions of the heart and skeletal muscles (Knüttel-Gustavsen and Harmeyer, 2011).

Carnitine, a dietary quaternary amine, is biosynthesised from N^{ε}-trimethyllysine (TML), which is an alpha-amino acid bearing a quaternary ammonium compound. TML is thought to originate from the hydrolysis of TML-containing proteins (e.g. myosin, histones, calmodulin and cytochrome c) (Servillo et al., 2018a). An enzyme, N^{ε}-trimethyllysine hydroxylase further converts TML into 3-hydroxy-TML (HTML) followed by cleaving into glycine and 4-N-trimethylamino-butyraldehyde (TMABA) by HTML aldolase (HTMLA; EC 4.1.2.'X'). TMABA is then dehydrogenated to 4-N- trimethyl aminobutyrate (TMABT) (γ-butyrobetaine) by TMABA dehydrogenase (TMABA-DH; EC 1.2.1.47). This is followed by hydroxylation of TMABT by γ-butyrobetaine dioxygenase (BBD; EC 1.14.11.1) to carnitine (Mestre Prates and Mateus, 2002, Vaz and Wanders, 2002, Servillo et al., 2018a). The synthesis of L-carnitine occurs almost exclusively in animal liver and the compound is then stored in skeletal muscle, which is reported to contain ~ 200 times more carnitine than blood plasma (Mestre Prates and Mateus, 2002).

Skeletal muscles form the main reservoir for synthesised L-CA in animals, which can either be free, short-chain acyl-carnitine or long-chain acyl-carnitine. Meat obtained from different species and different parts of the carcass have different concentrations of L-CA (Shimada et al., 2004). Higher levels of L-CA are associated with higher myoglobin content and type of myofibril, with red meat reported to possess elevated L-CA levels compared with white meat (for example, thigh meat has higher L-CA than chicken breast). However, L-CA is water-soluble; hence, differences between meat types can be influence by potential losses during storage and preparation methods (Shimada et al., 2004, Rigault et

al., 2008). The concentration of L-CA in meat is also influenced by the animal age where meat from older animals has a higher concentration than younger animals (Shimada et al., 2004). These differences due to age, muscle type, and species will have significant implications for TMA, and subsequently TVB-N, production in meat during post-mortem storage. Research reported in the literature (Section 10) has been limited to pork and chicken and was limited to pork loins and chicken breast meat. This clearly highlights the lack of information on other commercial meat species and cuts, which require further research.

Gut microbiota metabolises L-CA to trimethylamine (TMA) and (3R)-3-hydroxy-4-oxobutanoate using a glycyl radical containing enzyme, aerobic carnitine monooxygenase (CntA; EC 1.14.13.239) (Jameson et al., 2018). TMA is subsequently oxidized to trimethylamine-N-oxide (TMAO) by host hepatic enzymes, flavin monooxygenases (FMOs) (Alisson-Silva et al., 2016, Chen et al., 2019a). Catabolism of L-CA is only possible for microbes that can use it as a source of C-N (Koeth et al., 2013).

In meat, carnitine can exist in free and bound forms, mostly acetyl- and acyl-carnitine forms. The majority of carnitine is in free form, which might be easily accessible for TMA producing microorganisms. Total carnitine in beef and pork muscles remains stable on heating, except that about 50 percent of the carnitine is exuded with the fluids during cooking using various methods such as microwave cooking (1 min at 1000 W microwave power), boiling in water (boiling, 10-30 min) and pan-frying (200 °C, 2.5 min each side) (Knüttel-Gustavsen and Harmeyer, 2011).

7.1.3 Formation of TMA and TMA-N-O via choline metabolism pathways

Choline is a beta-hydroxyethyl trimethylammonium hydroxide that naturally occurs in all plants and animals obtain it through their diet. Human and animals require choline as a source of amino moieties that act as precursors for the synthesis of phospholipids' phosphatidylcholine, sphingomyelin, lysophosphatidylcholine and choline plasmalogen, all which are essential components of membrane structures (Zeisel, 1990, Mestre Prates and Mateus, 2002). From an animal nutrition point of view, choline is a key requirement in the animal's diet, being essential for liver function (Mestre Prates and Mateus, 2002). Choline is also a precursor for the biosynthesis of acetylcholine – a neurotransmitter and plays a role muscle contraction and lipid transport (Zeisel, 1990).

When supplied in the diet, digestive enzymes and microbes in the intestinal lumen facilitate the release of choline from phospholipids such as phosphatidylcholine, sphingomyelin and plasmalogen that are present in high concentrations in lecithin. Pancreatic secretions and enzymes by

microorganisms present in the intestines are capable of degrading lecithin to choline (Zeisel, 1990). Phospholipase (Ai) cleaves the alpha–fatty acid while phospholipase B degrades both the fatty acids. Glycerophosphocholine diesterase (L-3-glycerophosphocholine glycerophosphohydrolase) catalyses the conversion of glycerophosphocholine to glycerophosphate and free choline (Zeisel, 1990). Upon release, the methyl groups (trimethyl groups) in choline are then converted to trimethylamine by rumen microbiota (Neill et al., 1978). The metabolic pathways for the release of TMA from choline involve anaerobic glycyl radical-containing choline-TMA lyase (EC:4.3.99.4) (CutC), which contains a catalytic choline utilisation polypeptide glycyl radical enzyme (GRE) and an associated activating protein (CutD), which is encoded by a gene from a gene cluster encoding bacterial micro-compartment proteins (Cracium and Balskus, 2012, Brugere et al., 2014, Jameson et al., 2018, Kelly et al., 2019). CutC and CutD mainly participate in the cleavage of C−N bond that generates TMA and acetaldehyde (Cracium and Balskus, 2012).

Biosynthesis of TMA occurs mostly in the intestines where TMA-producing microorganisms exist (Niizeki et al., 2002). Rumen microbes possessing CutC and CutD gene sets such as *Olsenella umbonata,* Actinobacteria, *Coriobacteriaceae,* and *Caecibacter* (*Firmicutes*, *Veillonellaceae*), have been isolated from sheep and have been shown to be involved in the formation of mono-, di-, and tri-methylamines (Kelly et al., 2019).

The source of TMAO in tilapia (*Oreochromis niloticus*) tissues was investigated by dietary supplementation (Niizeki et al., 2002). Quaternary ammonium choline, carnitine, glycine betaine or phosphatidylcholine were individually supplemented in different diets and the formation of TMAO in the muscles was examined (Niizeki et al., 2002). The study demonstrated the generation of TMAO through the consumption of radio labelled choline only and the ability of TMAO to be transported to and stored in the muscles (Niizeki et al., 2002). The use of penicillin decreased the amount of TMA in the intestines. Furthermore, the TMAOse activity was detected in the liver and kidney of the fish. Collectively, this study demonstrated production of TMAO and its storage in muscle through dietary choline and that the process involves the gut bacteria and TMAOse activity. To the authors' knowledge, there are no such studies have been reported for ruminants. While there are clear differences between fish and ruminants in terms of digestion and that there is no certainty that a dietary effect would occur in such species, this contention can only be confirmed through research.

Concerning the role of bacteria in the formation of TMA, the metabolic pathway leading to

biosynthesis of TMA from choline differs from the synthesis using dietary TMAO (Landfald et al., 2017). The biosynthesis of TMA from choline and carnitine does not generate energy required for bacterial growth. However, in the dietary pathway, TMAO is an electron acceptor in oxidative respiration, which yields high levels of ATP for the growth of bacteria (Landfald et al., 2017). Microbes require TMA for carbon supply and utilize it as a precursor for TMAO with subsequent conversion to methane by methanogenic archaea (Brugere et al., 2014, Sintermann et al., 2014). The underlying genetics and biochemical mechanisms of the formation of TMA from choline have been reported (Cracium and Balskus, 2012).

Non-enzymatic oxidation of alkylamines occurs by two well-documented pathways in biological systems (Colby and Zatman, 1973). One pathway involves initial incorporation of oxygen to form trimethylamine N-oxide. The bacteria associated with this pathway were reported as *Pseudomonas aminovorans* and *Hyphomicrobium vulgare* NQ (Large et al., 1972), which are capable of producing TMA mono-oxygenase required to catalyse the reaction as follows:

$$(\boldsymbol{CH_3})_3\boldsymbol{N} + \boldsymbol{O_2} + \boldsymbol{NAD(P)H} + \boldsymbol{H^+} \rightarrow (\boldsymbol{CH_3})_3\boldsymbol{NO} + \boldsymbol{NAD(P)^+} + \boldsymbol{H_2O}$$

TMA mono-oxygenase was also reported in pig liver microsomes. Microbial enzymes linked with demethylation of TMAO to dimethylamine and formaldehyde (i.e. trimethylamine mono-oxygenase and trimethylamine N-oxide demethylase) are essentially from microbial sources (Colby and Zatman, 1973).

7.1.4 Formation of TMA and TMA-N-O via δ-valerobetaine pathway

Delta (δ)–Valerobetaine (N,N,N,-trimethyl-5-aminovaleric acid), a derivative of the amino acid 'glycine', is a known precursor for TMAO formation (Servillo et al., 2018a). Betaine acts as an organ osmolyte in animals and accumulates in cells and organelles to provide an osmoprotective effect upon exposure to osmotic and ionic stress. This helps to protect enzymes and cell membranes from inactivation by inorganic ions (Eklund et al., 2005). In addition, betaine reduces the energy requirement in cells during the pumping of ions across membranes when exposed to hyperosmotic medium. δ-valerobetaine is produced by rumen microbiota through the degradation of dietary N^{ε}-trimethyllysine and glycine (sourced from plant materials) (Servillo et al., 2018a, Salzano et al., 2019) or degradation of lysine via the catalysis of gamma-butyrobetaine dioxygenase (EC:1.14.11.1) (Huws et al., 2018). Animals can also obtain betaine from plants including sugar beet (high levels), wheat, peas, Lucerne, fish meal, oats, barley, maize, and soybean meal (Eklund et al., 2005). Some weeds common in

paddocks may contain a high choline content (e.g. Lambsquarters (*Chenopodium album*)) that was reported to have 330 mg/100 g and ranked 2nd on the USDA list after quinoa that had 630 mg/100 g. the concentrations of δ-valerobetaine in meat from ruminant meat are higher than meat from non-ruminants (Salzano et al., 2019).

Biosynthesis of TMA from choline by liver mitochondria occurs via betaine aldehyde that acts as an intermediate product during the conversion process (Zeisel, 1990). This process produces NADH that is utilized for ATP production. The biosynthesis occurs through three enzymatic pathways (Figure 6). Betaine acts both as an intermediate for TMA production and an agent in the conversion of phosphatidylcholine, which can further be degraded to choline (Zeisel, 1990). Niizeki et al. (2002) showed that dietary glycine betaine was responsible for elevated levels of TMAO in the fish muscle. Similar to choline-based diets, glycine betaine led to the accumulation of TMAO, but at approximately half the choline efficiency.

Bacteria genus *Methanosphaera* and *Methanobrevibacter* present in the ruminant's gut were reported to produce aldehyde dehydrogenase, which catalyses the utilisation of betaine aldehyde to produce TMA (Kelly et al., 2019).

7.1.5 Other sources of proatherogenic TMA-N-O

Ergothioneine, sinapine and sphingomyelins, which are components of animal feeds, can be catabolised to TMA a precursor for TMAO (Mitchell et al., 2002).

7.2 Application as freshness indicators

Trimethylamine (TMA) $(CH_3)_3N$) and trimethylamine N-oxide (TMAO-N), $(CH_3)_3NO$), are found in human and animal tissues and plasma, and it is believed that gut microbiota play the major role in their synthesis. Bacteria metabolise amino acids using decarboxylases and produce a range of biogenic amines (e.g. histamine from histidine, tyramine from tyrosine, and tryptamine from tryptophan) as well as TMA, DMA, cadaverine from lysine, and putrescine from ornithine via arginine (Ruiz-Capillas and Jimenez-Colmenero, 2005, Bota and Harrington, 2006). Putrescine is further converted into spermine or spermidine (Ruiz-Capillas and Jimenez-Colmenero, 2005) and is broken down by putrescine oxidase (EC 1.4.3.10) or diamine oxidase (EC 1.4.3.22) in the presence of water and oxygen to produce hydrogen peroxide, 4-aminobutanal and ammonia. Generally, biogenic amines are known to take part in several biological functions in living organisms, including formation of amino acids and maintenance of synapses (Okado et al., 2001). Specifically, TMA is thought to participate in

osmoregulation and countering the destabilising effects of the high intracellular and extracellular concentrations of urea (Landfald et al., 2017). However, these compounds have wide range of negative health effects such as allergy, toxic effects and cardiovascular diseases (Ruiz-Capillas and Jimenez-Colmenero, 2005, Wang et al., 2019).

TMAO acts as an electron acceptor to aid bacteria in degrading animal proteins, which has been identified as one of the pathways for biosynthesis of TMA. Similar to seafood, TMA is regarded as the precursor of off-flavour in pork. TMA concentration in meat is influenced by animal-related factors including the breed, gender, age, diet and carcass handling, which have been shown to cause different aroma exhibited by different meat cuts from different animals (Hamid et al., 2014). The authors found pork from a native Chinese pig breed to have less TMA than pork from European breeds. In addition, they reported that pork from normal male pigs had higher TMA concentration than pork from females and castrated males. The increase in TMA matched the reduction in the flavour and acceptability of the meat, indicating the contribution of TMA to quality changes among other factors, but in ruminants the thresholds where such effects occur are not known.

Trimethylamine (TMA) and its derivative dimethylamine (DMA) are very important compounds that are directly linked to TVB-N and the freshness of seafood. There are thousands of publications that documented the role of TMA and its derivatives in freshness and sensory properties of seafood. On the contrary, there are few publications documenting the presence of TMA and TMAO in beef, lamb and pork (discussed below), but it is generally assumed that TMA and DMA are present in meat (Bota and Harrington, 2006, Cai et al., 2011, Xiao-wei et al., 2016). A number of studies have reported the availability of TMA in chicken (Balamatsia et al., 2006, Balamatsia et al., 2007, Economou et al., 2009). Fewer studies reported the concentrations of TMA in fresh pork (Øverland et al., 1999, Hamid et al., 2014, Wang et al., 2014a, Xiao-wei et al., 2016, Chen et al., 2019a). The formation of TMA in cadaver has been reported in *post-mortem* muscles (Liu et al., 2008, Xia et al., 2016). To the best of the authors' knowledge, there are no studies reporting the concentration of TMA and TMA-O in beef, mutton, chevon and venison. However, several clinical studies described the formation of TMA and TMA-O in the gut, and mechanisms of assimilation into the plasma. The role of diet on the TMA /TMAO-N concentration in the plasma and general health effects has also been reported. The presence of TMA in human muscle was confirmed using ^{1}H NMR (Kumbhare et al., 2014) and therefore, it is reasonable to assume that TMA and TMA-O are present in other muscles such as in beef and lamb, but the basal concentration and changes during postmortem storage are yet to be established.

Aside from the differences caused by the breed and gender of the pigs, TMA concentrations have been reported to vary with the organs, diet and post-harvest handling of meat. Chen et al. (2019a) reported TMA values of 1.5 and 3.5 mg/100 g in the livers of animals supplemented with either soybean or rapeseed diets, respectively. However, the colon and cecum of the pigs reached levels of 10.0 mg/100 g and 5.0 mg/100 g, respectively.

7.3 Microbiological mediated pathways for TVB-N and TMA synthesis

Given the importance of TMA and TVB-N in meat freshness and in human health (discussed below), it is important to identify potential sources of TVB-N in animals and in meat. Saleem et al. (2012) reported that an increase in gut pH was accompanied by an increase in amine-containing compounds (methylamine and putrescine) during rumen digestion. The animal gut degrades plant material in nitrogen (N) cycle producing volatile reduced nitrogen compounds, for example, ammonia (NH_3) and aliphatic amines (e.g. trimethylamine) (Sintermann et al., 2014). The liberated N in the gut is absorbed in the bloodstream and transported to the liver for synthesis of urea. The increase in urea concentration has a damaging effect on several biochemical processes since urea can destabilize the macromolecular structures and inhibits cellular functions of several biomolecules (Velasquez et al., 2016). To counteract these negative effects of urea, TMAO is produced to protect and restore the native structure of proteins, which maintain their biological functions (Lin and Timasheff, 1994, Yancey and Siebenaller, 1999).

The gut microflora consists of a wide range of microorganisms such as anaerobic bacteria, protozoa, fungi, methanogenic archaea and phage (Huws et al., 2018). Some of these microorganisms are responsible for the production of TMAO and TMA production, and it is logical to suggest that differences in the animals' microbiota composition will result in differences in the amount of these methylamines (Huws et al., 2018). The above information suggests that diet and the gut microorganism composition will dictate the production of pre-slaughter TVB-N and TMA-O in the animals (Sintermann et al., 2014). This will affect the stability of meat generated from these animals.

The current progress in rumen metabolomics can help identify metabolites associated with the increase of TVB-N levels in live animals (Huws et al., 2018). Metabolites of microbial origin are precursors for animal products (meat, milk). Metabolites can also be used as biomarkers for the characteristics and activities of gut microbiome (e.g. increased concentration of phosphatidylcholine can indicate flourishing protozoa flora) (Saleem et al., 2012).

A close similarity between the human gut microflora and the rumen microbiota in terms of the production of ruminal TMA has been reported (Kelly et al., 2019). The strains identified to be TMA-producing include phyla *Actinobacteria*, *Firmicutes* and *Proteobacteria* that comprise 18 strains that use the CutC-CutD pathway mentioned earlier (Figure 6). The documented choline TMA lyase producing bacteria in the rumen of cows, sheep, buffaloes, goats showed strong evidence of TMA production in the animal gut (Kelly et al., 2019). *Olsenella sp*. and *Caecibacter spp*. were reported to be involved in TMA release from plant-derived choline in ovine whereas *Olsenella*, *Caecibacter*, and *Eubacterium* were involved in the bovine rumen (Kelly et al., 2019). Another group of bacteria that plays an important role in determining the concentration of the methylamines in ruminants is the Methylotrophs. These bacteria methanogens are bacterial species capable of utilising methanol, methylamines or dimethyl-sulphide. They are classified as obligate or facultative methylotrophs (Colby and Zatman, 1973, Anthony, 1982). Two main genera of bacteria identified as rumen methanogens are *Methanobrevibacter*, and *Methanosphaera* that encompasses the core rumen methylotrophic methanogens (Kelly et al., 2019). These bacteria deplete TMA in the intestine and halt its transportation to the liver where it can be oxidised to TMAO. Another class of bacteria *Methanomassiliicoccales* found in the rumen utilises TMA to produce methane. These bacteria have enzymes including TMA monooxygenase and TMAO demethylase that allow the utilisation of methylamine, dimethylamine and trimethylamine and utilize them as energy sources.

7.4 Specific enzymatic pathways for TVB-N synthesis and degradation

Formation of total volatile bases occurs in animals both *pre*- and *post-mortem*. The enzymes responsible for this formation are naturally available in the gut or in the liver of the animals.

7.4.1 TMA monooxygenase

TMA from the animal gut is easily absorbed and migrates to the liver where it is transformed into the odourless trimethylamine N-oxide (TMAO) through an oxidation process by TMA monooxygenase (EC 1.14.13.148, also referred to as Flavin monooxygenase 3 (FMO3)) (Niizeki et al., 2002, Jameson et al., 2018, Servillo et al., 2018a). FMO3 is a mammalian enzyme reported to enable bacteria to use TMA as a sole source of carbon energy. The enzyme is endogenously produced in bacteria (*Pseudomonas aminovorans*) and animals. The equation below shows the reaction scheme for the production of TMA-O from TMA (Lickteig et al., 2009).

N,N,N trimethylamine + NADPH + H^+ + O_2 $\rightarrow$ N,N,N trimethylamine N-oxide + $NADP^+$ + H_2O...eq 1

7.4.2 TMAO Oxidoreductase

Trimethylamine oxide oxidoreductase (EC 1.7.2.3) is a microbial enzyme that is responsible for the breakdown of TMAO to TMA, a volatile base. Bacterial species implicated in this conversion process include *Pseudomonas spp*., which is commonly flourish and grow in meat during *post-mortem* storage (Medic et al., 2018). Due to the psychotropic nature of *Pseudomonas*, TMA production continues during chilled storage of meat. The production is characterised by an initial lag phase, which then changes to an exponential phase during storage (Haard and Simpson, 2000). Several intestinal microorganisms also contain TMAO reductase, which enables them to reduce TMAO to TMA under micro-aerobic conditions (Niizeki et al., 2002).

7.4.3 Trimethylamine oxide aldolase (TMAOase)/ (Trimethylamine N-oxide demethylase

TMAO demethylase (EC 4.1.2.32) degrade TMAO to dimethylamine (DMA) and formaldehyde (FA) in the absence of oxygen (Haard and Simpson, 2000). The DMA and FA causes considerable damage to muscle foods, especially to texture. FA promotes protein denaturation, lipid oxidation and causes crosslinking of myosin in chilled or frozen meat, which was linked to TMAO aldolase activity (Nielsen and Nielsen, 2012). The activity of trimethylamine oxide aldolase (TMAOase) has been reported in ruminant meat and pork (Nowshad et al., 2018).

Degradation of TMAO produces equimolar quantities of formaldehyde and dimethylamine (DMA) and the process continues to occur during *post-mortem* storage. Formaldehyde is an aliphatic aldehyde molecule that is required in minute quantities in certain physiological processes such as the folate cycle (Trézl et al., 1997), and the production of formate that promotes the synthesis of nucleotides (Burgos-Barragen et al., 2017). Aside from demethylation activity, formaldehyde can also be generated from serine-glycine interconversion catalysed by pyridoxal phosphate and enzyme transhydroxymethylase (Trézl et al., 1997, Nowshad et al., 2018). It has been suggested that the accumulation of DMA in seafood is not strongly associated with significant quality changes since it is a secondary amine and is less reactive (Santos-Yap, 1996). On the other hand, the accumulation of formaldehyde can cause rapid deterioration of meat quality (as presented in the next sections). Due to equimolar production of formaldehyde and DMA, it has been suggested that the content of DMA can be measured as an indicator of formaldehyde production to predict textural changes in fish (Santos-Yap, 1996).

7.4.4 Trimethylamine dehydrogenase

Trimethylamine dehydrogenase (EC 1.5.8.2) is an iron-sulphur flavoprotein where bacterial ferredoxin-type (4Fe/4S) cluster covalently links to 6-hydroxyflavin mononucleotide (FMN). The enzyme catalyses the oxidative demethylation of trimethylamine to dimethylamine and formaldehyde (Rohlfs and Hille, 1994, Lu et al., 2003). The TMA oxidation process is thought to occur through an intermediary imine moiety, which dissociates immediately on detachment from the enzyme.

7.4.5 Dimethylamine monooxygenase (EC 1.14.13.238)

The enzyme, characterized from several bacterial species such as *Aminobacter*, *Cyberlindnera jadinii* and *Ruegeria pomeroyi*, is involved in a pathway for the degradation of methylated amines. It is composed of three subunits, one of which is a ferredoxin, and contains heme iron and an FMN cofactor.

7.4.6 Methylamine dehydrogenase (EC 1.4.9.1)

Methylamine dehydrogenase is a soluble quinone responsible for the oxidation of methylamine to formaldehyde and ammonia (Pierdominici-Sottile et al., 2006). Its structure contains a quinone cofactor called tryptophan tryptophylquinone (TTQ). Its oxidative process begins with a reduction step of quinone carbon atom C6 of tryptophan tryptophylquinone (TTQ) to form amino quinol. An oxidation step follows where two electrons are channelled to an electron acceptor returning the original quinone status of the enzyme (Pierdominici-Sottile et al., 2006).

7.4.7 Other enzymes

By-products of TMA/TMAO degradation must be eliminated from the body of the animal to reduce chances of cytotoxicity development. Enzymes such as formaldehyde dehydrogenase (FDH, EC 1.2.1.46) complete the process of TMA/TMAO degradation by degrading the by-products of the oxidation process. Formaldehyde is potentially toxic and carcinogenic due to its reactivity to DNA. FDH is produced by microbes including *Pseudomonads sp*., *Acinetobacter sp*., *Bacillus sp*. and *Escherichia sp*. (Colby and Zatman, 1973, Zhang et al., 2013a). The FDH glutathione-dependent type oxidises formaldehyde to yield free formate (Liao et al., 2013). The glutathione-independent type oxides formaldehyde through the intermediate compound s-formylglutathione. A reductase reaction finally results in hydrolysis of glutathione sulphonamide to glutathione sulphinic acid and ammonia (Kubienová et al., 2013).

8.0 ANALYSIS OF TMA/TMAO AND TVB-N

Several methods have been reported for the measurement of TVB-N including organoleptic tests and distillation methods (high temperature, reduced pressure, and distillation at a temperature slightly higher than room temperature with capturing of the volatiles using a Conway cell) (Wang and Duncan, 2017, Saenz-Garcia et al., 2020, Senapati and Sahu, 2020, Tantratian and Kaephen, 2020). Also, the use of high-performance liquid chromatography (HPLC), gas chromatography-flame ionisation detection (GC-FID), GC–MS, capillary electrophoresis (CE), flow-injection analysis, amperometric bi-enzyme electrodes, and metalloporphyrin-coated quartz microbalance sensor array (electronic nose) ion mobility spectrometry (IMS) has been documented (Karpas et al., 2002, Wang et al., 2013, Chang et al., 2015, Sun et al., 2018). The reported results are affected by the analytical procedure, especially those involving the use of a distillation step where the use of high temperature and strong alkaline causes decomposition of nitrogen-containing substances. TVB-N determination measures the concentration of ammonia, trimethylamine and dimethylamine (Choe et al., 2017) and is generally perceived as a reflection of the level of protein decomposition in meat, therefore used as an indicator for meat deterioration.

The most common method for TVB-N determination is the distillation method where the muscle tissue is heated with alkali to form ammonia from amides. Ammonia is liberated either using direct distillation under heating in the presence of alkali using steam distillation system such as Antona apparatus. Due to the likely erroneous results from steam distillation, the liberation of the TVB at room temperature through mixing with alkaline is captured by an acid placed in a microdiffusion cell “Conway cell”. This latter method has the advantage of being carried at low temperature and has been the most popular in fish studies (Howgate, 2010a) for the measurements of TVB-N and TMA. However, a steam distillation method using the Kjeldahl system has been adopted as the standard by many laboratories (Cai et al., 2011, Huang et al., 2015a, Chen et al., 2019c) and as the baseline test for the developed TVB-N regulations. While the use of meat samples has been the most common, the use of meat juice was suggested as a better alternative than the raw meat for biogenic amines detection (Bota and Harrington, 2006). The flow-injection analysis (FIA) is based on partitioning the bases generated from an alkalised extract through a Polytetrafluoroethylene membrane into a carrier stream containing bromothymol blue indicator. The above mentioned, methods are collectively regarded as conventional methods. These methods suffer from potential decomposition of the TVB-N during distillation and lack of standardisation for TMA and TVB methods. They are also time-consuming,

labour-intensive and destructive procedures. Therefore, many researchers have developed rapid and non-destructive methods. For example, Xia et al. (2016) used a rat model to compare steam distillation using the Kjeldahl method and new conductometric titration using a conductivity meter for determination of TVB-N in skeletal muscles. The study showed that TVB-N values could successfully predict the *post-mortem* protein decomposition of animal tissues after 24 h storage at 24 °C and 80% relative humidity.

8.1 New indirect/rapid methods for the determination of TVB-N

Unlike conventional methods used for the determination of TVB-N, non-invasive/ non-destructive methods have attracted a lot of interest due to their high reliability, being used directly on the sample without the need to conduct sample preparation, and fast determination of several properties simultaneously. Several technologies have been reported for this purpose, computer vision, infrared spectroscopy (VIS–near-infrared, mid-infrared, far-infrared spectroscopy and short-wave near infrared), E-nose, NMR spectroscopy, Raman spectroscopy, and hyper-spectroscopy have been reported (Kamruzzaman et al., 2015, Cheng et al., 2017, Su et al., 2017, Xiong et al., 2017, Taheri-Garavand et al., 2019). Due to the high interest in the biological effects of TVB-N and TMA on the quality of food products and on health, a new generation of rapid methods of determination have been proposed (Table 3b). Many of these methods have been described as a cheap, safe, rapid and non-destructive option for rapid detection of TVB-N and unsafe levels of bacteria spoilage. Since loss in meat quality due to bacterial activity also causes changes in internal and external physicochemical attributes (chemical changes such as pH value, structure modification such as texture/tenderness, and colour change), they collect information on changes in multiple properties, which could provide a better strategy for the measurement of freshness. Therefore, sensors that are capable of detecting certain substances and products of biochemical/microbial activities have been developed to measure the freshness of meat (Shi et al., 2018a). The use of non-destructive systems for the evaluation of the quality of fish, meat and poultry.

8.1.1 Glucose sensor

When muscle glucose decreases during storage, microbial enzymes revert to the breakdown of amino acids and proteins for energy supply (Umuhumuza and Sun, 2011). Thus, the depletion of glucose has been explored to develop a rapid TVB-N detection technique. Several types have been proposed that varied in their chemistry and thus their potential use in direct contact with foods. A thin-film polymer Glucose oxidase-immobilised electrode was developed for amperometric sensing of

glucose by electrochemical copolymerisation of thiophene, thiophene-3-acetic acid, and dicyclopentadienyl iron-1,4-dienylmethyl-2-(thiophen-3-yl)acetate. The glucose oxidase utilises oxygen and glucose to produce hydrogen peroxide (equations below). Either the loss of oxygen or the production of hydrogen peroxide could be measured to determine the amount of glucose utilised in the reaction:

$$\beta - \mathrm{D} - \mathrm{Glucose} + \mathrm{GOD(FAD)} \rightarrow \mathrm{GOD(FADH_2)} + \mathrm{d} - \mathrm{glucono} - \delta - \mathrm{lactone}$$

$$\mathrm{GOD(FADH_2)} + \mathrm{O_2} \rightarrow \mathrm{GOD(FAD)} + \mathrm{H_2O_2}$$

$$2\mathrm{Med_{red}} \rightarrow 2\mathrm{Med_{ox}} + 2\mathrm{e^-(at\ electrode)}$$

The electrons could be detected using reagent less probes such as polymeric redox mediators (small molecules that have low redox potential and can shuttle the electrons between the enzyme and the electrode surface). Several types of ferrocene-based and probe designs have been reported (such as poly(vinyl ferrocene-co-hydroxyethyl methacrylate), poly(N-acryloyl pyrrolidine-co-vinyl ferrocene), acryl amide copolymers, ferrocene-based polyamides, and poly(glycidyl methacrylate-co-vinyl ferrocene) (Saito and Watanabe, 1998, Abasiyanik and Senel, 2010, Şenel and Abasıyanık, 2010, Periasamy et al., 2011, Şenel et al., 2013). Two groups of conductive polymers have been widely used, polyaniline and multi-walled carbon nanotubes. Both polymers have their own advantages and disadvantage in terms of their molecular structure, electronic, chemical, and mechanical properties, environmental stability, redox properties and reversible nature of electrical conductivity.

8.1.2 Colorimetric sensors and digital imaging

Digital imaging and computer vision have been proposed for the detection and evaluation of quality changes by determining features such as colour, shape, surface texture and size (Huang et al., 2014). Several techniques integrated the use of combinations of the following methods to predict the quality and TVB-N in muscle foods; digital image processing, modelling techniques, colorimetric sensor, E-nose, and NIR.

The digital image involves the acquisition of cell pictures with a microscope at different time intervals, which is followed by a translation of RGB image to grey image and obtaining the binary edge cell image after digital image processing. Data were extracted for several cell morphological characteristic parameters from the binary edge cell image. A prediction model is then developed from the characteristic parameters as input and the value of TVB-N as output using neural network

modelling (Chang et al., 2015).

Xiao et al. (2014a) proposed an on-line computer vision system for the determination of pork freshness. The authors developed a colour region ration (CRR) feature extraction method that was correlated to TVB-N by probabilistic neural network algorithm. The freshness of pork stored at 26 ˚C over 3 days. The TVB-N content of the samples was negatively correlated with the CRR with R2 of 0.9683. Similarly, Taheri-Garavand et al. (2019) described a computer vision combined with artificial intelligence techniques that were able to predict chicken TVB-N content at a correlation coefficient of 0.7542. A combined approach that employed near-infrared spectroscopy, E-nose and computer vision was more power in predicting the freshness of pork than a single technique (Huang et al., 2014). Several different modelling approach and techniques have been used due to inherent differences in the extracted characteristic parameters among different meat types may vary from one meat type to another (Huang et al., 2014). The use of colour changes may be a successful strategy to develop computer vision systems for TVB-N determination in red meat due to the successful use of this approach with pork (Xiao et al., 2014a) and tilapia fish (Shi et al., 2018a). In this latter study, a machine vision system based on colour changes in the gills and pupils was successfully developed for simultaneous prediction of TVB-N, TVC and TBA contents of tilapia during storage at 4 °C and high correlations (R^2 = 0.98–0.99) were reported (Shi et al., 2018a).

A widely used approach for the evaluation of the freshness of muscle foods is colorimetric sensor array method (CSAM) that employ dyes sensitive to the volatile organic compounds, e.g. porphine compounds, methyl red, bromocresol green and bromophenol blue) and develop non-specific interactions with the dyes (Li et al., 2017). The developed CSAM is then analysed using image analysis and chemometric techniques to enable classification of products. The method was successfully adapted to classify the freshness of fish, chicken and pork (Chen et al., 2014a, Morsy et al., 2016, Xu et al., 2018). The method was used for the evaluation of fish freshness using image processing, and a correlation coefficient of 0.86 was found for the prediction of TVB-N (Huang et al., 2015b). The contents of TVB-N in chicken and pork were determined using CSAM consisted of 12 dyes responsive to VOCs and pH indicators (Khulal et al., 2015, Khulal et al., 2016a) and in combination with an optical sensor (Khulal et al., 2017). The use of backpropagation artificial neural network (BPANN) and an adaptive boosting (AdaBoost) algorithm (AdaBoost–BPANN) resulted in prediction (R = 0.89 and 0.90, for chicken and pork, respectively). A similar approach was used for the evaluation of chicken freshness (Chen et al., 2014a) with 100% successful classification. The CSAM was used to develop a portable E-

nose system for the evaluation of pork freshness (Chen et al., 2014b). The authors used linear discriminant analysis (LDA) and backpropagation artificial neural network (BP-ANN) to develop appropriate models and obtained > 97% discrimination rate. The technique was compared with hyperspectral imaging and a combination of both techniques to predict TVB-N content in pork (Li et al., 2015b) and different data processing and analysis (backpropagation adaptive boosting (BP-AdaBoost) and principal component analysis and backpropagation artificial neural network (PCA-BPANN) algorithms). The BP-AdaBoost and use of an integrated HIS and colorimetric sensors provided the best predictive power (R^2 = 0.93). The above information indicates that CSAM is a compelling technique on its own or in combination with other technologies and provide several advantages compared with conventional methods. The technique has the potential of determining multiple quality properties in the same analysis. For example, the TVC was successfully predicted using CSAM and backpropagation adaptive boosting (BP-AdaBoost) algorithm with a determination coefficient of 0.81 (Xu et al., 2018).

Colour sensors that employ single compound have been reported where the compound used to exhibit different colours upon interaction with the VOCs or due to change in pH. Alizarin (1% w/v) was used in a cellulose-chitosan matrix to develop a film to investigate in that detected TVB-N at concentration 20.53 mg N/100g of minced beef when alizarin colour changed from brown to purple (Ezati et al., 2019). This level of detection is higher the Chinese standard for pork, but in the absence of any data on acceptable freshness threshold, it will be difficult to assess the usefulness of the available detection system. The use of natural colour sensors has been increasingly reported. (Sun et al., 2020) designed a colour sensor made of blueberry-derived anthocyanins and degradable Poly-L-Lactic acid to investigate the freshness of mutton. The principle of the sensor is the colour change of the anthocyanins from pink to colourless as ammonia concentration increases during post-mortem storage. The authors demonstrated the effectiveness of the system and proposed its use as a useful indicator in intelligent packaging to monitor the freshness of meat. Several other natural dyes and products were reported such as *Arnebia euchroma* root, *Lycium ruthenicum* Murr., and hibiscus extracts, where changes in TVB-N and TVC were consistent with colour changes (Huang et al., 2019, Liu et al., 2019, Peralta et al., 2019). Rapid detection of TMA was reported by Xiao-wei et al. (2016) using a nano porous colorimetric sensor array that was developed using titanium oxide (TiO_2) and nano porous film.

8.1.3 Hyperspectral imaging

Hyperspectral imaging (HIS) technique has been investigated as a non-destructive approach of food quality and safety evaluation (Chen et al., 2013, Huang et al., 2019, Peralta et al., 2019, Sun et al., 2020). The technique is advantageous with the integration of computer imaging and spectral technique in the near infra-red (NIR) region to acquire both spatial and spectral information (Barbin et al., 2013, Chen et al., 2013, Zhao et al., 2019a). The technique was reported to be beneficial to determine TVB-N in meat (Cheng et al., 2014) and provide real-time monitoring of meat quality (Li et al., 2015b). Hyperspectral imaging (HSI) alone or integrated with other systems have been used for the determination of pork (Barbin et al., 2013, Chen et al., 2013, Tao and Peng, 2014), beef (Liu and Gan, 2016, Zhao et al., 2019a) and lamb (Xiong et al., 2014) quality targeting various biochemical and physical quality indices.

HSI is based on the principle that the surface colour and texture of meat changes gradually (Li et al., 2015b). These changes can be detected by HSI using Vis-NIR, and spatial distribution of chemical components is then used to generate maps of a concentration gradient. This is followed by extraction of hyperspectral microscopic images, which are then correlated with the levels of the chemical determined using standard methods such as TVB-N, TBARS and colour. Processing of HSI images involves subtraction of initial images from images obtained at various time points and attribute information, e.g. texture and RGB colour, are extracted and correlated using special techniques such as principle component analysis with the determined spoilage metabolites or change in the quality property. Fingerprint information on the correlations among the metabolites, physical, chemical and sensory attributes are used for the optimization of secondary rapid HSI method and used for prediction of the quality attributes (Li et al., 2015b). The hyperspectral imaging employs several chemometric and statistical techniques (e.g. principal component analysis, uninformative variable elimination, genetic algorithms and artificial intelligence algorithms, regression coefficient analysis, successive projections algorithm) for the selected variables of interest and identifies the most suitable spectral regions (Cheng et al., 2017, Su et al., 2017, Xiong et al., 2017). Several modelling approaches are commonly used to develop predictive models (e.g. artificial neural network, multiple linear regression, partial least squares regression, and least squares-support vector machine (LS-SVM). Peng et al. (2011) investigated the spoilage of beef rump steaks during storage at 8 ˚C using hyperspectral scattering profiles in the region of 400-1100 nm and strong prediction model (R^2 = 0.95) of log TVC was generated. Lee et al. (2018) determined the level of TVB-N using hyperspectral fluorescence imaging technique and

prediction model generated by least squares support vector machines analysis was developed. A high coefficient of determination for TVB-N content was reported (R^2 = 0.967). Grau et al. (2011) used visible and short-wavelength near-infrared to predict the freshness and quality of packaged sliced chicken breast. Multiple wavelengths (413, 426, 449, 460, 473, 480, 499, 638, 942, 946, 967, 970 and 982 nm) were found to be suitable to construct models correlated with TVC, ATP degradation and TVB-N value. The combined use of hyperspectral imaging (HSI) and colorimetric sensors (Li et al., 2015b) and acousto-optical tunable filter (AOTF)-based visible/near-infrared spectral (Wang et al., 2013) provided the capability to investigate several quality properties (TVB-N content, texture and colour) in pork.

8.1.4 Indicator dye

Dye indicator techniques (Table 3b) are designed for rapid visual detection that could be used in intelligent packing. Many of these techniques are based on pH changes due to reaction with volatile compounds. Chemo-responsive dyes have active sites that interact with the analytes and have the capacity to couple to an intense chromophore (Li et al., 2015b). The dye can either be pH indicators (e.g. Brønsted acidic or basic dyes), metal ion containing dyes (e.g. Lewis acid/base dyes) or zwitterionic solvatochromic dyes (dyes with large permanent dipoles). Examples of metal ion containing dyes include porphyrins and their metal complexes.

Cao et al. (2019) developed pH-sensitive cassia gum hydroxypropyl triethylamine grafting groups incorporating bromothymol blue. The spoilage of pork and chicken as indicated by colour changes (from yellow to green-blue) were correlated to triethylamine and TVB-N contents as measured by standards methods.

8.2 Detection of specific volatile bases

8.2.1 Trimethylamine

Several traditional methods are available in the literature that describes the measurement of TMA and these have been reviewed (Howgate, 2010a). A method coined “Conway method” has been used in the literature in many studies. The method is using Conway microdiffusion cell, hence the same name used as with the TVB-N, but the sample preparation and induction is different. This method involves the use of 1 ml of sample juice (described for fish mostly and obtained by mincing the samples and pressing it) that is mixed with formalin (0.5 ml to complex with ammonia) in a Conway cell. The solution pH is made alkaline by the addition of saturated potassium carbonate. This will liberate the

volatile bases that are received in an acid that is placed in a separate compartment in the cell. The diffusion process is allowed to progress for a fixed time, and excess acid is to calculate the amount bound with the diffused bases. The methods are criticized for being unspecific for TMA (Howgate, 2010a). A more common method is the “Picrate Method” that has been used as a standard method and referred to in the literature as “Dyer method”. The method is based on the mixing of sample muscle extract with formaldehyde to complex with ammonia, and an alkaline solution is used to increase release the bases. The free bases are then extracted into an organic solvent and reacted with picrate salt to develop a colour that could be measured spectrophotometrically.

Other methods reported in the literature include distillation with formaldehyde, flow injection analysis, gas-liquid chromatography, high-pressure liquid chromatography, capillary electrophoresis, enzymatic and biosensors (Howgate, 2010a). An ion mobility spectrometry was also reported for the determination of TMA (Bota and Harrington, 2006).

The use of chemical sensors to detect TMA in the headspace of packaged fish have been reported (Smits et al., 2012). A nano porous colorimetric sensor array prepared using TiO_2 probe had a detection threshold of 10 ppb TMA in Yao-meat storage at 4 °C (Xiao-wei et al., 2016). The nanopores gave high uniformity of detection and high correlation (0.84-0.89) between the measured and the predicted VOCs of Yao-meat.

8.2.2 Formaldehyde

Formaldehyde is naturally accumulated in meat due to post-mortem enzymatic reactions, and it has been used as a freshness indicator in muscle foods, especially fish (Nielsen and Jørgensen, 2004, Immaculate and Jamila, 2018). Progressive accumulation of ammonia in un-iced fish (10.64-18.75 mg/kg) was shown to be higher than iced fish (0.001-0.32 mg/kg), reflecting the differences in shelf life and consumers acceptability of the product (Immaculate and Jamila, 2018). Formaldehyde concentration was found correlate well with the microbiological and sensory quality of meat (Trézl et al., 1997). This is expected since the accumulation of formaldehyde contributes to off flavour and can cause cross-linking of proteins leading to modification of meat texture. Formaldehyde can reduce the solubility of myofibrillar proteins by reacting with amino acids, terminal amino groups and various low molecular weight compounds leading to denaturation and cross-linking of proteins (Sikorski and Kolakowska, 1994, Santos-Yap, 1996, Xiong, 1997).

The degradation of TMAO is the leading source for the accumulation of formaldehyde can

occur in frozen stored meat as the activity of trimethylamine oxide aldolase (TMAOase) was detected in certain fish species (Nielsen and Jørgensen, 2004, Nowshad et al., 2018). To the best of the authors' knowledge, this enzyme has not been characterised in red meat. However, formaldehyde has been quantified in poultry and red meat (Nowshad et al., 2018). The concentration of formaldehyde in raw beef and poultry samples ranged between 8.2 to 8.5 mg/kg and the cooked samples were half these concentrations. Mutton samples had 15.2 mg/kg. It is interesting to mention that formaldehyde concentrations in plant and milk products are many folds higher than those found in meat (Trézl et al., 1997, Nowshad et al., 2018). Trézl et al. (1997) investigated levels of formaldehyde in meat samples using a hydralazine-formaldehyde method that has a limit of detection 0.5 µg/L. The results showed higher formaldehyde content in sausages and ham than fresh meat and cooked poultry products. This may indicate that the microbiological activities may be the primary suspected source of the demethylation process that lead to the formation of formaldehyde.

Formaldehyde can also be detected using a spectrophotometric method (absorbance reading at 415 nm) after reacting TCA extract samples (pH 6.0-6.5) with Nash reagent (2,4-pentanedione (0.2%), Acetic acid (0.1 M), Ammonium acetate (3.89 M)) (Immaculate and Jamila, 2018, Nowshad et al., 2018). Meng et al. (2014) proposed a colorimetric-based formaldehyde sensor using an Ormosil (organically modified silica) coating containing methyltriehoxysilane and polydimethylsiloxane prepared through a sol-gel process, which immobilised a natural dye – rose anthocyanin and hydroxylamine sulphate. The reaction between formaldehyde and hydroxylamine sulphate produced acidic protons, which lowered the pH of the coating to ~ 1.5. The reaction turned the rose anthocyanin from hemiketal form (pale yellowish) to falvylium form (deep rose) with the test showing high specificity for formaldehyde at sensing time of 5 min and detection limit of 0.06 ppm (Meng et al., 2014).

9.0 RELATIONSHIP BETWEEN TVB-N AND HUMAN HEALTH

The relationship between diet and gut microbiota has been implicated in several metabolic, chronic, and inflammatory diseases. Previous studies investigated the adverse health effects of multiple dietary components such as high intakes of saturated fat, salt, cholesterol, and heterocyclic compounds that were heavily investigated in meat products (Shekelle et al., 1981, Dawber et al., 1982, Sterzel et al., 1988, Adamson and Thorgeirsson, 1995, De Stefani et al., 1997). Recent clinical studies have been focussed on other compounds that are present in meat. For example, many studies reported

a strong relationship between the consumption of a diet rich in choline (such as red meat, eggs and dairy) and impaired immune function and complex disease phenotypes including obesity and insulin resistance, renal complications, cardiovascular diseases (CVD), ulcerative colitis (UC), cancer, and inflammatory bowel disease (IBD) (Wang et al., 2011, Koeth et al., 2013, Wang et al., 2014b, Wilson et al., 2015, Guertin et al., 2017). A large number of studies linked TMAO and its intermediate compound TMA with these diet-related diseases. A recent meta-analysis of published clinical trials covering 2369 patients demonstrated a strong association between TMAO and increased incidences of major cardiac events (MACE) in patients with existing coronary heart diseases (Yao et al., 2020). The study identified a maximum dose level of 5.1 µmol/L TMAO concentration for prognosis, which is slightly higher than the expected concentration of plasma TMAO in normal healthy individuals (0.5 – 5 µM) (Subramaniam and Fletcher, 2018). Significant dietary sources of TMA are phosphatidylcholine, choline, betaine and L-carnitine, which are found in red meat, eggs, liver, kidney, peas, beans, peanuts, soya and brassicas (i.e. Brussels sprouts, broccoli, cabbage, and cauliflower) (Yao et al., 2020). It is also found in milk from cows predominantly fed on wheat-based diets (Scully, 2014). Dietary fat was reported not having an association with plasma TMAO or its metabolites (Boutagy et al., 2015, Wang et al., 2019). Compared to egg and red meat diets, consumption of fish has been reported to yield 46-62, 8-14, and 4-2 times higher levels of plasma TMAO, TMA and DMA ($P < 0.001$) (Figure 7), which peaked at 2 h and lasted for up to 6 h following consumption of a meal (Cho et al., 2017).

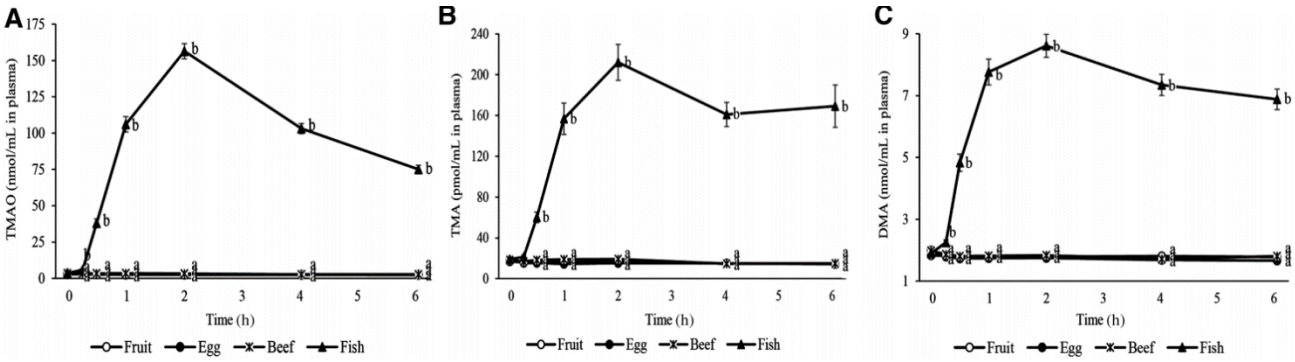

Figure 7

Effects of the study meals on plasma concentrations of trimethylamine-*N*-oxide (TMAO) (A), trimethylamine (TMA) (B), and dimethylamine (DMA) (C) across the 6-h study period. Different letter superscripts show a significant effect of study meal at each time point (one-way ANOVA, Tukey–Kramer post hoc test). Values are mean ± SEM, *n* = 40 per study meal. Adapted from Cho et al. (2017).

As indicated in the section on the biogenesis of TMAO, endogenous enzymes and those from gut microbiota metabolize choline, carnitine and betaine to yield TMA, which is assimilated and converted by hepatic enzymes to TMAO (Koeth et al., 2013, Tang et al., 2013). Two hepatic flavin mono-oxygenase family members, FMO1 and FMO3, oxidise TMA and produce TMAO. However, FMO3 was found to yield ten times more TMAO that FMO1, therefore, its activity has been widely reported (Wang et al., 2011). Not only is TMAO an indicator of meat spoilage, but it has also been listed as a proatherogenic and a risk factor for CVD. Numerous species of colonic bacteria (both anaerobes and facultative anaerobes) are involved in the conversion of dietary phosphatidylcholine and carnitine to TMA including, *Clostridia*, *Proteus*, *Shigella*, and *Aerobacter* (Colby and Zatman, 1973, Sandhu and Chase Jr, 1986, Tsoy et al., 2009, Khatri et al., 2012, Foti et al., 2013, Zhang et al., 2013a).

The underlying risks, including mortality from diseases caused by elevated TMAO levels are discussed in the next sections. The growing interest in gut metabolites-disease relationships has led to the development of prediction tools for several diseases using TMA, TMAO, and related metabolites (Wang et al., 2011, Missailidis et al., 2016, Chelu and Li, 2018, Winther et al., 2019, Zheng et al., 2019). Among those, TMA and TMAO were used as potential predictors of an individual's risk of developing CVD, coronary and kidney complications, insulin resistance, and diabetes. These prediction tools have also been a subject of recent patents granted to Hazen et al. (2016) and (2019) of the Cleveland Clinic Foundation (Cleveland, OH, US). The patents aimed at improving the efficiency of early diagnosis by using TMA-related compounds as markers for determining the risk of acquiring metabolic-related diseases in the ensuing one to three years, which emphasizes the importance of understanding and quantifying TVB-N content in meat.

9.1 Risks arise from the accumulation of TMA/TMAO

9.1.1 Association of TMAO with cardiovascular diseases

Insights from animal models have shown a positive correlation between TMAO levels and development of CVD. Wang et al. (2011) established a relationship between gut flora dependent metabolism of dietary phosphatidylcholine (PC) and CVD pathogenesis. The study investigated the association between plasma levels of dietary phosphatidylcholine and CVD risk in atherosclerosis-prone mice fed a regular chow diet incorporated with varied choline or TMAO levels (0.08-0.09%, 0.5%, 1% total choline or 0.12% TMAO). Plasma levels of TMAO were positively correlated with atherosclerotic plaque formation, with a female mice group showing more sensitive response compared to the male mice (Wang et al., 2011, Bennett et al., 2013). The association between dietary

metabolites and CVD complications has continuously been focused on the role of FMO3 as the primary enzymatic source of TMAO in humans. Wang et al. (2011) reported a strong positive correlation between the expression level of FMO3 with plasma TMAO and the development of atherosclerotic lesions. This observation was subsequently supported by the work of Bennett et al. (2013), who reported a significant increase in TMAO levels due to hepatic FMO3 overexpression in mice model. In that study, the risk of developing atherosclerotic complications was positively correlated (11% contribution) with elevated TMAO levels. Numerous clinical studies in humans have also shown a strong correlation between elevated TMAO levels and CVD and coronary artery calcification. The studies involving healthy individuals on supplemented diets (Tang et al., 2013, Choi et al., 2015) or patients, both identified through new diagnosis or have undergone treatment for various illnesses, reported strong effects for choline, carnitine, betaine and TMAO on CVD. Perhaps the other important conclusion that could be reached literature regarding the relationship between TMAO and diet (meat-based) is that the TMAO concentration is related to the activity of gut flora and that the increase in TMAO may be subject to individual differences in gut microbes (Bennett et al., 2013, Cho et al., 2017). It is important to mention that TMAO absorbed directly from the diet will play a major role that is not related to the activity of gut microbiota (Cho et al., 2017).

Although the pathways for the formation of TMAO and the link to disease promotion are well documented, limited studies are exploring the mechanism by which TMAO causes major illnesses. In an attempt to elucidate the mechanism of action of TMAO in promoting CVD, the mechanisms of pathogenesis of CVD (e.g. atherosclerosis) were explored at the molecular level (Koeth et al., 2013, Mondal, 2016). Mondal (2016) used heterodyne-detected vibrational sum-frequency generation (HD-VSFG) spectroscopy to investigate the water structure at zwitterionic phosphatidylcholine (PC) lipid monolayer−water interface (mimic of endothelial membrane-blood interface). The study revealed that TMAO contributed to the development of atherosclerosis in part by promoting cholesterol accumulation within macrophages. TMAO and TMA can alter interfacial properties of other macromolecules, including the exposed surface charges and residues through electrostatic interference. For instance, interfacial water becomes H-up oriented (away from the surface) in the presence of TMAO in the aqueous phase. TMAO was reported to increase the relative influence of the anionic phosphate by preferentially screening the cationic choline at the zwitterionic phosphatidylcholine lipid interface where the phosphate and choline groups occur simultaneously (Mondal, 2016). Collectively, these actions influence cellular metabolism of sterol and suppress reverse

cholesterol transport (RCT), which also changes the composition of bile acids (Wang et al., 2011, Koeth et al., 2013). The RCT is a mechanism in which cholesterol efflux from macrophages occurs and returning it to the liver for excretion, barring the process promotes atherosclerosis (Rader, 2003). The potential for serum TMAO to cause atherosclerosis is, therefore manifested based on its ability to affect the influx/efflux of lipids on the arterial wall (Mondal, 2016).

TMAO can also affect electrostatic interactions involving cationic or anionic serum compounds, which influences their orientation at lipid-monolayer interfaces, which would modify the electrostatic interactions at the endothelial cell membrane–blood interface (artery wall), potentially affecting the influx/efflux of fatty deposits on artery walls (Wang et al., 2011, Koeth et al., 2013, Tripolt et al., 2015, Mondal, 2016). Elevated plasma TMAO concentrations were reported to increase (58-62%) the risk of major adverse cardiovascular events (MACE) in patients with Coronary heart disease (CHD) (Yao et al., 2016). TMAO (> 5.1 µmol/L) potentially accelerated pathological progress of CVD and occurrence of MACE including death by promoting atherogenesis, thrombosis and vascular inflammation activities. TMAO can also promote platelet hyper-responsiveness to thrombosis and vascular inflammation, macrophage scavenger receptor expression and macrophage foam-cell formation (Wang et al., 2011, Koeth et al., 2013, Yao et al., 2016).

9.1.2 Trimethylamine N-Oxide and Kidney Disease and renal functions

Elevated levels of TMAO in patients with chronic kidney diseases have been linked to increased risk of mortality (Kim et al., 2016, Missailidis et al., 2016). Wang et al. (2019) investigated the impact of chronic dietary patterns of TMAO levels on metabolism and renal excretion in 113 healthy volunteers. Participants were fed a high or low –saturated fat diet with 25% calories from protein from either red meat, white meat or nonmeat protein sources for 4 weeks. TMA and TMAO levels were measured at the end of the trial revealed that chronic red meat consumption led to a significant increase in the urine TMAO and its reduced fractional renal excretion. Red meat consumption resulted in elevated levels of dietary precursors TMAO formation and increased the microbial degradation of carnitine into TMA/TMAO. Mueller et al. (2015) showed that the plasma levels of TMAO were confounded by impaired kidney function and poor metabolic control. The study used a liquid chromatography-high resolution mass spectrometry (LC-HRMS) technique to determine plasma concentrations of TMAO, betaine and choline in 339 patients undergoing tests for suspected coronary artery disease. A positive correlation was established between decreasing renal function and increased levels of TMAO and choline (Spearman's rho: 0.281; $P < 0.001$). Patients with a history of acute

myocardial infarction exhibited lower plasma levels of choline compared with those who did not have a history. Another recent study reported a strong correlation between plasma TMAO levels and measured glomerular filtration rate (mGFR) in patients with chronic kidney disease, CKD (Pelletier et al., 2019). In the study, plasma levels of TMA, TMAO, choline, betaine, and carnitine were determined using liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) among 124 control, CKD and haemodialysis (HD) patients. The study showed a negative correlation between plasma concentration and MGFR ($R^2 = 0.39$), implying that TMAO plasma levels could adversely affect mFGR in CKD patients. These findings corroborate earlier documented results showing a positive correlation between elevated plasma TMAO levels with increased renal interstitial fibrosis and expression of kidney injury molecule-1 (Sun et al., 2017) and impaired kidney function accompanied with poor metabolic control (Mueller et al., 2015).

9.1.3 Trimethylamine N-Oxide and Diabetes

High TMAO levels have also been associated with the pathogenesis of type 2 diabetes (Li et al., 2015c) or promotion of coronary heart disease (CHD) in diabetic subjects (Li et al., 2015a, Dong et al., 2018). Li et al. (2015c) studied the effects of elevated TMAO on the risk of type 2 diabetes (T2D). A total of 5466 diabetic subjects, 3655 CVD, 8675 cancer and 4009 implausible dietary data were followed over a period of 24-26 years (1986-2010). The study showed that an increase of dietary choline by 100 mg (regular diet with choline-containing component) increased the risk of type 2 diabetes by 17% (95% confidence interval 13-22). Although the study by Li et al. (2015c) did not observe significant interactions between phosphatidylcholine and age or BMI, a similar study (small sample and data points) by Dambrova et al. (2016) observed independent association between TMAO and age, and diabetes status and BMI. Dambrova et al. (2016) investigated the association between TMAO and plasma levels of L-carnitine in relation to diabetes among 12-20 week old mice and patients undergoing percutaneous coronary intervention. Diabetic mice exhibited 10 times higher TMAO levels, higher body weight, and insulin resistance at 8 weeks compared to non-diabetic subjects. Further evaluation of data from 191 patients undergoing percutaneous coronary intervention showed that higher TMAO levels independent of L-carnitine were dependent on age, diabetes status and BMI and were positively correlated with CVD. A more recent study attempted to alleviate the influence of TMAO on T2D (Baugh et al., 2018). In this study controlled diets (55% carbohydrate, 30% fat) were supplemented with 10 g/day of either an inulin supplement or maltodextrin placebo for 6 weeks and consumed by overweight/obese adults (n = 18). The authors did not find significant differences

between fasting or postprandial TMAO or TMA moieties between the inulin and placebo groups at baseline or plasma TMAO or TMA moiety concentrations following inulin or placebo, which showed that inulin did not play a significant role in regulating TMAO in the blood.

9.1.4 Trimethylamine N-Oxide and Cancer

The relationship between TMAO and carcinogenesis has been reported in numerous studies (Kim et al., 2010, Bae et al., 2014, Xu et al., 2015, Guertin et al., 2017, Liu et al., 2018, Griffin et al., 2019). Kim et al. (2010) showed that plasma levels of TMAO among other urinary metabolites (e.g. Hippurate, 3-indoxylsulfate, 2-oxoglutarate, and citrate) might be used as biomarkers for development of gastric tumours in a mouse model. Associations have also been identified between elevated plasma TMAO levels and primary liver cancer (PLC) (Liu et al., 2018), proximal, distal, and rectal colon cancers (Guertin et al., 2017). Liu et al. (2018) investigated the association between TMAO and the risk of primary liver cancer (PLC) on 671 newly diagnosed PLC patients (671 control). The study measured the level of TMAO and choline using high-performance liquid chromatography with on-line electrospray ionization tandem mass spectrometry (HPLC-MS/MS). In the study, the PLC group exhibited higher serum TMAO levels than the controls, which showed increased PLC risk for individuals with high TMAO. Choline levels showed an inverse relationship with PLC risk, which was stronger among non-smokers compared to smokers. The inverse relationship between choline and PLC risk was attributable to the fact that other dietary components, e.g. L-carnitine contributed to the high TMAO levels. It is notable that, despite such a strong association between TMAO and negative health effects, the studies may be limited by single-point measurements where body conditions may be influenced by external factors that affect the measurement. In addition, the effect of the organ malfunction due to the disease (e.g. the effect of liver cancer on FMO3 production and activity) on enhancement or suppression of the measured compounds, e.g. TMAO may not have been considered.

Xu et al. (2015) constructed epigenetic interaction networks (EINS) to study associations between TMAO and colorectal cancer using chemical-gene, disease-gene and protein-protein interaction data from multiple large-scale data resources. The data, which was validated with TMAO-related links with CVD confirmed that TMAO was related to metabolic syndromes and development of cancers. Other chronic conditions have also been associated with elevated plasma TMAO, for instance, Wilson et al. (2015) observed correlation between plasma TMAO inflammatory bowel disease (IBD) including (Crohn's disease (CD) and ulcerative colitis (UC)). The study conducted on 479 subjects (373 non-IBD and 106 IBD) showed TMAO levels were lower in IBD patients, perhaps relating to the altered

performance of gut microbiota, which affected TMAO production.

9.1.5 Mitigating the effects of TMAO accumulation

A number of suggestions have been developed to attempt to mitigate TMAO effects, which has been seen as a means of reducing disease risk for patients experiencing other complications (Wang et al., 2019). Avoidance of TMA precursors in the diet was suggested as a way of overcoming the problem (Mitchell et al., 2002) and minimizing the risk of developing CVD. Brugere et al. (2014) proposed arresting the development of TMAO in the gut using a microbe *Methanomassiliicoccus luminyensis B10*, which utilises methyl compounds including TMA and produces an inert molecule (e.g. hydrogen) for methanogenesis. Interventions by populating the gut with different diets (Saleem et al., 2012) or selected species of protozoa or yeasts (*Saccharomyces cerevisiae*) to control the level and type of metabolic products have also been suggested (Tripathi and Karim, 2011, Morgavi et al., 2015). Protozoa are thought to control the host metabolome by modulating the bacterial community or by their own metabolic activities. Another mitigation strategy suggested the stoppage of TMA transportation to the liver where it can be oxidised to TMAO (Morgavi et al., 2015) through manipulating the composition of the gut microbiota.

9.2 Risk from the accumulation of formaldehyde

Formaldehyde (FAD) is a product of TMA degradation, either by sequential oxygenation and demethylation of the oxygenated form to yield DMA and FAD. FAD can also be formed through direct N-demethylation of TMA to give DMA and formaldehyde, catalysed by TMA dehydrogenase (EC 1.5.8.2) (Colby and Zatman, 1973). The health risk from accumulation of other TVB-N products was also studied (Norliana et al., 2009). Accumulation of endogenous formaldehyde in meat could be toxic to humans. Toxic levels of FAD in the blood were reported at a level of 2 µg/g (Heck et al., 1985). Formaldehyde can be metabolised by S-(hydroxymethyl) glutathione dehydrogenase (EC 1.1.1.284) to hydroxymethylglutathione, which is subsequently metabolised to formate by formaldehyde dehydrogenase (2,6-dichloro- phenol-indophenol-dependent) (EC 1.2.1.46). Failure to be metabolized by formaldehyde dehydrogenase could lead to harmful interactions, including the formation of protein-protein cross-linkages and linkages between proteins and single-stranded DNA. Formaldehyde affects the solubility of myofibrillar proteins due to cross-linking and denaturation, which may affect normal body function (Sikorski and Kolakowska, 1994, Xiong, 1997).

10.0 SUMMARY OF MEAT RESEARCH REPORTING TVB-N

The topics of extended shelf-life and product freshness have long been a focus for meat researchers. Within these themes and based on its application as an objective metric, much of our knowledge pertaining to TVB-N and its accuracy, application or usefulness as a freshness biomarker has been gained. This is an essential point because TVB-N is rarely the sole focus of investigation, and our understanding of its relationship with the freshness of meat products is consequently lacking. It is further apparent that TVB-N tests for spoilage or freshness are more frequent for some meat types, whereas for others the use of TVB-N is rare. Here, we have provided a summary of meat research that has reported TVB-N, categorised by meat type and by default, highlighting paucities that necessitate further investigation.

10.1 Beef

Beef is an important global commodity. Its market access and value is supported by assurances of freshness and end-consumer acceptance, often following a substantial interval, post-slaughter. Researchers have already applied TVB-N to objectively determine beef freshness (Table 4) with it widely observed that TVB-N levels will increase with post-slaughter time and ageing. Further, these increases are often reflected by similar changes or trends in other spoilage biomarkers. This outcome is illustrated by several recent studies.

Qian et al. (2018) compared the effects of sub-zero frozen storage for up to 24 weeks on beef quality and freshness. In this study, the colder frozen storage temperatures resulted in the lowest TVB-N levels, which demonstrated that TVB-N could accumulate even when microbial proliferation was substantively inhibited. We should note that beef samples were held at -9 °C using low field nuclear magnetic resonance (Qian et al., 2018) and this which would impact on the extent of true freezing and actual 'liquid water fraction' (Rogers, 2007). Further, because all samples were sourced from a single beef carcass there was no true replication, and so we cannot be confident in broader representation of these findings. Yet, a study of Chinese Yellow cattle *longissimus lumborum* muscles (LL) compared storage temperatures and also observed that TVB-N accumulated during 24 weeks of frozen storage at -18 °C (Lu et al., 2019). Whether this outcome would remain significant if sample portions had been allocated to storage treatment within each loin is academic, but it is noted that shear force and therefore proteolysis differences were evident in frozen samples (Lu et al., 2019). This same study also reported that the rate of TVB-N accumulation increased with temperature, from frozen < 'super-

chilled' (-4 °C) < conventional chilled (2 °C) storage.

Frank et al. (2019) assessed the cold-chain between China and Australia, and the impact of up to 140 days at -0.5 °C on the shelf-life of Australian beef striploins. This study reported that storage temperature fluctuations of export samples had a significant impact on TVB-N levels when compared to control samples that were held at a more consistent temperature. As a result, control beef was observed to remain fresh even after 140 days, whereas exported beef was not fresh after 84 days (TVB-N < 15 mg/100 g) (Frank et al., 2019). A follow-up study of these same samples reported that both free amino acids and peptide carnosine contents increased until 84 days of cold-storage and then plateaued (Frank et al., 2020). These non-volatile components are indicative of proteolysis and suggest this peak drove the TVB-N results, an observation supported by the absence of any aerobic plate count or LAB differences between 120 and 140 days of cold storage (Frank et al., 2020). It is of further interest that these results have been confirmed: with a total of 60 Australian beef striploins purchased upon delivery to China, sectioned and then held chilled for an additional 15 weeks (Chen et al., 2019c). This study found that TVC and LAB increases with storage times were somewhat similar to TVB-N increases, and that final TVB-N levels of 14.8 ± 0.04 mg/100 g were indicative of acceptable freshness (TVB-N < 15 mg/100) (Chen et al., 2019c). An interesting aspect of this latter study was its verification of freshness using subjective odour assessment that used the same ten trained panellists at each measurement interval, which could conceivably introduce an element of bias into the assessment.

A study with beef tenderloins found that TVB-N levels increased more so with aerobic storage and exceeded freshness guidelines (TVB-N < 20 mg/100 g) after nine days when compared with vacuum-packaged beef (Mansur et al., 2019). This outcome is of interest because sensory aroma scores for the aerobic beef samples were likewise considered to be unacceptable after nine days. We should note that aerobic storage exceeded microbial limits (CSIRO, 1995, Rodas-Gonzalez et al., 2011, Mills et al., 2014) after seven days (TVC < log7 cfu/g) and vacuum packaged beef was acceptable up to 21 days when the experiment finished. This supports the idea that beef aroma may have a relationship with TVB-N and not TVC, particularly as vacuum-packaged beef sensory aroma scores were acceptable after eleven days (Mansur et al., 2019). Research with 'super-chilled' Chinese beef striploin held chilled for up to 20 weeks likewise demonstrated a similarity between odour intensity and freshness scores to TVB-N values (Chen et al., 2020), although the trained panellists were again unblinded to the treatment. This same study found that abattoirs could impact on TVB-N results, with an acceptable shelf-life of twelve weeks or nine weeks dependent on the abattoir. The authors propose this

difference to be the result of different hygienic practices (Chen et al., 2020) – with TVC found to exceed log 7 cfu/g at only one abattoir and then after six weeks. Alternatively, it could be the result of a breed effect, as Simmental and Luxi Yellow Cattle carcasses were included in this experiment without reference to their representation at each abattoir. Nonetheless, this suggests a clear discrepancy between the changes to TVB-N and TVC across increasing storage duration.

The relationship between TVB-N and microbial loads becomes more apparent when we consider studies that have aimed to extend beef shelf-life by inhibiting microbial proliferation. Lyu et al. (2016) for example, found that in beef *longissimus lumborum* and *Psoas major* muscles, TVB-N levels increased in a 46 day storage period: those that were vacuum packaged had the highest final TVB-N levels, and TVB-N levels declined as MAP ozone concentration increased. The authors attribute this outcome to the sterilisation effect of ozone, thus reducing the microbial contributions to proteolysis and amine generation. While encouraging, it should be noted that ozone pre-treatment resulted in perceptible malodours and therefore (based on the authors' advice that humans can detect ozone at level > 0.02 ppm), if applied to ensure beef freshness, a pre-treatment 1.5 h of 5% ozone and 95% carbon monoxide was recommended (Lyu et al., 2016). Alternatively, the addition of a chitosan coating prior to vacuum packaging was found to result in lower TVB-N levels for beef aged for 45 days (Duran and Kahve, 2020). This outcome was proposed to be the result of its polycationic and therefore, antimicrobial effect. When we compare the microbial proliferation rates in this study, total mesophilic aerobic bacteria and TVB-N were observed to share a similar trend across the ageing period. However, the similarities were more apparent between TVB-N and LAB. Another study used multiple linear regression models to compare Pearson coefficients of correlation and found that TVB-N and total plate count for beef aged aerobically at 0 or 4 °C was ca. 0.96 and 0.97, respectively (Byun et al., 2003). Furthermore, the strength of this relationship was lower between TVB-N and sample psychotropic bacterial counts (Byun et al., 2003). If we then consider aerobic microbes as indicative of oxidative potential, these outcomes become obvious: protein oxidation and protein degradation (proteolysis and associated TVB-N synthesis) have an antagonistic relationship (Lund et al., 2011).

Building on this point, Koh et al. (2019) suggested that higher quality Hanwoo beef (QG++) had lower TVB-N levels after 28 days at 2 °C than lower quality beef (QG3) because of its lesser content of protein and therefore lower susceptibility to proteolysis, due to its higher intramuscular fat content. This same study found a significant interaction between TVB-N content and ageing time, however this data was omitted from Koh et al. (2019), although it was referenced. Indeed, the design elements of

this study were not included into the statistical analysis (i.e. each loin was divided into eleven equal portions that were then randomly assigned to the different ageing period, hence 'loin' could be a block or random term) and the associated variation may ultimately impact on their conclusion (Koh et al., 2019).

Other studies also support a relationship between TVB-N and microbial loads in beef. However, we should first consider their experimental design elements (representative sampling, reproducible analyses, robust statistics, etc.) against their broader interpretations. For example, using samples sourced from ~ two beef fillets, Azarifar et al. (2020) attributed TVB-N increases with storage time of beef fillets to microbial spoilage and observed that TVB-N and TVC exceeded acceptable limits of 16.5 mg/100 g and log 7 cfu/g respectively, after 12 days at 4 °C and then only for the control samples not packaged using gelatine-CMC films that incorporated chitin nanofibers and *Trachyspermum ammi* essential oils. Likewise, Han et al. (2014) used the loins from two beef carcasses to compare antimicrobial film efficacies to conclude that it was the antimicrobial activity of the films that retarded TVB-N formation and that concentrations of 1% rhubarb ethanol extract and 0.08% cinnamon essential oil provided improved preservation and the impact of these compounds on product (mal)odour. This study did not include the statistical model terms used to confirm these results. Jiao et al. (2020) also failed to include details of the statistical model they used to test beef rounds treated with kiwifruit phenolic extract and other active compounds. Nonetheless, this study reported that although total phenolic component and therefore antimicrobial capacity of epicatechin was ~ double that of kiwifruit extract: the latter is recommended because of the formers negative impact on beef flavour (Jiao et al., 2020). Nondescript 'boneless beef' coated, with slightly acidic electrolysed water (SAEW), distilled water, tea polyphenols or nothing (controls), were used to conclude that TVB-N increases across a 16 day storage period were reflected, within the treatment, by increases to TVC (Sheng et al., 2018). Further, SAEW was recommended to extend beef shelf-life, although statistical models did not include any random or blocking terms (one-way anova) and results were interpreted using an unreferenced TVB-N 'maximum allowable upper limit for beef' of 20 mg/100 g (Sheng et al., 2018).

The determination of TVB-N via non-destructive means has emerged as a focus for meat scientists, as it offers a means to monitor and manage beef in real-time and based on its 'freshness'. This includes the research of Cheng et al. (2019) that used low-field magnetic resonance imaging (MRI) to determine the storage times of beef *semimembranosus* muscle samples, with TVB-N an important contributor to this outcome because of its found correlations (partial least squares regression) with pH

and TBARS. Multi-element viscoelastic models with six parameters were found to predict TVB-N levels with optimal accuracy, although a total of 50 beef eye round samples were used (38 calibration set and 12 test set) and were trimmed prior to assessment. This questions the practical applications of the methodology, with samples representing a range of 8.7 to 19.1 mg/100 g TVB-N and therefore unable to provide the reader confidence in its representation of samples outside of this range (e.g. Table 4; (Li et al., 2018, Li et al., 2019b, Li et al., 2019c). Zhao et al. (2018) found that DNA yield had a negative correlation to TVB-N in long-term frozen beef (total 66 months). Additional scrutiny of these findings was limited by the absence of a statistical analysis section, and information pertaining to the packaging type, variation to storage temperature, and the muscle type or portion sampled. Further, it is unclear as to whether this correlation between DNA yield and TVB-N content is coincidental, as correlations are not indicative of causation, and therefore, these findings may be the result of collinearity. Further, appraisal of the figures suggests that TVB-N levels were effectively static after 11 months of frozen storage, and the same was not true for DNA yield (Zhao et al., 2018).

Liu et al. (2020) used front-face synchronous fluorescence spectroscopy to classify beef freshness and found the excitation wavelength peak near 290 nm to have the strongest association with TVB-N levels for Simmental beef loin and rump samples, measured across 25 days of chilled storage. Interestingly, this peak region has already been associated with amino acids and conjugated Schiff base compounds, which are typically generated by protein degradation, as per the TVB-N compounds. Nonetheless, there was found to be an accuracy of 87% for the classification of samples as either fresh, acceptable or spoilt (Liu et al., 2020), which suggests further investigation with additional reference populations is necessary. The same could be true for TVB-N evaluation using e-nose technology, as Hong et al. (2012) demonstrate when using samples held for four days at 8 °C as a calibration set for the test set of samples held for 14 days at 2 °C; and as Xiao et al. (2014a) demonstrates by the omission of the number of samples tested or replication of their assessment of beef striploins. The principle of e-nose assessment of TVB-N is in theory sound, with organic volatiles also shown to increase with proteolysis (storage temperature) and storage periods (Franke and Beauchamp, 2017, Frank et al., 2020, Kilgannon et al., 2020). However, more robust investigation is recommended to confirm this application and its practical suitability for the beef sector.

Table 4

A summary of storage parameters on beef mean total volatile basic-nitrogen (TVB-n) levels and observed impact on freshness. Please note that the freshness threshold used may vary between studies and ~ signifies that TVB-n values were extrapolated from figures.

Storage parameters	Initial timepoint		Final timepoint		Freshness remarks and threshold	Reference
	Description	TVB-n content	Description	TVB-n content		
Beef, chilled at 4 °C 1. Control 2. Gelatine-carboxymethyl cellulose film (Gel-CMC) 3. Gel-CMC + 2% chitin nanofibril (CHNF) + 0.24% *Trachyspermum ammi* essential oil (TAEO) 4. Gel-CMC + 2% CHNF + 1.00% TAEO 5. Gel-CMC + 4% CHNF + 0.24% TAEO 6. Gel-CMC + 4% CHNF + 1.00% TAEO	24 h post-slaughter	6.1 mg/100 g	12 days	1. ~ 20.0 mg/100 g 2. ~ 19.5 mg/100 g 3. ~ 18.5 mg/100 g 4. ~ 12.5 mg/100 g 5. ~ 13.0 mg/100 g 6. 11.1 mg/100 g	1. Spoilt after 9 days 2. Spoilt after 12 days 3. Spoilt after 12 days 4. Acceptable freshness at 12 days 5. Acceptable freshness at 12 days 6. Acceptable freshness at 12 days (used TVB-n > 16.5 mg/100 g threshold)	Azarifar et al. (2020)
Beef, aerobic 1. Chilled at 0 ° C 2. Chilled at 4 °C	Day of retail purchase	7.0 mg/100 g	1. 22 days 2. 12 days	1. 32.3 mg/100 g 2. 29.9 mg/ 100g	1. Spoilt after 10 days 2. Spoilt after 8 days (used TVB-n > 16.5 mg/100 g threshold)	Byun et al. (2003)
Beef, anaerobic, (Super)chilled at -1 °C 1. Abattoir A 2. Abattoir B	24 h post-slaughter	11.1 mg/100 g	140 days	1. 20.1 mg/100 g 2. 18.4 mg/100 g	1. Spoilt after 63 days 2. Spoilt after 42 days (used TVB-n > 15 mg/100 g threshold)	Chen et al. (2020)
Beef, anaerobic, (Super)chilled at -1 °C	~ 38 days post-slaughter	10.4 mg/100 g	140 days	15.7 mg/100 g	Spoilt after 105 days (used TVB-n > 15 mg/100 g threshold)	Chen et al. (2019c)
Beef, aerobic, chilled at 4 °C	Day of retail purchase	9.8 mg/100 g	10 days	29.8 mg/100 g	Spoilt at 10 days (TVB-n threshold not provided)	Cheng et al. (2019)

Storage parameters	Initial timepoint		Final timepoint		Freshness remarks and threshold	Reference
	Description	TVB-n content	Description	TVB-n content		
Beef, anaerobic, chilled at 4 °C 1. Vacuum packaging (VP) 2. Chitosan coating + VP	Day of retail purchase	5.4 mg/100 g	45 days	1. 10.7 mg/100 g 2. 7.5 mg/100 g	No comparison provided	Duran and Kahve (2020)
Beef, anaerobic, (Super)chilled at -1 °C	84 days post-slaughter	4.8 mg/100 g	140 days	7.2 mg/100 g	Acceptable freshness at 140 days (used TVB-n > 15 mg/100 g threshold)	Frank et al. (2020)
Beef, anaerobic, (Super)chilled at -1 °C 1. Abattoir (Australia) 2. Abattoir (China 1) 3. Abattoir (China 2) 4. Abattoir (China 3)	84 days post-slaughter	1. 4.8 mg/100 g 2. 16.0 mg/100 g 3. 14.8 mg/100 g 4. 17.0 mg/100 g	140 days	1. 7.7 mg/100 g 2. 19.4 mg/100 g 3. 19.6 mg/100 g 4. 20.6 mg/100 g	1. Acceptable freshness at 140 days 2. Spoilt at 84 days 3. Spoilt after 84 days 4. Spoilt at 84 days (used TVB-n > 15 mg/100 g threshold)	Frank et al. (2019)
Beef, chilled at 4 °C 1. Control 2. 0.5% rhubarb ethanol extract (REE) + 0.04% cinnamon essential oil (CEO) 3. 1% REE + 0.08% CEO 4. 2% REE + 0.16% CEO 5. 5% REE + 0.32% CEO	Day of retail purchase	4.6 mg/100 g	12 days	1. ~ 37.5 mg/100 g 2. ~ 31.5 mg/100 g 3. 17.4 mg/100 g 4. 26.1 mg/100 g 5. 23.4 mg/100 g	1. Spoilt after 8 days 2. Spoilt at 12 days 3. Acceptable freshness at 12 days 4. Acceptable freshness at 12 days 5. Acceptable freshness at 12 days (used TVB-n > 30 mg/100 g threshold)	Han et al. (2014)
Beef, aerobic, chilled at 8 °C	Day of retail purchase	~ 13.0 mg/100 g	14 days	~ 47.0 mg/100 g	No comparison provided	Hong et al. (2012)
Beef, (Super)chilled at -1 °C 1. Control 2. Active packaging (allyl isothiocyanate and chitosan)	12 h post-slaughter	~ 6.0 mg/100 g	1. 21 days 2. 25 days	1. ~ 35.0 mg/100 g 2. ~ 23.0 mg/100 g	1. Spoilt after 11 days 2. Spoilt after 18 days (used TVB-n > 15 mg/100 g threshold)	Huang et al. (2017b)

Storage parameters	Initial timepoint		Final timepoint		Freshness remarks and threshold	Reference
	Description	TVB-n content	Description	TVB-n content		
Beef, aerobic, chilled at 4 °C 1. Control 2. Young kiwifruit polyphenols coating 3. Epicatechin coating 4. Potassium sorbate coating	Not defined. Timepoint 0	9.8 mg/100 g	7 days	1. 17.1 mg/100 g 2. 13.2 mg/100 g 3. 13.7 mg/100 g 4. 14.8 mg/100 g	1. Spoilt after 6 days 2. Acceptable freshness at 7 days 3. Acceptable freshness at 7 days 4. Acceptable freshness at 7 days (used TVB-n > 15 mg/100 g threshold)	Jiao et al. (2020)
Beef, anaerobic, chilled at 2 °C	4 days post-slaughter	Unknown	28 days	11.8-13.3 mg/100 g	Acceptable freshness at 28 days (used TVB-n > 20 mg/100 g threshold)	Koh et al. (2019)
Beef, aerobic, chilled at 4 °C	Day of retail purchase	9.4 mg/100 g	13 days	19.6 mg/100 g	Spoilt at 9 days and after 12 days (used TVB-n > 15 mg/100 g threshold)	Li et al. (2019b) Li et al. (2018)
Beef, MAP (60% O_2 + 40% CO_2), chilled at 4 °C 1. Loin 2. Rump	48 h post-slaughter	1. ~ 6.5 mg/100 g 2. ~ 6.5 mg/100 g	25 days	1. ~16.0 mg/100 g 2. ~16.0 mg/100 g	No comparison provided	Liu et al. (2020)
Beef, anaerobic 1. (Super)chilled at -1 °C 2. Chilled at 2 °C 3. Frozen at -18 °C	48 h post-slaughter	9.1 mg/100 g	1. 168 days 2. 56 days 3. 168 days	1. 17.6 mg/100 g 2. 18.8 mg/100 g 3. ~ 11.5 mg/100 g	1. Spoilt after 84 days 2. Spoilt after 28 days 3. Acceptable freshness at 168 days (used TVB-n > 15 mg/100 g threshold)	Lu et al. (2019)
Beef, anaerobic, chilled at 0 °C 1. Control 2. 100% CO (pre-treatment) 3. 2% O_3 + 98% CO (pre-treatment) 4. 5% O_3 + 97% CO (pre-treatment)	48 h post-slaughter	~ 6.5 mg/100 g	46 days	1. ~ 34.5 mg/100 g 2. ~ 26.0 mg/100 g 3. ~ 22.0 mg/100 g 4. ~ 20.0 mg/100 g 5. ~ 20.0 mg/100 g	No comparison provided	Lyu et al. (2016)

Storage parameters	Initial timepoint		Final timepoint		Freshness remarks and threshold	Reference
	Description	TVB-n content	Description	TVB-n content		
5. 10% O_3 + 90% CO (pre-treatment)						
Beef, chilled at 4 °C 1. Aerobic packaging 2. Vacuum packaged	Day of retail purchase	5.4 mg/100 g	1. 11 days 2. 21 days	1. 30.1 mg/100 g 2. 19.1 mg/100 g	1. Spoilt after 9 days 2. Acceptable freshness at 21 days (used TVB-n > 20 mg/100 g threshold)	Mansur et al. (2019)
Beef, aerobic, chilled at 4 °C 1. Control 2. Water coating 3. Tea polyphenols coating 4. Slightly acidic electrolysed water coating	Day of retail purchase	1. 8.4 mg/100 g 2. 8.4 mg/100 g 3. 8.1 mg/100 g 4. 8.2 mg/100 g	16 days	1. ~ 45.0 mg/100 g 2. ~ 38.0 mg/100 g 3. ~ 18.0 mg/100 g 4. ~ 13.5 mg/100 g	1. Spoilt after 8 days 2. Spoilt after 12 days 3. Acceptable freshness at 16 days 4. Acceptable freshness at 16 days (used TVB-n > 20 mg/100 g threshold)	Sheng et al. (2018)
Beef, packaging unknown 1. Chilled at 4 °C 2. (Super)chilled at -1 °C 3. Frozen at -6 °C 4. Frozen at -9 °C 5. Frozen at -12 °C 6. Frozen at -18 °C	Not defined. Timepoint 0	~ 5.0 mg/100 g	1. 14 days 2. 24 days 3. 84 days 4. 126 days 5. 168 days 6. 168 days	1. ~24.0 mg/100 g 2. 15.0 mg/100 g 3. 15.0 mg/100 g 4. 14.7 mg/100 g 5. 12.4 mg/100 g 6. 11.0 mg/100 g	1. Spoilt after 9 days 2. Spoilt after 24 days 3. Spoilt after 84 days 4. Spoilt after 126 days 5. Acceptable freshness at 168 days 6. Acceptable freshness at 168 days (used TVB-n > 15 mg/100 g threshold)	Qian et al. (2018)
Beef, anaerobic (vials) 1. Stored at room temperature 2. Chilled at 4 °C	Day of retail purchase	6.9 mg/100 g	1. 2 days 2. 9 days	1. 51.0 mg/100 g 2. 48.3 mg/100 g	1. Spoilt at 1 day 2. Spoilt after 2 days (used TVB-n > 15 mg/100 g threshold)	(Xiao et al., 2014b)
Beef, packaging unknown, frozen at -20 °C	3 h post-slaughter	~ 6.0 mg/100 g	~ 2007.5 days	~ 32.5 mg/100 g	Spoilt after ~ 30 days (used TVB-n > 15 mg/100 g	Zhao et al. (2018)

11.2 Lamb and sheep meat

Lamb and sheep meat is often held frozen or chilled to preserve its quality, safety and nutritional elements throughout the supply chain (Leygonie et al., 2012, Ponnampalam et al., 2016). However, scrutiny of literature has demonstrated that there is scant research using TVB-N to validate lamb and sheep meat freshness (Table 5). Those few studies available are somewhat limited in their transference to industry and export conditions typical for these meat products. For example, the effect of different active packaging types on minced lamb freshness was tested using TVB-N (Alizadeh-Sani et al., 2020). The authors of this study concluded that rosemary oil and titanium oxide-based active packaging could inhibit microbial- and enzymatic-driven proteolysis and in doing so restrict the accumulation of TVB-N (Alizadeh-Sani et al., 2020). But this finding was based on the analysis of pseudo-replicates of minced lamb, and interpretations of acceptable freshness were made using an arbitrary TVB-N threshold first proposed for minced camel meat (Khezrian and Shahbazi, 2018). These factors and the possible difference between minced and whole cut products limit broader extrapolations. Hu et al. (2011) instead, dipped fresh mutton into different concentrations of M. *officinalis* extract and investigated its effect on shelf-life when held aerobically at 7 °C for up to 12 days. Results of this study demonstrated suppression of TVB-N accumulation. However, this outcome merits consideration as at day 0 of the experiment, all TVB-N levels were < 1 mg/100 g and after three days at 7 °C the TVB-N content of control samples had risen less than would be expected (to ~ 10 mg/100 g). That said, sensory evaluation found mutton with levels of TVB-N > 15 mg/100 g were still of acceptable quality (Hu et al., 2011). This cautions against the transference of TVB-n guidelines between different types of meat and a need to develop a lamb and sheep meat specific threshold.

Table 5

A summary of storage parameters on lamb and sheep meat mean total volatile basic-nitrogen (TVB-n) levels and observed impact on freshness. Please note that the freshness threshold used may vary between studies and ~ signifies that TVB-n values were extrapolated from figures.

Storage parameters	**Initial timepoint**		**Final timepoint**		**Freshness remarks and threshold**	**Reference**
	Description	TVB-n content	Description	TVB-n content		
Lamb mince, chilled at 4 °C 1. Control PET film packaging 2. Active biopolymer packaging (rosemary oil + TiO_2)	Day of retail purchase	7.6 mg/100 g	15 days	1. 33.6 mg/100 g 2. 15.4 mg/100 g	1. Spoilt after 6 days 2. Acceptable freshness at 15 days (used TVB-n > 25 mg/100 g threshold)	Alizadeh-Sani et al. (2020)
Mutton, aerobic, chilled at 4 °C 1. Control 2. 1% *Magnolia officinalis* extract (MOE) 3. 2% MOE 4. 4% MOE 5. 6% MOE	Not defined. Timepoint 0	1.1 mg/100 g	12 days	1. 56.7 mg/100 g 2. ~ 21.5 mg/100 g 3. ~ 9.5 mg/100 g 4. ~ 6.5 mg/100g 5. ~ 6.0 mg/100 g	No comparison provided	Hu et al. (2011)

11.3 Pork

Pork and pig meat is consumed as many different forms, being processed as ham, bacon, etc. or purchased fresh for home preparation (Sosniki, 2016). When focussing on fresh pork products, their preservation, assurances of freshness, and targeted distribution, we can observe that TVB-N has been applied as an objective metric (Table 6).

For example, Zhao et al. (2015) used vacuum packaged pork leg samples to observe (but not discuss) that TVB-N increased across a three week chilled storage period, a trend that was reflected in TVC, but with some variance. Fan et al. (2019) tested the preservative effect of POE on pork tenderloins that were aged for up to nine days at 4 °C. There was found to be no difference between POE concentrations on TVB-N content, although the rate of TVB-N accumulation was greater for control samples as these achieved unacceptable levels > 15 mg/100 g after five days (Fan et al., 2019). This was proposed to result from POE inhibition of spoilage bacteria, a point supported by the TVC results for these same samples. A similar outcome was observed using black pepper essential oil (BPEO), with pork LL TVB-N levels found to increase across a nine-day ageing period at 4 °C, but at a lesser rate when first sprayed with BPEO (Zhang et al., 2016). The authors of this study attributed these variations to the differences in microbial loads rather than chemical deterioration, and from the results, this could be interpreted as BPEO specific inhibition of *Pseudomonas* sp., and *Enterobacteriaceae*. This outcome is of interest when we consider Huang et al. (2014), wherein pork loin samples were inoculated with different microbial species – this included *Bacillus fusiformis*, *Acinetobacter guillouiae*, *Pseudomonas koreensis*, and *Brochothrix thermosphacta*. It was found that the TVB-N of inoculated samples was higher than control (uninoculated) samples, and that the increase across an eleven day chilled storage period was dependent on the inoculum selected (Huang et al., 2014). These findings are encouraging, but actual microbial profiles or loads were not confirmed in the experimental samples and therefore we cannot infer the basal microbial loads or relationship between microbial proliferation and TVB-N levels. However, Li et al. (2019a) observed that at the same point at which chilled pork TVB-N values dramatically increased, its microbial profile was dominated by *Pseudomonas* sp., *Acinetobacter* sp. and *Photobacterium* sp. which therefore affirms the capacity for TVB-N levels to be influenced by specific microbial types. This suggests, therefore, that TVB-N may not always be an accurate biomarker for TVC.

Sub-zero freezing temperatures inhibit the proliferation of many microbial types, so evaluation of TVB-N changes within frozen storage may provide some insight into the TVB-N attributable to

microbial loads. The effect of frozen storage on pork TVB-N has attracted some conflicting outcomes. Custodio et al. (2018), for example, reported that TVB-N levels declined across a frozen storage period (at -18 °C) of 135 days for both loin and leg muscle samples. The authors did not provide context for this result, although we note that TVB-N levels in the chilled samples increased for eight days and then plateaued, although these included substantial variance around the mean values. Zequan et al. (2019) instead observed TVB-N levels of both *biceps femoris* and LL muscles to increase with 18 weeks of frozen storage, irrespective of pork being PSE-like or control. These latter outcomes suggests that TVB-N accumulation is somewhat independent to microbial loads as -18 °C should be enough to restrict their proliferation (Coombs et al., 2017b). That said, research with lamb meat demonstrated that microbial loads could change across frozen storage periods of up to 12 months (Coombs et al., 2017a). Instead, the accumulation of TVB-N during frozen storage may be indicative of a continuation of tenderisation or proteolytic activities (Medic et al., 2018), with it worth noting the availability of unfrozen water fractions at sub-zero freezing temperatures > -22 °C (Rahelic et al., 1985) wherein enzymatic reactions can still occur.

Methods to determine pork TVB-N levels have been developed to be non-destructive. For instance, laser light-scattering images were used to categorise pork as fresh, stale or spoilt (Li et al., 2016a). This study found that adaptive boosting orthogonal linear discriminate analysis was best to support modelling, with outcomes proving useful at categorising pork samples as either fresh or spoilt: potentially the result of a limited calibration and test set of 20 and 10 pork fillets respectively (Li et al., 2016a). E-nose technology has also been applied to pork samples, with models that include 'nitrogen oxides, aromatic and sulphur organic compounds, alcohols and partially aromatic compounds' found to provide the best prediction for vacuum packaged pork TVB-N levels (Li et al., 2016b). This model profile was different for MAP and pallet packaging and resulted in slightly different levels of precision (Li et al., 2016b), and we observe that the range of TVB-n was also different between packaging types and for the most part, spoilt samples (TVC > log 7 cfu/g) were absent from this study. Chen et al. (2019b) developed colorimetric labels that visibly change as pork leg samples became spoilt. This was designed to change from green-to-red when TVB-N levels exceeded 15 mg/100 g and sensory scores became unacceptable (< 5) with both of these outcomes shown to occur simultaneously after five days of storage at 5 °C. A similar device, instead developed using rosella anthocyanins, was likewise found to change colour at the time point when pork TVB-N exceeded 15 mg/100 g (Zhang et al., 2019a). It should be considered, however, that the detection capacities and applications of these intelligent

packaging devices would be impacted if the TVB-N ‘rejection limit’ of 15 mg/100 g is changed.

Table 6

A summary of storage parameters on pork mean total volatile basic-nitrogen (TVB-n) levels and observed impact on freshness. Please note that the freshness threshold used may vary between studies and ~ signifies that TVB-n values were extrapolated from figures.

Storage parameters	**Initial time point**		**Final time point**		**Freshness remarks and threshold**	**Reference**
	Description	TVB-n content	Description	TVB-n content		
Pork, aerobic 1. Chilled at 0 ° C 2. Chilled at 4 °C	Day of retail purchase	8.0 mg/100 g	1. 22 days 2. 12 days	1. 32.5 mg/100 g 2. 31.5 mg/ 100g	1. Spoilt after 10 days 2. Spoilt after 6 days (used TVB-n > 16.5 mg/100 g threshold)	Byun et al. (2003)
Pork, aerobic, chilled at 5 °C	Day of retail purchase	4.2 mg/100 g	8 days	29.0 mg/100 g	Spoilt after 6 days (used TVB-n > 15 mg/100 g threshold)	Chen et al. (2019b)
Pork, aerobic 1. Leg, chilled at 5 °C 2. Loin, chilled at 5 °C 3. Leg, frozen at -18 °C 4. Loin, frozen at -18 °C	24 h post-slaughter	1. 9.0 mg/100 g 2. 15.4 mg/100 g 3. 22.0 mg/100 g 4. 24.2 mg/100 g	1. 16 days 2. 16 days 3. 180 days 4. 180 days	1. 29.5 mg/100 g 2. 32.9 mg/100 g 3. 11.1 mg/100 g 4. 3.6 mg/100 g	No comparison provided	Custodio et al. (2018)
Pork, aerobic, chilled at 4 °C 1. Control 2. 0.25% *Portulaca oleracea* L. extract (POE) 3. 0.50% POE 4. 1.00% POE	Day of retail purchase	1. ~ 8.0 mg/100 g 2. ~ 7.5 mg/100 g 3. ~ 7.5 mg/100 g 4. ~ 6.5 mg/100 g	9 days	1. ~ 18.5 mg/100 g 2. 14.0 mg/100 g 3. 12.7 mg/100 g 4. 11.9 mg/100 g	1. Spoilt after 5 days 2. Acceptable freshness at 9 days 3. Acceptable freshness at 9 days 4. Acceptable freshness at 9 days (used TVB-n > 15 mg/100 g threshold)	Fan et al. (2019)

Storage parameters	Initial time point		Final time point		Freshness remarks and threshold	Reference
	Description	TVB-n content	Description	TVB-n content		
Pork, aerobic, chilled at 4 °C 1. *B. fusiformis* inoculation 2. *A. guillouiae* inoculation 3. *P. koreensis* inoculation 4. B. thermosphacta inoculation 5. Control	24 h post-slaughter	1. 5.4 mg/100 g 2. 5.3 mg/100 g 3. 6.3 mg/100 g 4. 5.4 mg/100 g 5. 4.5 mg/100 g	11 days	1. 31.5 mg/100 g 2. 28.9 mg/100 g 3. 35.0 mg/100 g 4. 29.7 mg/100 g 5. 26.9 mg/100 g	No comparison provided	(Huang et al., 2014)
Pork, aerobic, chilled at 4 °C 1. Retail outlet 1 2. Retail outlet 2 3. Retail outlet 3 4. Retail outlet 4 5. Retail outlet 5 6. Retail outlet 6	Day of retail purchase	1. ~ 4.0 mg/100 g 2. ~ 6.5 mg/100 g 3. ~ 6.5 mg/100 g 4. ~ 6.0 mg/100 g 5. ~ 9.0 mg/100 g 6. ~ 9.0 mg/ 100 g	9 days	1. ~ 10.0 mg/100 g 2. ~ 12.5 mg/100 g 3. ~ 17.5 mg/100 g 4. ~ 17.5 mg/100 g 5. ~ 21.0 mg/100 g 6. ~ 13.0 mg/100 g	1. Acceptable freshness at 9 days 2. Acceptable freshness at 9 days 3. Spoilt after 7 days 4. Spoilt after 9 days 5. Spoilt after 7 days 6. Acceptable freshness at 9 days (used TVB-n > 15 mg/100 g threshold)	Li et al. (2019a)
Pork, aerobic (petri dish), chilled at 4 °C	Day of retail purchase	~ 10.5 mg/100 g	5 days	~ 17.5 mg/100 g	Spoilt after 5 days (TVB-n > 15 mg/100 g threshold)	Li et al. (2016a)
Pork, chilled at 5 °C 1. Pallet packaging 2. Vacuum packaging 3. MAP (40% CO_2 + 40% O_2 + 20% N_2)	24 h post-slaughter	~ 11.0 mg/100 g	1. 9 days 2. 14 days 3. 16 days	1. 28.0 mg/100 g 2. 24.2 mg/100 g 3. 21.1 mg/100 g	No comparison provided	Li et al. (2016b)

Storage parameters	Initial time point		Final time point		Freshness remarks and threshold	Reference
	Description	TVB-n content	Description	TVB-n content		
Pork, anaerobic, frozen at -18 °C 1. Longissimus lumborum, PSE-like 2. Longissimus lumborum, control 3. Biceps femoris, PSE-like 4. Biceps femoris, control	24 h post-slaughter	1. ~ 10.0 mg/100 g 2. ~ 9.5 mg/100 g 3. ~ 10.0 mg/100 g 4. ~ 10.0 mg/100 g	126 days	1. ~ 15.5 mg/100 g 2. ~ 14.0 mg/100 g 3. ~ 14.0 mg/100 g 4. ~ 13.0 mg/100 g	Acceptable freshness at 126 days (used TVB-n > 15 mg/100 g threshold)	Zequan et al. (2019)
Pork, aerobic (petri dish), stored at 25 °C	Day of retail purchase	7.5 mg/100 g	2.5 days	30.8 mg/100 g	Spoilt after 1 day (used TVB-n > 15 mg/100 g threshold)	Zhang et al. (2019a)
Pork, aerobic, chilled at 4 °C 1. Control 2. 0.1% black pepper essential oil (BPEO) 3. 0.5% black pepper essential oil (BPEO)	30 min post-slaughter	~ 11.0 mg/100 g	9 days	1. ~ 26.5 mg/100 g 2. ~ 21.0 mg/100 g 3. ~16.5 mg/100 g	1. Spoilt after 9 days 2. Acceptable freshness at 9 days 3. Acceptable freshness at 9 days (used TVB-n > 26 mg/100 g threshold)	Zhang et al. (2016)
Pork, aerobic, chilled at 4 °C	Day of retail purchase	~7.5 mg/100 g	14 days	~ 24.0 mg/100 g	Spoilt after 8 days (used TVB-n > 15 mg/100 g threshold)	Zhai et al. (2020)
Pork, anaerobic, chilled at 0 °C	20 h post-slaughter	5.7 mg/100 g	21 days	8.8 mg/100 g	No comparison provided	Zhao et al. (2015)

11.4 Fish and seafood

There has been an abundance of research pertaining to the relationship between TVB-N levels and the freshness of fish and other seafood products (Kirk and Sawyer, 1991, Connell, 1995, Gimenez et al., 2002, Azam et al., 2004, Castro et al., 2006, Hamaguchi et al., 2007, Limo et al., 2009, Howgate, 2010b, Shi et al., 2020, Xiong et al., 2020). From these studies, we can observe the widespread application of TVB-N to determine fish freshness – although there remains contrary positions as to its value as an indicator of fish freshness (e.g. Chytiri et al. (2004) and Tejada and Huidobro (2002)). This has not restricted the development of TVB-N standards, but these are limited to specific fish species and product types (e.g. whole fish, fillets, slaughter method, etc.). We should adhere to this point when considering the application of TVB-N standards across other meat types – often having a substantially lesser body of research than that evident for fish products.

11.5 Chicken and poultry meat

Chicken and poultry meat are widely eaten, with consumers demanding fresh products that are both safe and of high eating quality. TVB-N has therefore been applied to provide an objective metric to quantify chicken freshness. Indeed, the TVB-N content of chicken meat is shown to increase with storage time, temperatures and muscle type (Table 7), with Silva and Gloria (2002) finding that the rate of TVB-N accumulation across 15 days storage at 4 °C was greater for thigh meat when compared to the breast – although this observation may be confounded the different microbial loads for each cut. Further, this study observed that TVB-N values were significantly different only after the expiration dates for the chicken samples and were therefore useful only to detect the later stages of deterioration (Silva and Gloria, 2002). It was interesting to note that similar, but not identical trends to TVB-N, observed for other bioactive amine quality indices originally proposed for tuna (Mietz and Karmas, 1977, Veciana-Nogues et al., 1997) were calculated for the same chicken samples. When we transfer the acceptable limits for these indices (bioactive amines < 50 mg/kg, Veciana-Nogues et al. (1997)), we can observe that breast meat was unacceptable after 15 days (when TVB-n was equal to 34.4 mg/100 g) whereas thigh meat remained acceptable, although its TVB-N level was 46.5 mg/100 g and there was substantial variance around these mean values.

In an investigation into the effect of chilled storage temperature on the freshness of chicken meat, significant correlations were identified between TVB-N and sensory panel assessment, microbial loads of *Pseudomonas* spp., TVC, LAB and *Enterobacteriaceae* (in descending order) and pH (Ghollasi-

Mood et al., 2017). Based on these correlations, the authors identified TVB-N levels of 25.5 mg/100 g as the point of sensory panel rejection and suggested this as a critical limit. It was observed that this study compared its TVB-N results to that of Abu-Ruwaida et al. (1994), which did not investigate TVB-N, include a description of the statistical models applied to the data nor acknowledge that simple correlations are not indicative of causation. Alternatively, Balamatsia et al. (2006) observed that the TVB-N level of irradiated chicken samples was lower for non-irradiated samples stored aerobically, and did not exceed 37.0 mg/100 g TVB-N when held for up to 21 days at 4 °C. These samples were considered to be of acceptable freshness – an observation supported by positive odour, taste and appearance sensory scores when irradiation was 2.0 kGy. The pathway for this outcome was likely the impact of irradiation on microbial populations, with data showing alignment between TVB-N and the proliferation of aerobic, LAB, *Brochothrix thermosphacta* and *Enterobacteriaceae*, wherein numbers at 21 days declined as the level of irradiation increased. Alginate coatings with lactoperoxiase systems have also been applied to inhibit microbial proliferation and TVB-N accumulation in chicken breast fillets (Yousefi et al., 2018). In this study, the coatings were shown to prevent TVB-N levels from exceeding 15 mg/100 g for up to eight days, (thereafter, chicken meat is considered to be 'stale') and therefore more than the four days achieved with uncoated control samples (Yousefi et al., 2018). The authors propose this to be the result of reduced rates of decarboxylation of amino acids from enzymatic or microbial-mediated pathways and observe that the coatings had different efficacies towards the inhibition of different microbial types. This latter observation supports the differences in TVB-N content after 12 days and suggests that its accumulation may be dependent on the specific microbial profiles.

Non-destructive methods to determine the TVB-N content of chicken meat have been developed. These include capacitive gas sensors designed to identify spoilt chicken breast meat (Senapati and Sahu, 2020). This study found the sensor could detect changes to volatile NH_3, TMA, H_2S and ethanol which were correlated to TVB-N and therefore, could determine TVB-N levels. The rate of TVB-N accumulation increased with temperature, as samples were held at either 4 °C, 15 °C or 25 °C. However, we are unaware of the number of chicken breasts used in this study (Senapati and Sahu, 2020). It was of interest that the authors compared chicken TVB-N values against the limits for pork and beef described in Byun et al. (2003) rather than TVB-N guidelines developed for chicken or poultry meats. Colorimetric sensors for intelligent packaging (Khulal et al., 2016a) and hyperspecial imaging (Khulal et al., 2016b) were both found to predict TVB-N levels in chicken samples. Both studies used

the same 15 aged chicken samples, that had a TVB-N range of 5.7-42.7 mg/100 g, and considered unacceptable freshness to be when TVB-N > 15 mg/100 g. We observe a disadvantage to colorimetric sensors being the unrestrictive access to the information. For instance, the customer will observe that the chicken meat is not entirely fresh by the marginal colour change and therefore avoid its purchase (Holman et al., 2018). The proposed colorimetric sensors of Khulal et al. (2016b) includes 12 different dots of chemical responsive dye that are quantified using chemometric methods, and we would expect that these aspects would prevent unwanted or erroneous interpretations of chicken meat freshness. The same cannot be applied to the colorimetric sensor for TVB-N using chicken breasts, outlined in Rukchon et al. (2014).

There are few studies that have used TVB-N to investigate duck freshness. This paucity is demonstrated in the evaluation of the TVB-N content of duck meat using visible and NIR spectroscopy (Qiao et al., 2017). This study held the breast muscles from 61 ducks at 4 °C for up to 16 days, to achieve the calibration and test sets and a TVB-n sample range of 6.8-17.1 mg/100 g (Qiao et al., 2017). The absence of information pertaining to duck meat and its acceptable levels of TVB-N or its relationship to other freshness parameters makes it difficult to glean practical insight from the reported capacity to spectroscopically predict duck meat TVB-N. We would anticipate that more critical appraisal of this and other similar studies could be made if specific guidelines for duck meats were available.

11.6 Other types of meat

An investigation of rabbit meat found TVB-N levels to be temperature dependent, with LL samples chilled at 4 °C for up to 14 days observed to have TVB-N levels of 79.1 mg/100 g (Wang et al., 2020) (Table 7). From this study, we observed that the TVC for these same samples exceeded log 7 cfu/g when TVB-N was ~ 55 mg/100 g. This TVB-N level may therefore be considered as the upper limit for rabbit meat freshness – although, additional research would be advisable.

The impact of several types of active packaging on minced camel meat shelf-life was measured using TVB-N, with the authors proposing the arbitrary threshold of 25 mg/100 g (Khezrian and Shahbazi, 2018) (Table 8). We observe that the control sample TVC > log 7 cfu/g and was therefore unacceptable after four days (Maqsood et al., 2016). This was the same time point at which TVB-N exceeded the proposed threshold (25 mg/100 g) and as a result, could be the basis for the applied threshold. This was not clarified in the text. Moreover, caution may be advisable as the *semimembranosus* muscle mince was prepared as a single batch and experimental effects were not

replicated in this study (Khezrian and Shahbazi, 2018). Nonetheless, the concept to inoculate the mince to standardise the microbial profile of the samples is of interest, particularly to confirm a relationship between specific microbial types and TVB-N.

Table 7

A summary of storage parameters on chicken and poultry meat as well as other types of meat mean total volatile basic-nitrogen (TVB-n) levels and observed impact on freshness. Please note that the freshness threshold used may vary between studies and ~ signifies that TVB-n values were extrapolated from figures.

Storage parameters	**Initial timepoint**		**Final timepoint**		**Freshness remarks and threshold**	**Reference**
	Description	TVB-n content	Description	TVB-n content		
Chicken, aerobic, chilled at 4 °C 1. Control 2. Irradiated at 0.5 kGy 3. Irradiated at 1.0 kGy 4. Irradiated at 2.0 kGy	24 h post-slaughter	1. ~ 26.5 mg/100 g 2. ~ 25.0 mg/100 g 3. ~ 20.0 mg/100 g 4. ~ 15.0 mg/100 g	21 days	1. ~ 58.0 mg/100 g 2. ~ 36.0 mg/100 g 3. ~ 32.5 mg/100 g 4. ~ 28.0 mg/100 g	1. Spoilt after 5 days 2. Spoilt after 14 days 3. Spoilt after 17 days 4. Acceptable freshness at 21 days (used TVB-n > 28 mg/100 g threshold)	Balamatsia et al. (2006)
Chicken, aerobic, 1. Chilled at 0 °C 2. Chilled at 4 °C 3. Chilled at 10 °C 4. Stored at 15 °C	Day of slaughter	11.3 mg/100 g	1. 2 days 2. 5 days 3. 9.2 days 4. 13.3 days	1. ~ 36.5 mg/100 g 2. ~ 32.5 mg/100 g 3. ~ 32.0 mg/100 g 4. ~ 29.5 mg/100 g	1. Spoilt after 11 days 2. Spoilt after 6.9 days 3. Spoilt after 2.9 days 4. Spoilt after 2.3 days (used TVB-n > 23 mg/100 g threshold)	Ghollasi-Mood et al. (2017)
Camel mince, aerobic, chilled at 4 °C 1. Control 2. Carboxymethyl cellulose film (CMC) 3. CMC + 0.5% *Ziziphora clinopodioides* oil (ZEO) 4. CMC + 1.0% ZEO	24 h post-slaughter	8.1 mg/100 g	14 days	1. 41.6 mg/100 g 2. ~ 37.0 mg/100 g 3. ~ 31.0 mg/100 g 4. ~ 23.0 mg/ 100 g 5. ~ 19.5 mg/100 g	1. Spoilt after 4 days 2. Spoilt after 6 days 3. Spoilt after 12 days 4. Acceptable freshness at 14 days 5. Acceptable freshness at 14 days	Khezrian and Shahbazi (2018)

Storage parameters	Initial timepoint		Final timepoint		Freshness remarks and threshold	Reference
	Description	TVB-n content	Description	TVB-n content		
5. CMC + 2% ZEO					(used TVB-n > 25 mg/100 g threshold)	
Chicken, aerobic (container) 1. Chilled at 4 °C 2. Stored at 15 °C 3. Stored at 25 °C	Day of retail purchase	~ 11.5 mg/100 g	1. 5 days 2. 3.3 days 3. 1 day	1. ~ 26.0 mg/100 g 2. ~ 39.0 mg/100 g 3. ~ 39.5 mg/100 g	1. Acceptable freshness at 5 days 2. Spoilt after 2.5 days 3. Spoilt after 0.75 days (used TVB-n 25 mg/100 g threshold)	(Senapati and Sahu, 2020)
Chicken, packaging unknown, chilled at 4 °C 1. Breast 2. Thigh	Day of slaughter	1. 14.3 mg/100 g 2. 16.8 mg/100 g	15 days	1. 34.4 mg/100 g 2. 46.5 mg/ 100 g	No comparison given	Silva and Gloria (2002)
Rabbit, aerobic 1. Chilled at 4 °C 2. Frozen at -4 °C 3. Frozen at -12 °C 4. Frozen at -18 °C	24 h post-slaughter	7.6 mg/100 g	1. 14 days 2. 45 days 3. 45 days 4. 45 days	1. 79.1 mg/100 g 2. 33.9 mg/100 g 3. 25.3 mg/100 g 4. 19.4 mg/100 g	No comparison given	Wang et al. (2020)
Chicken, aerobic, chilled at 4 °C 1. Control 2. 0% alginate-lactoperoxidase coating (A-L) 3. 2% A-L coating 4. 4% A-L coating 5. 6% A-L coating	Day of retail purchase	13.7 mg/100	16 days	1. 32.9 mg/100 g 2. 31.8 mg/100 g 3. 25.5 mg/100 g 4. 22.0 mg/100 g 5. 21.1 mg/100 g	1. Spoilt after 4 days 2. Spoilt after 8 days 3. Spoilt after 8 days 4. Spoilt after 8 days 5. Spoilt after 16 days (used TVB-n > 15 mg/100 g threshold)	Yousefi et al. (2018)

11.0 TVB-N GUIDELINES AND FRESHNESS LIMITS

From Table 8, we observe there to be a range of different recommendations for TVB-N levels that assure meat product freshness. Of those found, national organisations tended to propose more conservative TVB-N limits. For example, the National Standards of the People's Republic of China (PRC) consistently state that 15 mg TVB-N per 100 g as the limit for 'fresh' red meat and poultry products. Both the Korean Ministry of Agriculture and Forestry and the Egyptian Organisation for Standardisation and Quality Control advice that 20 mg TVB-n per 100 g is the limit for 'fresh' livestock meat and poultry and rabbit meat, respectively. The European Union instead recommended three different TVB-N limits for fish freshness, these dependent on the species of interest. It is of interest that these TVB-N limits are often in contrast to those recommended by scientific studies. Balamatsia et al. (2006) for example, proposed the TVB-N values of 28-29 mg/100 g to be the upper limits for the initiation of spoilage for aerobically stored chicken meat, having based this limit on sensory odour analysis. Senapati and Sahu (2020) proposed a similar acceptable limit for human consumption of 25-28 mg/100 g TVB-N that should be applied to identify the spoilage status of chicken meat – although the authors did not provide a reference for this statement. Based on 'sensorial noticeability' of the onset of spoilage, Stephan et al. (1997) found that 40.3 mg/100 g TVB-N would be applicable to determine beef freshness. Regardless of their source, inconsistencies between these guidelines highlights the importance of TVB-N limits for specific meat type or species – otherwise there is the potential for misinterpretation or misrepresentation.

The observed range of available TVB-N limits allows stakeholders to select a limit that suits their narrative or agenda, rather than one that provides a true reflection of product freshness. This is evident from the range of TVB-N limits applied in Tables 5-8 to interpret the freshness of the meat products investigated. From these it is also apparent that the 15 mg/100 g TVB-N threshold is predominant – with researchers often citing the policies of the National Standards of the People's Republic of China. Indeed, technologies that apply non-destructive methods to quantify TVB-N will often categorise 'freshness' based on this same threshold (Khulal et al., 2016b, Li et al., 2018). Yet, investigation of these policies does not elucidate the scientific basis or source for this TVB-N limit or confirm its applications to different meat types (i.e. mutton, lamb, beef, poultry, etc.). This omission detracts from our confidence that it is evidence that is driving our interpretations of product freshness rather than potentially subjective policies. To counter this premise, it would be advisable to reference

the scientific research that underpins current TVB-N guidelines and undertaken additional research to corroborated TVB-N levels with perceivable quality and safety parameters indicative of ‘freshness’ and specific to different storage parameters and meat types. Provision of this comprehensive guidelines could help standardise the interpretation of research to extend the shelf-life of meat products and better inform market access and quality assurances.

Table 9

A summary of total volatile basic-nitrogen (TVB-N) guidelines and thresholds to define the freshness or spoilage of different types of meat. Please note that the organisation and corresponding policy have been included were available, otherwise the reference is the source of the included TVB-N threshold.

Meat type or species	TVB-n guidelines	Reference / Organisation and Policy
Fresh and frozen pork lean, cuts	Fresh: < 15 mg TVB-n per 100 g	National Standards of People's Republic of China (GB/T 9956.2-2008)
Fresh and frozen mutton carcass	Fresh: < 15 mg TVB-n per 100 g	National Standards of People's Republic of China (GB/T 9961-2008)
Fresh and frozen beef, quarters	Fresh: < 15 mg TVB-n per 100 g	National Standards of People's Republic of China (GB/T 9960-2008)
Fresh and frozen livestock and poultry products	Fresh: < 15 mg TVB-n per 100 g	National Health and Family Planning Commission of the People's Republic of China and The State Food and Drug Administration (GB 2707-2016) Standardized Administration of the People's Republic of China (GB 2707-2005) – repealed 2016
Fish	Fresh: < 16.5	Pearson (1976)
Fresh and frozen demi carcass pork	Fresh: < 20 mg TVB-n per 100 g	National Standards of People's Republic of China (GB 9959.1-2001)
Poultry and rabbit	Fresh: < 20 mg TVB-n per 100 g	Egyptian Organization for Standardization and Quality Control (EOS 1090/2005)
Livestock meat	Fresh: < 20 mg TVB-n per 100 g	Korean Ministry of Agriculture and Forestry (MFDS Notice No. 2015-94)
Fresh and frozen meat	Fresh: < 20 mg TVB-n per 100 g Stale: > 30 mg TVB-n per 100 g	FAO (1986)
White fish	Fresh: < 20 mg TVB-n per 100 g Stale: 20-40 mg TVB-n per 100 g Spoilt: > 40 mg TVB-n per 100 g	Kirk and Sawyer (1991)
Camel	Fresh: < 25 mg TVB-n per 100 g	Khezrian and Shahbazi (2018)
Fish[1]	Fresh: < 25 mg TVB-n per 100 g	European Union (EC No. 2074/2005) European Union (EC No. 95/149) – repealed 2006

Meat type or species	TVB-n guidelines	Reference / Organisation and Policy
Chicken	Fresh: < 25-28 mg TVB-n per 100 g	(Senapati and Sahu, 2020)
Chicken	Fresh: < 25.5 mg TVB-n per 100 g	Ghollasi-Mood et al. (2017)
Chicken	Fresh: <28-29 mg TVB-n per 100 g	Balamatsia et al. (2006)
Fish[2]	Fresh: < 30 mg TVB-n per 100 g	European Union (EC No. 2074/2005) European Union (EC No. 95/149) – repealed 2006
Fish	Fresh: < 30 mg TVB-n per 100 g	Connell (1995)
Cephalopods	Fresh: < 30 mg TVB-n per 100 g	Altissimi et al. (2017)
Fish[3]	Fresh: < 35 mg TVB-n per 100 g	European Union (EC No. 2074/2005) European Union (EC No. 95/149) – repealed 2006
Beef	Fresh: < 40.3 mg TVB-n per 100 g	Stephan et al. (1997)
Crustaceans	Fresh: < 42 mg TVB-n per 100 g	Altissimi et al. (2017)

[1](*Sebastes sp*., *Helicolenus dactylopterus*, *Sebastichthys capensis*). [2](*Pleuronectidae* sp.). [3](*Salmo salar*, *Merlucciidae sp*., *Gadidae sp*.)

12.0 CONCLUSIONS/RECOMMENDATIONS

This comprehensive review revealed several important aspects pertinent to meat quality and safety.

- Total volatile basic nitrogen (TVB-N) in meat is generated as a result of *post-mortem* biochemical and chemical activities due to exogenous and microbial enzymes.
- TVB-N is shown to increase with ageing period and other markers of spoilage (increase in TVC and oxidation by-products). This relationship has been found in fresh, frozen and processed meat products.
- The alignment of TVB-N concentration increases with time *post-mortem* are inconsistent and vary among meat from different species. This is largely influenced by the natural ability of the meat to support bacterial growth, especially those suspected for their involvement in the decomposition of nitrogen-containing compounds.
- The majority of the reported research in meat studies apply TVB-N as an independent metric to estimate the progression of spoilage, rather than its interrelationship with other spoilage metrics.
- There is paucity in good experimental design that provide reproducible insight into the mechanistic relationship between TVB-N generation and production, processing and preservation conditions.
- Generally, there is no specific TVB-N threshold values for beef and lamb to interpret the results against and most of the studies refer to values set for pork. When values reported for beef are compared against existing recommendations there is little relationship with other spoilage thresholds based on TBARS, microbial loads, colour, odour, etc.
- Most of the reported non-destructive systems are able to differentiate between spoiled and non-spoiled samples through chemometric classification systems. This is a limited scope for commercial utilisation.
- There are a range of TVB-N guidelines proposed to identify fresh or spoilt meat products. These are often specific to a species or meat type – with few available for red meat. In addition, many guidelines propose TVB-N thresholds without reference to the scientific basis or source of this recommendation.
- The level and type of biogenic amines present in meat is considered a health concern, owing to their known toxic effects. This may add another element to our understanding of the TVB-N of meat, as its precursors (TMA, TMAO-N and formaldehyde) have been associated with

dietary-related illnesses – such as, cardiovascular diseases, diabetes, cancer and renal complications

From these observations it is apparent that future investigation is necessary in the following areas:

- Investigation specific to red meat (beef and sheep meat) of TVB-N as a freshness biomarker must be undertaken to provide industry an evidence-based guideline for adoption within domestic and export markets.
- Investigations of spectroscopic and other non-invasive measurements of TVB-N should be undertaken to provide industry the tools for the rapid, inexpensive and objective assessment of red meat products adherence to appropriate guidelines of freshness.
- Investigations that confirm the association between TVB-N and other quality parameters should be undertaken to understand the implications of a TVB-N based threshold for freshness – e.g. would a TVB-N threshold disadvantage red meat product that is tender (as a result of proteolytic activity) or otherwise sensorial appealing?
- Investigation of microbial populations and the association of specific types of bacteria with TVB-N generation should be undertaken for red meat to determine whether TVB-N is a viable proxy for microbial safety considerations or otherwise.
- Investigation of the generation of TVB-N precursors *post-mortem* in red meat and their health implications upon ingestion should be undertaken for red meat to demonstrate safety and health outcomes.

13.0 BIBLIOGRAPHY

ABASIYANIK, M. F. & SENEL, M. 2010. Immobilization of glucose oxidase on reagentless ferrocene-containing polythiophene derivative and its glucose sensing application. *Journal of Electroanalytical Chemistry,* 639, 21-26.

ABU-RUWAIDA, A. S., SAWAYA, W. N., DASHTI, B. H., MURAD, M. & AL-OTHMAN, H. A. 1994. Microbiological quality of broilers during processing in a modern commercial slaughterhouse in Kuwait. *Journal of Food Protection,* 57, 887-892.

ADAMSON, R. H. & THORGEIRSSON, U. P. 1995. Carcinogens in foods: Heterocyclic amines and cancer and heart disease. *In:* LONGENECKER, J. B., KRITCHEVSKY, D. & DREZNER, M. K. (eds.) *Nutrition and Biotechnology in Heart Disease and Cancer.* Boston, USA: Springer.

AHMED, I., LIN, H., ZOU, L., LI, Z., BRODY, A. L., QAZI, I. M., LV, L., PAVASE, T. R., KHAN, M. U., KHAN, S. & SUN, L. 2018. An overview of smart packaging technologies for monitoring safety and quality of meat and meat products. *Packaging Technology and Science,* 31, 449-471.

AJAYKUMAR, V. J. & MANDAL, P. K. 2020. Modern concept and detection of spoilage in meat and meat products. *In:* BISWAS, A. K. & MANDAL, P. K. (eds.) *Advanced Evaluation Methods, Techniques, and Technologies.* Academic Press.

AKKOSE, A. & AKTAS, N. 2008. Determination of glass transition temperature of beef and effects on various cryoprotective agents on some chemical changes. *Meat Science,* 80, 875-878.

ALISSON-SILVA, F., KAWANISHI, K. & VARKI, A. 2016. Human risk of diseases associated with red meat intake: Analysis of current theories and proposed role for metabolic incorporation of a non-human sialic acid. *Molecular Aspects of Medicine,* 51, 16-30.

ALIZADEH-SANI, M., MOHAMMADIAN, E. & MCCLEMENTS, D. J. 2020. Eco-friendly active packaging consisting of nanostructured biopolymer matrix reinforced with TiO_2 and essential oil: Application for preservation of refrigerated meat. *Food Chemistry,* 322, 126782.

ALSHEJARI, A., KODOGIANNIS, V. S. & PETROUNIAS, I. 2015. A rapid detection of meat spoilage using FTIR and neuro-fuzzy systems. *In:* BI, Y., KAPOOR, S. & BHATIA, R. (eds.) *SAI Intelligent Systems Conference (IntelliSys).* Longdon, UK.

ALTISSIMI, S., MERCURI, M. L., FARAMBOAS, M., TOMMASINO, M., PELLI, S., BENEDETTI, F., BELLA, S.

D. & HAOUET, N. 2017. Indicators of protein spoilage in fresh and defrosted crustaceans and cephalopods stored in domestic conditions. *Italian Journal of Food Safety,* 6, 217-221.

ALUR, M. D., WARIER, S. B., KOKE, S. N. & NAIR, P. M. 1993. Role of bacterial proteolysis in the spoilage of irradiated flesh foods. *Journal of Food Biochemistry,* 17, 419-435.

ANTHONY, C. 1982. *The biochemistry of methylotrophs,* New York, Academic press.

ANUSANKARI, S., BALAJI GANESH, A., SUBASRI, R. & DEEPA, N. 2019. Optical determination of carbon dioxide and oxygen by a fluorescent membrane to evaluate the freshness of meat products. *Instrumentation Science & Technology,* 47, 640-665.

ARSALANE, A., EL BARBRI, N., TABYAOUI, A., KLILOU, A. & RHOFIR, K. 2019. The assessment of fresh and spoiled beef meat using a prototype device based on GigE Vision camera and DSP. *Journal of Food Measurement and Characterization,* 13, 1730-1738.

ATANASSOVA, S., VELEVA, P. & STOYANCHEV, T. 2018. Near-infrared spectral informative indicators for meat and dairy products, bacterial contamination, and freshness evaluation. *In:* HOLBAN, A. M. & GRUMEZESCU, A. M. (eds.) *Microbial contamination and food degradation. Handbook of Food Bioengineering*. Academic Press.

AZAM, K., ALI, M. Y., ASADUZZAMAN, M., BASHER, M. Z. & HOSSAIN, M. M. 2004. Biochemical assessment of selected fresh fish. *Journal of Biological Sciences,* 4, 9-10.

AZARIFAR, M., GHANBARZADEH, B., KHIABANI, M. S., BASTI, A. A. & ABDULKHANI, A. 2020. The effects of gelatin-CMC films incorporated with chitin nanofiber and *Trachyspermum ammi* essential oil on the shelf life characteristics of refrigerated raw beef. *International Journal of Food Microbiology,* 318, 108493.

BAE, S., ULRICH, C. M., NEUHOUSER, M. L., LMALYSHEVA, O., BAILEY, L. B., XIAO, L., BROWN, E. C., CUSHING-HAUGEN, K. L., ZHENG, Y., CHENG, T. Y., MILLER, J. W., GREEN, R., LANE, D. S., BERESFORD, S. A. & CAUDILL, M. A. 2014. Plasma choline metabolites and colorectal cancer risk in the Women's Health Initiative Observational Study. *Cancer Research,* 74, 7442-7452.

BALAMATSIA, C. C., PATSIAS, A., KONTOMINAS, M. G. & SAVVAIDIS, I. N. 2007. Possible role of volatile amines as quality-indicating metabolites in modified atmosphere-packaged chicken fillets: Correlation with microbiological and sensory attributes. *Food Chemistry,* 104, 1622-1628.

BALAMATSIA, C. C., ROGGA, K., BADEKA, A., KONTOMINAS, M. G. & SAVVAIDIS, I. N. 2006. Effect of low-dose radiation on microbiological, chemical, and sensory characteristics of chicken meat stored aerobically at 4°C. *Journal of Food Protection,* 69, 1126-1133.

BANOVIC, M., GRUNERT, K. G., BARREIRA, M. M. & FONTES, M. A. 2009. Beef quality perception at the point of purchase: A study from Portugal. *Food Quality and Preference,* 20, 335-342.

BARBIN, D. F., EL MASRY, G., SUN, D. W., ALLEN, P. & MORSY, N. 2013. Non-destructive assessment of microbial contamination in porcine meat using NIR hyperspectral imaging. *Innovative Food Science & Emerging Technologies,* 17, 180-191.

BAUGH, M. E., STEELE, C. N., ANGILETTA, C. J., MITCHELL, C. M., NEILSON, A. P., DAVY, B. M., HULVER, M. W. & DAVY, K. P. 2018. Inulin supplementation does not reduce plasma trimethylamine N-oxide concentrations in individuals at risk for type 2 diabetes. *Nutrients,* 10, 793.

BEKHIT, A. E. D., HOPKINS, D. L., FAHRI, F. T. & PONNAMPALAM, E. N. 2013. Oxidative processes in muscle systems and fresh meat: Sources, markers and remedies. *Comprehensive Reviews in Food Science and Food Safety,* 12, 565-597.

BENNETT, B. J., DE AGUIAR VALLIM, T. Q., WANG, Z., SHIH, D. M., MENG, Y., GREGORY, J., ALLAYEE, H., LEE, R., GRAHAM, M., CROOKE, R., EDWARDS, P. A., HAZEN, S. L. & LUSIS, A. J. 2013. Trimethylamine-N-oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation. *Cell Metabolism,* 17, 49-60.

BERDAGUE, J. L., MONTEIL, P., MONTEL, M. C. & TALON, R. 1993. Effects of starter cultures on the formation of flavour compounds in dry sausage. *Meat Science,* 35, 275-287.

BHAT, Z. F., MORTON, J. D., MASON, S. L. & BEKHIT, A. E. D. A. 2018. Role of calpain system in meat tenderness: A review. *Food Science and Human Wellness,* 7, 196-204.

BIAN, H., MA, J., GENG, Z., LIU, T., SUN, C., WANG, D., ZHANG, M. & XU, W. 2019. Changes of hydroxyl-linoleic acids during Chinese-style sausage processing and their relationships with lipids oxidation. *Food Chemistry,* 296, 63-68.

BIJI, K. B., RAVISHANKAR, C. N., MOHAN, C. O. & SRINIVASA GOPAL, T. K. 2015. Smart packaging systems for food applications: a review. *Journal of Food Science and Technology,* 52, 6125-35.

BOOTHE, D. D. H. & ARNOLD, J. W. 2002. Electronic nose analysis of volatile compounds from poultry

meat samples, fresh and after refrigerated storage. *Journal of the Science of Food and Agriculture,* 82, 315-322.

BOTA, G. M. & HARRINGTON, P. B. 2006. Direct detection of trimethylamine in meat food products using ion mobility spectrometry. *Talanta,* 68, 629-635.

BOTTA, J. R. 1995. *Evaluation of seafood freshness quality*, John Wiley & Sons.

BOUTAGY, N. E., NEILSON, A. P., OSTERBERG, K. L., SMITHSON, A. T., ENGLUND, T. R., DAVY, B. M., HULVER, M. W. & DAVY, K. P. 2015. Short-term high-fat diet increases postprandial trimethylamine-N-oxide in humans. *Nutrition Research,* 35, 858-864.

BRAUN, P., FEHLHABER, K., KLUG, C. & KOPP, K. 1999. Investigations into the activity of enzymes produced by spoilage-causing bacteria: a possible basis for improved shelf-life estimation. *Food Microbiology,* 16, 531-540.

BRENDA 2020. The comprehensive enzyme information system. https://www.brenda-enzymes.org/index.php.

BRUGERE, J. F., BORREL, G., GACI, N., TOTTEY, W., O'TOOLE, P. W. & MALPUECH-GRUGERE, C. 2014. Archaebiotics: Proposed therapeutic use of archaea to prevent trimethylaminuria and cardiovascular disease. *Gut Microbes,* 5, 5-10.

BURGOS-BARRAGEN, G., WIT, N., MEISER, J., DINGLER, F. A., PIETZKE, M., MULDERRIG, L., PONTEL, L. B., ROSADO, I. V., BREWER, T. F., CORDELL, R. L., MONKS, P. S., CHANG, C. J., VAZQUEZ, A. & PATEL, K. J. 2017. Mammals divert endrogenous genotoxic formaldehyde into one-carbon metabolism. *Nature,* 548, 549-554.

BYUN, J. S., MIN, J. S., KIM, I. S., KIM, J. W., CHUNG, M. S. & LEE, M. 2003. Comparison of indicators of microbial quality of meat during aerobic cold storage. *Journal of Food Protection,* 66, 1733-1737.

CAI, J., CHEN, Q., WAN, X. & ZHAO, J. 2011. Determination of total basic nitrogen (TVB-N) content and Warner-Bratzler shear force (WBSF) in pork using Fourier transformed near infrared (FT-NIR) spectroscopy. *Food Chemistry,* 126, 1354-1360.

CAMPBELL, L. L., JR. & WILLIAMS, O. B. 1951. The action of members of the genus Achromobacter on trimethylamine oxide and related compounds. *Journal of bacteriology,* 62, 249-251.

CAO, L., SUN, G., ZHANG, C., LIU, W., LI, J. & WANG, L. 2019. An intelligent film based on cassia gum containing bromothymol blue-anchored cellulose fibers for real-time detection of meat freshness. *Journal of Agricultural and Food Chemistry,* 67, 2066-2074.

CAO, Y., GU, W., ZHANG, J., CHU, Y.-J., YE, X., HU, Y. & CHEN, J. 2013. Effects of chitosan, aqueous extract of ginger, onion and garlic on quality and shelf life of stewed-pork during refrigerated storage. *Food Chemistry,* 141, 1655-1660.

CARLSON, D. B., MCFADDEN, J. W., D'ANGELO, A., WOODOWRTH, J. C. & DRACKLEY, J. K. 2007. Dietary L-carnitine affects periparturient nutrient metabolism and lactation in multiparous cows. *Journal of Dairy Science,* 90, 3422-3441.

CARRIZOSA, E., BENITO, M. J., RUIZ-MOYANO, S., HERNANDEZ, A., VILLALOBOS, M. D. C., MARTIN, A. & CORDOBA, M. G. 2017. Bacterial communities of fresh goat meat packaged in modified atmosphere. *Food Microbiology,* 65, 57-63.

CASABURI, A., NASI, A., FERROCINO, I., DI MONACO, R., MAURIELLO, G., BILLANI, F. & ERCOLINI, D. 2011. Spoilage-related activity of Carnobacterium maltaromaticum strains in air-stored and vacuum-packed meat. *Applied and Environmental Microbiology,* 77, 7382-7393.

CASTRO, P., PADRON, J. C. P., CANSINO, M. J. C., VELAZQUES, S. E. & DE LARRIVA, R. M. 2006. Total volatile base nitrogen and its use to assess freshness in European sea bass stored in ice. *Food Control,* 17, 245-248.

CHANG, H., LI, H. & HU, Y. 2015. An intelligent method of detecting pork freshness based on digital image processing. *2015 International Conference of Intelligent Computing and Internet of Things.* Chongquing, PRC.

CHELU, M. G. & LI, N. 2018. Biomarkers (plasma trimethylamine-N-oxide) to predict atrial fibrillation: Are we there yet? *International Journal of Cardiology,* 267, 116-117.

CHEN, H., PENG, L., PÉREZ DE NANCLARES, M., TRUDEAU, M. P., YAO, D., CHENG, Z., URRIOLA, P. E., MYDLAND, L. T., SHURSON, G. C., OVERLAND, M. & CHEN, C. 2019a. Identification of Sinapine-Derived Choline from a Rapeseed Diet as a Source of Serum Trimethylamine N-Oxide in Pigs. *Journal of Agricultural and Food Chemistry,* 67, 7748-7754.

CHEN, H., ZHANG, M., BHANDARI, B. & YANG, C. 2019b. Development of a novel colorimetric food

packaging label for monitoring lean pork freshness. *LWT - Food Science and Technology,* 99, 43-49.

CHEN, Q., HUI, Z., ZHAO, J. & OUYANG, Q. 2014a. Evaluation of chicken freshness using a low-cost colorimetric sensor array with AdaBoost-OLDA classification algorithm. *LWT - Food Science and Technology,* 57, 502-507.

CHEN, Q. S., ZHANG, Y. H., ZHAO, J. W. & HUI, Z. 2013. Nondestructive measurement of total volatile basic nitrogen (TVB-N) content in salted pork in jelly using a hyperspectral imaging technique combined with efficient hypercube processing algorithms. *Analytical Methods,* 5, 6382-6388.

CHEN, X., ZHANG, Y., YANG, X., HOPKINS, D. L., ZHU, L., DONG, P., LIANG, R. & LUO, X. 2019c. Shelf-life and microbial community dynamics of super-chilled beef imported from Australia to China. *Food Research International,* 120, 784-792.

CHEN, X., ZHU, L., LIANG, R., MAO, Y., HOPKINS, D. L., LI, K., DONG, P., YANG, X., NIU, L., ZHANG, Y. & LUO, X. 2020. Shelf-life and bacterial community dynamics of vacuum packaged beef during long-term super-chilled storage sourced from two Chinese abattoirs. *Food Research International,* 130, 108937.

CHEN, Y., PATEL, N. A., CROMBIE, A., SCRIVENS, J. H. & MURRELL, J. C. 2011. Bacterial flavin-containing monooxygenase is trimethylamine monooxygenase. *Proceedings of the National Academy of Sciences,* 108, 17791-17796.

CHEN, Y., YE, R. & LIU, J. 2014b. Effects of different concentrations of ethanol and isopropanol on physicochemical properties of zein-based films. *Industrial Crops and Products,* 53, 140-147.

CHENG, J.-H., SUN, D.-W., ZENG, X.-A. & PU, H.-B. 2014. Non-destructive and rapid determination of TVB-N content for freshness evaluation of grass carp (Ctenopharyngodon idella) by hyperspectral imaging. *Innovative Food Science & Emerging Technologies,* 21, 179-187.

CHENG, J. H., NICOLAI, B. & SUN, D. W. 2017. Hyperspectral imaging with multivariate analysis for technological parameters prediction and classification of muscle foods: A review. *Meat Science,* 123, 182-191.

CHENG, S., WANG, X., YANG, H. S., LIN, R., WANG, H. & TAN, M. 2019. Characterization of moisture migration of beef during refrigeration storage by low-field NMR and its relationship to beef quality. *Journal of the Science of Food and Agriculture,* 100, 1940-1948.

CHI, W., CAO, L., SUN, G., MENG, F., ZHANG, C., LI, J. & WANG, L. 2020. Developing a highly pH-sensitive

κ-carrageenan-based intelligent film incorporating grape skin powder via a cleaner process. *Journal of Cleaner Production,* 244, 118862.

CHO, C. E., TAESUWAN, S., MALYSHEVA, O. V., BENDER, E., TULCHINSKY, N. F., YAN, J., SUTTER, J. L. & CAUDILL, M. A. 2017. Trimethylamine-N-oxide (TMAO) response to animal source foods varies among healthy young men and is influenced by their gut microbiota composition: A randomized controlled trial. *Molecular Nutrition and Food Research,* 61.

CHOE, J., KIM, Y. H. B., KIM, H. Y. & KIM, C. J. 2017. Evaluations of physicochemical and anti-oxidant properties of powdered leaves from lotus, shepherd's purse and goldenrod in restructured duck/pork patties. *Journal of Food Science and Technology,* 54, 2494-2502.

CHOI, I., LEE, J. Y., LACROIX, M. & HAN, J. 2017. Intelligent pH indicator film composed of agar/potato starch and anthocyanin extracts from purple sweet potato. *Food Chemistry,* 218, 122-128.

CHOI, Y. S., CHANG, Y., LEE, J. E., CHUN, S., CHO, J., SUNG, E., SUH, B. S., RAMPAL, S., ZHAO, D., ZHANG, Y., PASTOR-BARRIUSO, R., LIMA, J. A., SHIN, H., RYU, S. & GUALLAR, E. 2015. Egg consumption and coronary artery calcification in asymptomatic men and women. *Atherosclerosis,* 241, 305-312.

CHYTIRI, S., CHOULIARA, I., SAVVAIDIS, I. N. & KONTOMINAS, M. G. 2004. Microbiological, chemical and sensory assessment of iced whole and filleted aquacultured rainbow trout. *Food Microbiology,* 21, 157-165.

CLARK, D. S. & BURKI, T. 1972. Oxygen requirements of strains of Pseudomonas and Achromobacter. *Canadian Journal of Microbiology,* 18, 321-326.

COLBY, J. & ZATMAN, L. J. 1973. Trimethylamine metabolism in obligate and facultative methylotrophs. *The Biochemical Journal,* 132, 101-112.

CONNELL, J. J. 1995. *Control of fish quality,* London, UK, Fishing News Books Ltd.

COOMBS, C. E. O., HOLMAN, B. W. B., COLLINS, D., FRIEND, M. A. & HOPKINS, D. L. 2017a. Effects of chilled-then-frozen (up to 52 weeks) on lamb m. longissimus lumborum quality and safety. *Meat Science,* 134, 86-97.

COOMBS, C. E. O., HOLMAN, B. W. B., FRIEND, M. A. & HOPKINS, D. L. 2017b. Long-term red meat preservation using chilled and frozen storage combinations: A review. *Meat Science,* 125, 84-94.

COZZOLINO, D., BARLOCCO, N., VADELL, A., BALLESTEROS, F. & GALLIETA, G. 2003. The use of visible and near-infrared relectrance spectroscopy to predict colour on both intact and homogenised pork muscle. *LWT - Food Science and Technology,* 36, 195-202.

COZZOLINO, D. & MURRAY, I. 2002. Effect of sample presentation and animal muscle species on the analysis of meat by near infrared reflectance spectroscopy. *Journal of Near Infrared Spectroscopy,* 10, 37-44.

CRACIUM, S. & BALSKUS, E. P. 2012. Microbial conversion of choline to trimethylamine requires a glycyl radical enzyme. *Proceedings of the National Academy of Sciences,* 109, 21307-21312.

CRYAN, J. F., O'RIORDAN, K. J., COWAN, C. S. M., SANDHU, K. V., BASTIAANSSEN, T. F. S., BOEHME, M., CODAGNONE, M. G., CUSSOTTO, S., FULLING, C., GOLUBEVA, A. V., GUZZETTA, K. E., JAGGAR, M., LONG-SMITH, C. M., LYTE, J. M., MARTIN, J. A., MOLINERO-PEREZ, A., MOLONEY, G., MORELLI, E., MORILLAS, E., O'CONNOR, R., CRUZ-PEREIRA, J. S., PETERSON, V. L., REA, K., RITZ, N. L., SHERWIN, E., SPICHAK, S., TEICHMAN, E. M., VAN DE VOUW, M., VENTURA-SILVA, A. P., WALLACE-FITZSIMONS, S. E., HYLAND, N., CLARKE, G. & DINAN, T. G. 2019. The microbiota-gut-brain axis. *Physiological Reviews,* 99, 1877-2013.

CRYAN, J. F., O'RIORDAN, K. J., SANDHU, K., PETERSON, V. & DINAN, T. G. 2020. The gut microbiome in neurological disorders. *The Lancet Neurology,* 19, 179-194.

CSIRO 1995. Australian standard for hygienic production of meat for human consumption. Collingwood, AUS: Commonwealth Scientific and Industrial Research Organisation (CSIRO).

CUCCI, P., N'GATTA, A. C. K., SANGUANSUK, S., LEBERT, A. & AUDONNET, F. 2020. Relationship between Color and Redox Potential (Eh) in Beef Meat Juice. Validation on Beef Meat. *Applied Sciences,* 10, 3164.

CUSTODIO, F. B., VASCONCELOS-NETO, M. C., THEODORO, K. H., CHRISTE, R. C. & GLORIA, M. B. A. 2018. Assessment of the quality of refrigerated and frozen pork by multivariate exploratory techiques. *Meat Science,* 139, 7-14.

DAMBROVA, M., LATKOVSKIS, G., KUKA, J., STRELE, I., KONRADE, I., GRINBERGA, S., HARTMANE, D., PUGOVICS, O., ERGLIS, A. & LIEPINSH, E. 2016. Diabetes is associated with higher trimethylamine N-oxide plasma levels. *Experimental and Clinical Endrocrinology and Diabetes,* 124, 251-256.

DAVE, D. & GHALY, A. E. 2011. Meat spoilage mechanisms and preservation techniques: A critical

review. *American Journal of Agricultural and Biological Sciences,* 6, 486.

DAVIES, E. S. & GARDNER, C. D. 1996. *Oxygen indicating composition*.

DAVIES, M. J. 2016. Protein oxidation and peroxidation. *Biochemical Journal,* 473, 805-825.

DAWBER, T. R., NICKERSON, R. J., BRAND, F. N. & POOL, J. 1982. Eggs, serum cholesterol, and coronary heart disease. *The American Journal of Clinical Nutrition,* 36, 617-625.

DE MEY, E., DE KLERCK, K., DE MAERE, H., DEWULF, L., DERDELINCHX, G., PEETERS, M. C., FRAEYE, I., VANDER HEYDEN, Y. & PAELINCK, H. 2014. The occurence of N-nitrosamines, residual nitrite and biogenic amines in commercial dry fermented sausages and evaluation of their occasional relation. *Meat Science,* 96, 821-828.

DE STEFANI, E., RONCO, A., MENDILAHARSU, M., GUIDOBONO, M. & DENEO-PELLEGRINI, H. 1997. Meat intake, heterocyclic amines, and risk of breast cancer: A case-control study in Uruguay. *Cancer Epidemiology Biomarkers & Prevention,* 6, 573-581.

DE VRIEZE, J., PINTO, A. J., SLOAN, W. T. & IJAZ, U. Z. 2018. The active microbial community more accurately reflects the anaerobic digestion progress: 16S rRNA (gene) sequencing as a predictive tool. *Microbiome,* 6, 63.

DEVINE, C. E., GRAHAM, R. B., LOVATT, S. & CHRYSTALL, B. B. 1995. Red Meats. *In:* JEREMIAH (ed.) *Freezing effects on food quality.* Taylor & Francis Inc.

DOMÍNGUEZ-ARAGÓN, A., OLMEDO-MARTÍNEZ, J. A. & ZARAGOZA-CONTRERAS, E. A. 2018. Colorimetric sensor based on a poly(ortho-phenylenediamine-co-aniline) copolymer for the monitoring of tilapia (Orechromis niloticus) freshness. *Sensors and Actuators B: Chemical,* 259, 170-176.

DOMÍNGUEZ, R., PATEIRO, M., GAGAOUA, M., BARBA, F. J., ZHANG, W. & LORENZO, J. M. 2019. A comprehensive review on lipid oxidation in meat and meat products. *Antioxidants,* 8, 429.

DONG, D., JIAO, L., LI, C. & ZHAO, C. 2019. Rapid and real-time analysis of volatile compounds released from food using infrared and laser spectroscopy. *TrAC Trends in Analytical Chemistry,* 110, 410-416.

DONG, Z., LIANG, Z., GUO, M., HU, S., SHEN, Z. & HAI, X. 2018. The association between plasma levels of trimethylamine N-oxide and the risk of coronary heart disease in Chinese patients with or without

type 2 diabetes mellitus. *Disease Markers,* 2018, 1-7.

DOULGERAKI, A. I., ERCOLINI, D., VILLANI, F. & NYCHAS, G. J. E. 2012. Spoilage microbiota associated to the storage of raw meat in different conditions. *International Journal of Food Microbiology,* 157, 130-141.

DURAN, A. & KAHVE, H. I. 2020. The effect of chitosan coating and vacuum packaging on the microbiological and chemical properties of beef. *Meat Science,* 162, 107961.

ECONOMOU, T., POURNIS, N., NTZIMANI, A. & SAVVAIDIS, I. N. 2009. Nisin–EDTA treatments and modified atmosphere packaging to increase fresh chicken meat shelf-life. *Food Chemistry,* 114, 1470-1476.

EFSA PANEL ON BIOLOGICAL HAZARDS 2016. Growth of spoilage bacteria during storage and transport of meat. *EFSA Journal,* 14, e04523.

EKLUND, M., BAUER, E., WAMATU, J. & MOSENTHIN, R. 2005. Potential nutritional and physiological functions of betaine in livestock. *Nutrition Research Reviews,* 18, 31-48.

ERCOLINI, D., FERROCINO, I., LA STORIA, A., MAURIELLO, G., GIGLI, S., MASI, P. & VILLANI, F. 2010. Development of spoilage microbiota in beef stored in nisin activated packaging. *Food Microbiology,* 27, 137-143.

ERCOLINI, D., RUSSO, F., NASI, A., FERRANTI, P. & VILLANI, F. 2009. Mesophilic and psychrotrophic bacteria from meat and their spoilage potential in vitro and in beef. *Applied and Environmental Microbiology,* 75, 1990-2001.

ERCOLINI, D., RUSSO, F., TORRIERI, E., MASI, P. & VILLANI, F. 2006. Changes in the spoilage-related microbiota of beef during refrigerated storage under different packaging conditions. *Applied and Environmental Microbiology,* 72, 4663-4671.

ERKAN, N. 2007. Sensory, chemical, and microbiological attributes of sea bream (Sparus aurata): effect of washing and ice storage. *International Journal of Food Properties,* 10, 421-434.

ESTELLES-LOPEZ, L., ROPODI, A., PAVLIDIS, D., FOTOPOULOU, J., GKOUSARI, C., PEYRODIE, A., PANAGOU, E., NYCHAS, G. J. & MOHAREB, F. 2017. An automated ranking platform for machine learning regression models for meat spoilage prediction using multi-spectral imaging and metabolic profiling. *Food Research International,* 99, 206-215.

ESTÉVEZ, M. 2011. Protein carbonyls in meat systems: A review. *Meat Science,* 89, 259-279.

ESTÉVEZ, M., GERAERT, P. A., LIU, R., DELGADO, J., MERCIER, Y. & ZHANG, W. 2020. Sulphur amino acids, muscle redox status and meat quality: More than building blocks–Invited review. *Meat Science,* 163, 108087.

EZATI, P., TAJIK, H. & MORADI, M. 2019. Fabrication and characterization of alizarin colorimetric indicator based on cellulose-chitosan to monitor the freshness of minced beef. *Sensors and Actuators B: Chemical,* 285, 519-528.

FALONY, G., VIEIRA-SILVA, S. & RAES, J. 2015. Microbiology Meets Big Data: The Case of Gut Microbiota-Derived Trimethylamine. *Annual Review of Microbiology.*

FAN, X. J., LIU, S. Z., LI, H. H., HE, J., FENG, J. T., ZHANG, X. & YAN, H. 2019. Effects of *Portulaca oleracea* L. extract on lipid oxidation and color of pork meat during refrigerated storage. *Meat Science,* 147, 82-90.

FANG, Z., ZHAO, Y., WARNER, R. D. & JOHNSON, S. K. 2017. Active and intelligent packaging in meat industry. *Trends in Food Science & Technology,* 61, 60-71.

FAO 1986. Food analysis: quality, adulteration and tests of identify. *In:* AUTHORITY, S. I. D. (ed.) *Manuals of food quality control.* Rome, ITA: Food and Agriculture Organisation of the United Nations (FAO)

FAVA, F. 2015. Gut Microbiota–Immune System Crosstalk: Implications for Metabolic Disease. *Diet-Microbe Interactions in the Gut.* Elsevier.

FENG, X. & AHN, D. U. 2016. Volatile profile, lipid oxidation and protein oxidation of irradiated ready-to-eat cured turkey meat products. *Radiation Physics and Chemistry,* 127, 27-33.

FENG, Y. Z. & SUN, D. W. 2013. Near-infrared hyperspectral imaging in tandem with partial least squares regression and genetic algorithm for non-destructive determination and visualization of Pseudomonas loads in chicken fillets. *Talanta,* 109, 74-83.

FENNEMA, D., PHILLIPS, I. R. & SHEPHARD, E. A. 2016. Trimethylamine and Trimethylamine N-Oxide, a Flavin-Containing Monooxygenase 3 (FMO3)-Mediated Host-Microbiome Metabolic Axis Implicated in Health and Disease. *Drug Metabolism and Disposition,* 44, 1839-1850.

FLETCHER, B., MULLANE, K., PLATTS, P., TODD, E., POWER, A., ROBERTS, J., CHAPMAN, J., COZZOLINO, D. & CHANDRA, S. 2018. Advances in meat spoilage detection: A short focus on rapid methods and technologies. *CyTA - Journal of Food,* 16, 1037-1044.

FONT-I-FURNOLS, M. & GUERRERO, L. 2014. Consumer preference, behavior and perception about meat and meat products: An overview. *Meat Science,* 98, 361-371.

FOTI, M., MÉDICI, R. & RUIJSSENAARS, H. J. 2013. Biological production of monoethanolamine by engineered Pseudomonas putida S12. *Journal of Biotechnology,* 167, 344-349.

FRANK, D., HUGHES, J., PIYASIRI, U., ZHANG, Y., KAUR, M., LI, Y., MELLOR, G. & STARK, J. 2020. Volatile and non-volatile metabolite changes in 140-day stored vacuum packaged chilled beef and potential shelf life markers. *Meat Science,* 161, 108016.

FRANK, D., ZHANG, Y., LI, Y., LUO, X., CHEN, X., KAUR, M., MELLOR, G., STARK, J. & HUGHES, J. 2019. Shelf life extension of vacuum packaged chilled beef in the Chinese supply chain. A feasibility study. *Meat Science,* 153, 135-143.

FRANKE, C. & BEAUCHAMP, J. 2017. Real-Time Detection of Volatiles Released During Meat Spoilage: a Case Study of Modified Atmosphere-Packaged Chicken Breast Fillets Inoculated with Br. thermosphacta. *Food Analytical Methods,* 10, 310-319.

FUNG, D. Y. C. 2009. Food Spoilage, Preservation and Quality Control. *In:* SCHAECHTER, M. (ed.) *Encyclopedia of Microbiology (Third Edition).* Oxford: Academic Press.

GALGANO, F., FAVATI, F., BONADIO, M., LORUSSO, V. & ROMANO, P. 2009. Role of biogenic amines as index of freshness in beef meat packed with different biopolymeric materials. *Food Research International,* 42, 1147-1152.

GALSTYAN, V., BHANDARI, M. P., SBERVEGLIERI, V., SBERVEGLIERI, G. & COMINI, E. 2018. Metal oxide nanostructures in food applications: Quality control and packaging. *Chemosensors,* 6, 1-16.

GALSTYAN, V., COMINI, E., PONZONI, A., SBERVEGLIERI, V. & SBERVEGLIERI, G. 2016. ZnO quasi-1D nanostructures: Synthesis, modeling, and properties for applications in conductometric chemical sensors. *Chemosensors,* 4, 1-6.

GHOLLASI-MOOD, F., MOHSENZADEH, M., HOUSAINDOKHT, M. R. & VARIDI, M. 2017. Microbial and chemical spoilage of chicken meat during storage at isothermal and fluctuation temperature under

aerobic conditions. *Iranian Journal of Veterinary Science and Technology,* 8, 38-46.

GIL, L., BARAT, J. M., BAIGTS, D., MARTÍNEZ-MÁÑEZ, R., SOTO, J., GARCIA-BREIJO, E., ARISTOY, M. C., TOLDRÁ, F. & LLOBET, E. 2011. Monitoring of physical-chemical and microbiological changes in fresh pork meat under cold storage by means of a potentiometric electronic tongue. *Food Chemistry,* 126, 1261-1268.

GIMENEZ, B., RONCALÉS, P. & BELTRÁN, J. A. 2002. Modified atmosphere packaging of filleted rainbow trout. *Journal of the Science of Food and Agriculture,* 82, 1154-1159.

GOULAS, A. E., CHOULIARA, I., NESSI, E., KONTOMINAS, M. G. & SAVVAIDIS, I. N. 2005. Microbiological, biochemical and sensory assessment of mussels (Mytilus galloprovincialis) stored under modified atmosphere packaging. *Journal of Applied Microbiology,* 98, 752-760.

GRAU, R., SÁNCHEZ, A. J., GIRÓN, J., IBORRA, E., FUENTES, A. & BARAT, J. M. 2011. Nondestructive assessment of freshness in packaged sliced chicken breasts using SW-NIR spectroscopy. *Food Research International,* 44, 331-337.

GRIFFIN, L. E., DJURIC, Z., ANGILETTA, C. J., MITCHELL, C. M., BAUGH, M. E., DAVY, K. P. & NEILSON, A. P. 2019. A Mediterranean diet does not alter plasma trimethylamine N-oxide concentrations in healthy adults at risk for colon cancer. *Food & Function,* 10, 2138-2147.

GROSSMAN, S., BERGMAN, M. & SKLAN, D. 1988. Lipoxygenase in chicken muscle. *Journal of Agricultural and Food Chemistry,* 36, 1268-1270.

GUERTIN, K. A., LI, X. S., GRAUBARD, B. I., ALBANES, D., WEINSTEIN, S. J., GOEDERT, J. J., WANG, Z., HAZEN, S. L. & SINHA, R. 2017. Serum trimethylamine n-oxide, carnitine, choline, and betaine in relation to colorectal cancer risk in the alpha tocopherol, beta carotene cancer prevention study. *Cancer Epidemiology Biomarkers and Prevention,* 26, 945-952.

GUTIERREZ, R., GARCIA, T., GONZALEZ, I., SANZ, B., HERNANDEZ, P. E. & MARTIN, R. 1998. Quantitative detection of meat spoilage bacteria by using the polymerase chain reaction (PCR) and an enzyme linked immunosorbent assay (ELISA). *Letters in Applied Microbiology,* 26, 372-6.

GUYON, C., MEYNIER, A. & DE LAMBALLERIE, M. 2016. Protein and lipid oxidation in meat: A review with emphasis on high-pressure treatments. *Trends in Food Science & Technology,* 50, 131-143.

HAARD, N. F. & SIMPSON, B. K. 2000. Seafood enzymes: Utilization and influence on postharvest

seafood quality, CRC Press.

HACISALIHOGLU, A., JONGEJAN, J. A. & DUINE, J. A. 1997. Distribution of amine oxidases and amine dehydrogenases in bacteria grown on primary amines and characterization of the amine oxidase from Klebsiella oxytoca. *Microbiology,* 143, 505-512.

HAMAGUCHI, P. Y., WENG, W. Y., KOBAYASHI, T., RUNGLERTKREIGKRAI, J. & TANAKA, M. 2007. Effect of fish meat quality on the properties of biodegradable protein films. *Food Science & Technology Research,* 13, 200-204.

HAMID, M. A., WANG, X., DING, X., WANG, C. & ZHAO, X. 2014. Measurement of trimethylamine concentration and evaluation of pig meat natural quality by a spectrophotometric method. *Frontiers of Agricultural Science and Engineering,* 1, 236-241.

HAN, C., WANG, J., LI, Y., LU, F. & CUI, Y. 2014. Antimicrobial-coated polypropylene films with polyvinyl alcohol in packaging of fresh beef. *Meat Science,* 96, 901-907.

HAZEN, S. L., WANG, Z. & LEVISON, B. S. 2016. Trimethylamine compounds as risk predictors of cardiovascular disease.

HAZEN, S. L., WANG, Z. & LEVISON, B. S. 2019. Trimethylamine-containing compounds for diagnosis and prediction of disease.

HECK, H. A., CASANOVA-SCHMITZ, M., DODD, P. B., SCHACHTER, E. N., WITEK, T. J. & TOSUN, T. 1985. Formaldehyde (CH_2O) concentrations in the blood of humans and Fischer-344 rats exposed to CH_2O under controlled conditions. *American Industrial Hygiene Association Journal,* 46, 1-3.

HOLMAN, B. W. B., FOWLER, S. M. & HOPKINS, D. L. 2020. Red meat (beef and sheep) products for an ageing population: A review. *International Journal of Food Science & Technology,* 55, 919-934.

HOLMAN, B. W. B., KERRY, J. P. & HOPKINS, D. L. 2018. A review of patents for the smart packaging of meat and muscle-based food products. *Recent Patents on Food, Nutrition & Agriculture,* 9, 3-13.

HONG, X., WANG, J. & HAI, Z. 2012. Discrimination and prediction of multiple beef freshness indexes based on electronic nose. *Sensors and Actuators B: Chemical,* 161, 381-389.

HOWGATE, P. 2010a. A critical review of total volatile bases and trimethylamine as indices of freshness of fish. Part 1. Determination. *Electronic Journal of Environmental, Agricultural and Food Chemistry,* 9,

29-57.

HOWGATE, P. 2010b. A critical review of total volatile bases and trimethylamine as indices of freshness of fish. Part 2. Formation of the bases, and application in quality assurance. *Electronic Journal of Environmental, Agricultural and Food Chemistry,* 9, 58-88.

HSIAO, H. I. & CHANG, J. N. 2017. Developing a microbial time–temperature indicator to monitor total volatile basic nitrogen change in chilled vacuum-packed grouper fillets. *Journal of Food Processing and Preservation,* 41, 13158.

HSU, H. H. & PAN, B. S. 1996. Effects of protector and hydroxyapatite partial purification on stability of lipoxygenase from gray mullet gill. *Journal of Agricultural and Food Chemistry,* 44, 741-745.

HU, Y., QIAO, J., ZHANG, X. & GE, C. 2011. Antimicrobial activity of *Magnolia officinalis* extracts *in vitro* and its effect on the preservation of chilled mutton. *Journal of Food Biochemistry,* 35, 425-441.

HUANG, L., ZHAO, J., CHEN, Q. & ZHANG, Y. 2014. Nondestructive measurement of total volatile basic nitrogen (TVB-N) in pork meat by integrating near infrared spectroscopy, computer vision and electronic nose techniques. *Food Chemistry,* 145, 228-236.

HUANG, L., ZHAO, J., ZHANG, Y. & CHEN, Q. 2012. Simultaneous multi-component analysis of pork meat during bacterial spoiling process by FT-NIR evaluated with a non-linear algorithm. *Analytical Methods,* 4, 3816-3823.

HUANG, Q., CHEN, Q., LI, H., HUANG, G., OUYANG, Q. & ZHAO, J. 2015a. Non-destructively sensing pork's freshness indicator using near infrared multispectral imaging technique. *Journal of Food Engineering,* 154, 69-75.

HUANG, S., LIU, B., GE, D. & DAI, J. 2017a. Effect of combined treatment with supercritical CO_2 and rosemary on microbiological and physicochemical properties of ground pork stored at 4 ^{o}C. *Meat Science,* 125, 114-120.

HUANG, S., XIONG, Y., ZOU, Y., DONG, Q., DING, F., LIU, X. & LI, H. 2019. A novel colorimetric indicator based on agar incorporated with Arnebia euchroma root extracts for monitoring fish freshness. *Food Hydrocolloids,* 90, 198-205.

HUANG, X., XIN, J. & ZHAO, J. 2011. A novel technique for rapid evaluation of fish freshness using colorimetric sensor array. *Journal of Food Engineering,* 105, 632-637.

HUANG, X. Y., LV, R. Q., YAO, L. Y., GUAN, C., HAN, F. K. & TEYE, E. 2015b. Non-destructive evaluation of total volatile basic nitrogen (TVB-N) and K-values in fish using colorimetric sensor array. *Analytical Methods,* 7, 1615-1621.

HUANG, Y., ZENG, X., ZHU, Q., LU, K., XU, Q. & YE, C. 2017b. Development of an active packaging with molecularly imprinted polymers for beef preservation. *Packaging Technology and Science,* 31, 213-220.

HUWS, S. A., CREEVEY, C. J., OYAMA, L. B., MIZRAHI, I., DENMAN, S. E., POPOVA, M., MUÑOZ-TAMAYO, R., FORANO, E., WATERS, S. M., HESS, M., TAPIO, I., SMIDT, H., KRIZSAN, S. J., YÁÑEZ-RUIZ, D. R., BELANCHE, A., GUAN, L., GRUNINGER, R. J., MCALLISTER, T. A., NEWBOLD, C. J., ROEHE, R., DEWHURST, R. J., SNELLING, T. J., WATSON, M., SUEN, G., HART, E. H., KINGSTON-SMITH, A. H., SCOLLAN, N. D., DO PRADO, R. M., PILAU, E. J., MANTOVANI, H. C., ATTWOOD, G. T., EDWARDS, J. E., MCEWAN, N. R., MORRISSON, S., MAYORGA, O. L., ELLIOTT, C. & MORGAVI, D. P. 2018. Addressing global ruminant agricultural challenges through understanding the rumen microbiome: Past, present, and future. *Frontiers in Microbiology,* 9.

IMMACULATE, J. & JAMILA, P. 2018. Quality characteristics including formaldehyde content in selected Sea foods of Tuticorin, southeast coast of India. *International Food Research Journal,* 25, 293-302.

IULIETTO, M. F., SECHI, P., BORGOGNI, E. & CENCI-GOGA, B. T. 2016. Meat Spoilage: A critical review of a neglected alteration due to ropy slime producing bacteria. *Italian Journal of Animal Science,* 14, 4011.

JAMESON, E., QUARESHY, M. & CHEN, Y. 2018. Methodological considerations for the identification of choline and carnitine-degrading bacteria in the gut. *Methods,* 149, 42-48.

JIAO, Y., QUEK, S. Y., GU, M., GUO, Y. & LIU, Y. 2020. Polyphenols from thinned young kiwifruit as natural antioxidant: Protective effects on beef oxidation, physicochemical and sensory properties during storage. *Food Control,* 108, 106870.

JIN, G., ZHANG, J., YU, X., LEI, Y. & WANG, J. 2011. Crude lipoxygenase from pig muscle: Partial characterization and interactions of temperature, NaCl and pH on its activity. *Meat Science,* 87, 257-263.

JURADO, Á., GARCÍA, C., TIMÓN, M. L. & CARRAPISO, A. I. 2007. Effect of ripening time and rearing

system on amino acid-related flavour compounds of Iberian ham. *Meat Science,* 75, 585-594.

KAMRUZZAMAN, M., ELMASRY, G., SUN, D. W. & ALLEN, P. 2012. Prediction of some quality attributes of lamb meat using near-infrared hyperspectral imaging and multivariate analysis. *Analytica Chimica Acta,* 714, 57-67.

KAMRUZZAMAN, M., MAKINO, Y. & OSHITA, S. 2015. Non-invasive analytical technology for the detection of contamination, adulteration, and authenticity of meat, poultry, and fish: A review. *Analytica Chimica Acta,* 853, 19-29.

KANG, S., WANG, H., XIA, L., CHEN, M., LI, L., CHENG, J., LI, X. & JIANG, S. 2020. Colorimetric film based on polyvinyl alcohol/okra mucilage polysaccharide incorporated with rose anthocyanins for shrimp freshness monitoring. *Carbohydrate Polymers,* 229, 115402.

KARLSSON, A., ENFÄLT, A. C., ESSÉN-GUSTAVSSON, B., LUNDSTRÖM, K., RYDHMER, L. & STERN, S. 1993. Muscle histochemical and biochemical properties in relation to meat quality during selection for increased lean tissue growth rate in pigs. *Journal of Animal Science,* 71, 930-938.

KARPAS, Z., TILMAN, B., GDALEVSKY, R. & LORBER, A. 2002. Determination of volatile biogenic amines in muscle food products by ion mobility spectrometry. *Analytica Chimica Acta,* 463, 155-163.

KELLY, W. J., LEAHY, S. C., KAMKE, J., SONI, P., KOIKE, S., MACKIE, R., SESHADRI, R., COOK, G. M., MORALES, S. E. & GREENING, C. 2019. Occurrence and expression of genes encoding methyl-compound production in rumen bacteria. *Animal Microbiome,* 1, 15.

KHATRI, N., KHATRI, I., SUBRAMANIAN, S. & RAYCHAUDHURI, S. 2012. Ethanolamine utilization in Vibrio alginolyticus. *Biology Direct,* 7, 45.

KHEZRIAN, A. & SHAHBAZI, Y. 2018. Application of nanocompostie chitosan and carboxymethyl cellulose films containing natural preservative compounds in minced camel's meat. *International Journal of Biological Macromolecules,* 106, 1146-1158.

KHULAL, U., LI, H., CHEN, Q., HUI, Z. & ZHAO, J. 2015. Quantifying of total volatile basic nitrogen (TVB-N) content in chicken using a colorimetric sensor array and nonlinear regression tool. *Analytical Methods,* 7, 5682-5688.

KHULAL, U., ZHAO, J., HU, W. & CHEN, Q. 2016a. Comparison of different chemometric methods in quantifying total volatile basic-nitrogen (TVB-n) content in chicken meat using a fabricated colorimetric

sensory array. *RSC Advances,* 6, 4663-4672.

KHULAL, U., ZHAO, J., HU, W. & CHEN, Q. 2016b. Nondestructive quantifying total basic nitrogen (TVB-N) content in chicken using hyperspectral imaging (HSI) technque combined with different data dimension reduction algorithms. *Food Chemistry,* 197, 1191-1199.

KHULAL, U., ZHAO, J., HU, W. & CHEN, Q. 2017. Intelligent evaluation of total volatile basic nitrogen (TVB-N) content in chicken meat by an improved multiple level data fusion model. *Sensors and Actuators B: Chemical,* 238, 337-345.

KILGANNON, A. K., HOLMAN, B. W. B., FRANK, D. C., MAWSON, A. J., COLLINS, D. & HOPKINS, D. L. 2020. Temperature-time combination effects on aged beef volatile profiles and their relationship to sensory attributes. *Meat Science,* 168, 108193.

KIM, D., LEE, S., LEE, K., BAEK, S. & SEO, J. 2017. Development of a pH indicator composed of high moisture-absorbing materials for real-time monitoring of chicken breast freshness. *Food Science and Biotechnology,* 26, 37-42.

KIM, K. B., YANG, J. Y., KWACK, S. J., PARK, K. L., KIM, H. S., RYU, D. H., KIM, Y. J., HWANG, G. S. & LEE, B. M. 2010. Toxicometabolomics of urinary biomarkers for human gastric cancer in a mouse model. *Journal of Toxicology and Environmental Health,* 73, 1420-1430.

KIM, R. B., MORSE, B. L., DJURDJEV, O., TANG, M., MUIRHEAD, N., BARRETT, B., HOLMES, D. T., MADORE, F., CLASE, C. M. & RIGATTO, C. 2016. Advanced chronic kidney disease populations have elevated trimethylamine N-oxide levels associated with increased cardiovascular events. *Kidney International,* 89, 1144-1152.

KIRK, R. S. & SAWYER, R. 1991. Flesh foods. *Pearson's chemical analysis of foods.* 9th ed. Singapore: Longman Group.

KNÜTTEL-GUSTAVSEN, S. & HARMEYER, J. 2011. The content of L-carnitine in meat after different methods of heat treatment. *British Food Journal,* 113, 1114-1126.

KOBATAKE, M., TONOGAI, Y. & ITO, Y. 1988. Time Courses for Viable Counts, pH and Total Volatile Basic Nitrogen in Squid Homogenates (*Ommastraphes bartrami*) Inoculated with Spoilage Yeast or Bacteria. *Journal of Food Protection,* 51, 971-975.

KODOGIANNIS, V. S. A Rapid Detection of Meat Spoilage using an Electronic Nose and Fuzzy-Wavelet

systems. Proceedings of SAI Intelligent Systems Conference, 2016. Springer, 521-539.

KOETH, R. A., WANG, Z., LEVISON, B. S., BUFFA, J. A., ORG, E., SHEEHY, B. T., BRITT, E. B., FU, X., WU, Y. & LI, L. 2013. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nature Medicine,* 19, 576.

KOH, K. C., CHUNG, K. Y., KIM, H. S., KANG, S. J., CHOI, C. B., JO, C. & CHOE, J. 2019. Determination of point of sale and consumption for Hanwoo beef based on quality grade and aging time. *Food Science of Animal Resources,* 39, 139-150.

KUBIENOVÁ, L., KOPEČNÝ, D., TYLICHOVÁ, M., BRIOZZO, P., SKOPALOVÁ, J., ŠEBELA, M., NAVRÁTIL, M., TÂCHE, R., LUHOVÁ, L. & BARROSO, J. B. 2013. Structural and functional characterization of a plant S-nitrosoglutathione reductase from Solanum lycopersicum. *Biochimie,* 95, 889-902.

KUCHA, C. T. & NGADI, M. O. 2020. Rapid assessment of pork freshness using miniaturized NIR spectroscopy. *Journal of Food Measurement and Characterization,* 14, 1105-1115.

KUMBHARE, D. A., ELZIBAK, A. H., AKBARI, A. & NOSEWORTHY, M. D. 2014. Advanced skeletal muscle MR imaging approaches in the assessment of muscular dystrophies. *International Journal of Physical Medicine & Rehabilitation,* 2, 104-172.

KUMUDAVALLY, K. V., SHOBHA, A., VASUNDHARA, T. S. & RADHAKRISHNA, K. 2001. Chromatographic analysis of cadaverine to detect incipient spoilage in mutton. *Meat Science,* 59, 411-415.

KUSWANDI, B., JAYUS, RESTYANA, A., ABDULLAH, A., HENG, L. Y. & AHMAD, M. 2012. A novel colorimetric food package label for fish spoilage based on polyaniline film. *Food Control,* 25, 184-189.

LALITHA, K. V., SONAJI, E. R., MANJU, S., JOSE, L., GOPAL, T. K. S. & RAVISANKAR, C. N. 2005. Microbiological and biochemical changes in pearl spot (Etroplus suratensis Bloch) stored under modified atmospheres. *Journal of Applied Microbiology,* 99, 1222-1228.

LANDFALD, B., VALEUR, J., BERSTAD, A. & RAA, J. 2017. Microbial trimethylamine-N-oxide as a disease marker: something fishy? *Microbial Ecology in Health and Disease,* 28, 1327309.

LARGE, P., BOULTON, C. A. & CRABBE, M. J. 1972. The reduced nicotinamide-adenine dinucleotide phosphate-and oxygen-dependent N-oxygenation of trimethylamine by Pseudomonas aminovorans. *Biochemical Journal,* 128, 137-138.

LEE, H., KIM, M. S., LEE, W. H. & CHO, B. K. 2018. Determination of the total volatile basic nitrogen (TVB-N) content in pork meat using hyperspectral fluorescence imaging. *Sensors and Actuators B: Chemical,* 259, 532-539.

LEYGONIE, C., BRITZ, T. J. & HOFFMAN, L. C. 2012. Impact of freezing and thawing on the quality of meat: Review. *Meat Science,* 91, 93-98.

LI, D., KIRSOP, J. & TANG, W. H. W. 2015a. Listening to our gut: contribution of gut microbiota and cardiovascular risk in diabetes pathogenesis. *Current Diabetes Reports,* 15, 63.

LI, F. & GUAN, L. L. 2017. Metatranscriptomic Profiling Reveals Linkages between the Active Rumen Microbiome and Feed Efficiency in Beef Cattle. *Applied and Environmental Microbiology,* 83.

LI, H., CHEN, Q., ZHAO, J. & WU, M. 2015b. Nondestructive detection of total volatile basic nitrogen (TVB-N) content in pork meat by integrating hyperspectral imaging and colorimetric sensor combined with a nonlinear data fusion. *LWT-Food Science and Technology,* 63, 268-274.

LI, H., SUN, X., PAN, W., KUTSANEDZIE, F., ZHAO, J. & CHEN, Q. 2016a. Feasibility study on nondestructively sensing meat's freshness using light scattering imaging technique. *Meat Science,* 119, 102-109.

LI, L., XIE, S., ZHU, F., NING, J., CHEN, Q. & ZHANG, Z. 2017. Colorimetric sensor array-based artificial olfactory system for sensing Chinese green tea's quality: A method of fabrication. *International Journal of Food Properties,* 20, 1762-1773.

LI, M., WANG, H., SUN, L., ZHAO, G. & HUANG, X. 2016b. Application of electronic nose for measuring total volatile basic nitrogen and total viable counts in packaged pork during refrigerated storage. *Journal of Food Science,* 81, M906-M912.

LI, N., ZHANG, Y., WU, Q., GU, Q., CHEN, M., ZHANG, Y., SUN, X. & ZHANG, J. 2019a. High-throughput sequencing analysis of bacterial community composition and quality characteristics in refrigerated pork during storage. *Food Microbiology,* 83, 86-94.

LI, R., CAI, L., GAO, T., LI, C., ZHOU, G. & YE, K. 2020. Comparing the quality characteristics and bacterial communities in meatballs with or without blown pack spoilage. *LWT-Food Science and Technology*, 109529.

LI, X., CHEN, X., YAO, Y., LI, N. & CHEN, X. 2014. High-stability quartz crystal microbalance ammonia

sensor utilizing graphene oxide isolation layer. *Sensors and Actuators B: Chemical,* 196, 183-188.

LI, Y., TANG, X., SHEN, Z. & DONG, J. 2019b. Prediction of total volatile basic nitrogen (TVB-N) content of chilled beef for freshness evaluation using viscoelasticity based on airflow and laser technique. *Food Chemistry,* 287, 126-132.

LI, Y., WANG, D. D., CHIUVE, S. E., MANSON, J. E., WILLETT, W. C., HU, F. B. & QI, L. 2015c. Dietary phosphatidylcholine intake and type 2 diabetes in men and women. *Diabetes Care,* 38, e13-e14.

LI, Y., WANG, W., LONG, Y., PENG, Y., LI, Y., CHAO, K. & TANG, X. 2018. A feasibility study of rapid nondestructive detection of total volatile basic nitrogen (TVB-N) content in beef based on airflow and laser ranging. *Meat Science,* 145, 367-374.

LI, Y., XU, Y., DONG, J., YANG, K., ZHANG, B. & TANG, X. 2019c. Application of multi-element viscoelastic models to freshness evalution of beef based on the viscoelasticity principle. *Journal of Texture Studies,* 50, 306-315.

LIAO, Y., CHEN, S., WANG, D., ZHANG, W., WANG, S., DING, J., WANG, Y., CAI, L., RAN, X. & WANG, X. 2013. Structure of formaldehyde dehydrogenase from Pseudomonas aeruginosa: the binary complex with the cofactor NAD+. *Acta Crystallographica Section F: Structural Biology and Crystallization Communications,* 69, 967-972.

LICKTEIG, A. J., RILEY, R., MELTON, R. J., REITZ, B. A., FISCHER, H. D. & STEVENS, J. C. 2009. Expression and characterization of functional dog flavin-containing monooxygenase 3. *Drug Metabolism and Disposition,* 37, 1987-1990.

LIMO, S., SINELLI, N., TORRI, L. & RIVA, M. 2009. Freshness decay and shelf life predictive modelling of European sea bass (Dicentrarchus labrax) applying chemical methods and electronic nose. *LWT - Food Science and Technology,* 42, 977-984.

LIN, T., WANG, J. J., LI, J. B., LIAO, C., PAN, Y. J. & ZHAO, Y. 2013. Use of acidic electrolyzed water ice for preserving the quality of shrimp. *Journal of Agricultural and Food Chemistry,* 61, 8695-8702.

LIN, T. Y. & TIMASHEFF, S. N. 1994. Why do some organisms use a urea-methylamine mixture as osmolyte? Thermodynamic compensation of urea and trimethylamine N-oxide interactions with protein. *Biochemistry,* 33, 12695-12701.

LIU, H., JI, Z., LIU, X., SHI, C. & YANG, X. 2020. Non-destructive determination of chemical and microbial

spoilage indicators of beef for freshness evalution using front-face synchronous fluorescence spectroscopy. *Food Chemistry,* 321, 126628.

LIU, J., WANG, H., GUO, M., LI, L., CHEN, M., JIANG, S., LI, X. & JIANG, S. 2019. Extract from Lycium ruthenicum Murr. Incorporating κ-carrageenan colorimetric film with a wide pH–sensing range for food freshness monitoring. *Food Hydrocolloids,* 94, 1-10.

LIU, Q., CAI, X., LIU, Y., ZHOU, L., YI, S. & LIU, L. 2008. Spectrophotometric determination of trimethylamine-nitrogen in cadaver tissues for the estimation of late postmortem interval: A pilot study. *Journal of Huazhong University of Science and Technology,* 28, 630-633.

LIU, S. & GAN, L. Determination of total volatile basic nitrogen (TVB-N) content in beef by hyperspectral imaging technique. MATEC Web of Conferences, 2016. EDP Sciences, 06007.

LIU, Z. Y., TAN, X. Y., LI, Q. J., LIAO, G. C., FANG, A. P., ZHANG, D. M., CHEN, P. Y., WANG, X. Y., LUO, Y. & LONG, J. A. 2018. Trimethylamine N-oxide, a gut microbiota-dependent metabolite of choline, is positively associated with the risk of primary liver cancer: a case-control study. *Nutrition & Metabolism,* 15, 81.

LU, X., NIKOLIC, D., MITCHELL, D. J., VAN BREEMEN, R. B., MERSFELDER, J. A., HILLE, R. & SILVERMAN, R. B. 2003. A mechanism for substrate-induced formation of 6-hydroxyflavin mononucleotide catalyzed by C30A trimethylamine dehydrogenase. *Bioorganic & Medicinal Chemistry Letters,* 13, 4129-4132.

LU, X., ZHANG, Y., ZHU, L., LUO, X. & HOPKINS, D. L. 2019. Effect of superchilled storage on shelf life and quality characteristics of *M. longissimus lumborum* from Chinese Yellow cattle. *Meat Science,* 149, 79-84.

LUND, M. N., HEINONEN, M., BARON, C. P. & ESTÉVEZ, M. 2011. Protein oxidation in muscle foods: A review. *Molecular Nutrition & Food Research,* 55, 83-95.

LYU, F., SHEN, K., DING, Y. & MA, X. 2016. Effect of pretreatment with carbon monoxide and ozone on the quality of vacuum packaged beef meats. *Meat Science,* 117, 137-146.

MA, Q., DU, L. & WANG, L. 2017. Tara gum/polyvinyl alcohol-based colorimetric NH3 indicator films incorporating curcumin for intelligent packaging. *Sensors and Actuators B: Chemical,* 244, 759-766.

MANSUR, A. R., SONG, E. J., CHO, Y. S., NAM, Y. D., CHOI, Y. S., KIM, D. O., SEO, D. H. & NAM, T. G.

2019. Comparative evaluation of spoilage-related bacterial diversity and metabolite profiles in chilled beef stored under air and vacuum packaging. *Food Microbiology,* 77, 166-172.

MAO, Y., HOPKINS, D. L., ZHANG, Y. & LUO, X. 2016. Consumer patterns and consumer attitudes to beef and sheep meat in China. *American Journal of Food and Nutrition,* 4, 30-39.

MARIUTTI, L. R. B. & BRAGAGNOLO, N. 2017. Influence of salt on lipid oxidation in meat and seafood products: A review. *Food Research International,* 94, 90-100.

MARTINS, W. F., LONGHI, D. A., DE ARAGÃO, G. M. F., MELERO, B., ROVIRA, J. & DIEZ, A. M. 2020. A mathematical modeling approach to the quantification of lactic acid bacteria in vacuum-packaged samples of cooked meat: Combining the TaqMan-based quantitative PCR method with the plate-count method. *International Journal of Food Microbiology,* 318, 108466.

MATINDOUST, S., FARZI, A., BAGHAEI NEJAD, M., SHAHROKH ABADI, M. H., ZOU, Z. & ZHENG, L.-R. 2017. Ammonia gas sensor based on flexible polyaniline films for rapid detection of spoilage in protein-rich foods. *Journal of Materials Science: Materials in Electronics,* 28, 7760-7768.

MAYR, D., MARGESIN, R., KLINGSBICHEL, E., HARTUNGEN, E., JENEWEIN, D., SCHINNER, F. & MÄRK, T. D. 2003. Rapid detection of meat spoilage by measuring volatile organic compounds by using proton transfer reaction mass spectrometry. *Applied and Environmental Microbiology,* 69, 4697-4705.

MCCULLOUGH, E. B., PINGALI, P. L. & STAMOULIS, K. G. 2012. Small farms and the transformation of food systems: an overview. *In:* HAAS, R., CANAVARI, M., SLEE, B., TONG, C. & ANURUGSA, B. (eds.) *Looking east, looking west. Organic and quality food marketing in Asia and Europe.* Wageningen Academic Publishers.

MEDIC, H., KUSEC, I. D., PLEADIN, J., KOZACINSKI, L., NJARI, B., HENGL, B. & KUSEC, G. 2018. The impact of frozen storage duration on physical, chemical and microbiological properties of pork. *Meat Science,* 140, 119-127.

MENG, Q., HAN, T., WANG, G., ZHENG, N., CAO, C. & XIE, S. 2014. Preparation of a natural dye doped Ormosil coating for the detection of formaldehyde in the optical gas sensor. *Sensors and Actuators B: Chemical,* 196, 238-244.

MESTRE PRATES, J. A. & MATEUS, C. M. R. P. 2002. Functional foods from animal sources and their physiologically active components. *Revue de Medecine Veterinaire,* 153, 155-160.

MET, A. & YEŞILÇUBUK, N. Ş. 2017. Comparison of Two Volatile Sampling Techniques Based on Different Loading Factors in Determination of Volatile Organic Compounds Released from Spoiled Raw Beef. *Food Analytical Methods,* 10, 2311-2324.

MIETZ, J. L. & KARMAS, E. 1977. Chemical quality index of canned tuna as determined by HPLC. *Journal of Food Science,* 44, 515-517.

MILLS, J., DONNISON, A. & BRIGHTWELL, G. 2014. Factors affecting microbial spoilage and shelf-life of chilled vacuum-packed lamb transported to distant markets: A review. *Meat Science,* 98, 71-80.

MIN, B. & AHN, D. U. 2005. Mechanism of lipid peroxidation in meat and meat products - A review. *Food Science and Biotechnology,* 14, 152-163.

MIN, B., NAM, K. C., CORDRAY, J. & AHN, D. U. 2008. Endogenous factors affecting oxidative stability of beef loin, pork loin, and chicken breast and thigh meats. *Journal of Food Science,* 73, 439-446.

MISSAILIDIS, C., HÄLLQVIST, J., QURESHI, A. R., BARANY, P., HEIMBÜRGER, O., LINDHOLM, B., STENVINKEL, P. & BERGMAN, P. 2016. Serum trimethylamine-N-oxide is strongly related to renal function and predicts outcome in chronic kidney disease. *PloS one,* 11, 0141738.

MITCHELL, A. D., CHAPPELL, A. & KNOX, K. L. 1979. Metabolism of betaine in the ruminant. *Journal of Animal Science,* 49, 764-774.

MITCHELL, S. C., ZHANG, A. Q. & SMITH, R. L. 2002. Chemical and biological liberation of trimethylamine from foods. *Journal of Food Composition and Analysis,* 15, 277-282.

MOHRI, S., CHO, S. Y., ENDO, Y. & FUJIMOTO, K. 1990. Lipoxygenase activity in sardine skin. *Agricultural and Biological Chemistry,* 54, 1889-1891.

MONDAL, J. A. 2016. Effect of trimethylamine N-oxide on interfacial electrostatics at phospholipid monolayer–water interfaces and its relevance to cardiovascular disease. *The Journal of Physical Chemistry Letters,* 7, 1704-1708.

MORGAVI, D. P., RATHAHAO-PARIS, E., POPOVA, M., BOCCARD, J., NIELSEN, K. F. & BOUDRA, H. 2015. Rumen microbial communities influence metabolic phenotypes in lambs. *Frontiers in Microbiology,* 6, 1060.

MORSY, M. K., ZOR, K., KOSTESHA, N., ALSTRØM, T. S., HEISKANEN, A., EL-TANAHI, H., SHAROBA, A.,

PAPKOVSKY, D., LARSEN, J. & KHALAF, H. 2016. Development and validation of a colorimetric sensor array for fish spoilage monitoring. *Food Control,* 60, 346-352.

MUELA, E., SAÑUDO, C., CAMPO, M. M., MEDEL, I. & BELTRÁN, J. A. 2012. Effect of freezing method and frozen storage duration on lamb sensory quality. *Meat Science,* 90, 209-215.

MUELLER, D. M., ALLENSPACH, M., OTHMAN, A., SAELY, C. H., MUENDLEIN, A., VONBANK, A., DREXEL, H. & VON ECKARDSTEIN, A. 2015. Plasma levels of trimethylamine-N-oxide are confounded by impaired kidney function and poor metabolic control. *Atherosclerosis,* 243, 638-644.

MYHAN, R., MARKOWSKI, M., DASZKIEWICZ, T., ZAPOTOCZNY, P. & SADOWSKI, P. 2015. Non-linear stress relaxation model as a tool for evaluating the viscoelastic properties of meat products. *Journal of Food Engineering,* 146, 107-115.

NEILL, A. R., GRIME, D. W. & DAWSON, R. M. C. 1978. Conversion of choline methyl groups through trimethylamine into methane in the rumen. *Biochemical Journal,* 170, 529-535.

NIELSEN, M. & NIELSEN, H. 2012. Seafood enzymes. *Food biochemistry and food processing*, 247-262.

NIELSEN, M. K. & JØRGENSEN, B. M. 2004. Quantitative relationship between trimethylamine oxide aldolase activity and formaldehyde accumulation in white muscle from gadiform fish during frozen storage. *Journal of Agricultural and Food Chemistry,* 52, 3814-3822.

NIEMINEN, T. T., VIHAVAINEN, E., PALORANTA, A., LEHTO, J., PAULIN, L., AUVINEN, P., SOLISMAA, M. & BJÖRKROTH, K. J. 2011. Characterization of psychrotrophic bacterial communities in modified atmosphere-packed meat with terminal restriction fragment length polymorphism. *International Journal of Food Microbiology,* 144, 360-366.

NIIZEKI, N., DAIKOKU, T., HIRATA, T., EL-SHOURBAGY, I., SONG, X. & SAKAGUCHI, M. 2002. Mechanism of biosynthesis of trimethylamine oxide from choline in the teleost tilapia, Oreochromis niloticus, under freshwater conditions. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology,* 131, 371-386.

NIP, W.-K., NOLLET, L. M. L., GOPINADHAN, P. & BENJAMIN K., S. 2006. Food biochemistry and food processing. *In:* Y. H., H. (ed.) First edition ed. Victoria, Australia: Blackwell Publishing Ltd.

NISHIMURA, T. 1998. Mechanism involved in the improvement of meat taste during postmortem aging. *Food Science and Technology International,* 4, 241-249.

NORLIANA, S., ABDULAMIR, A. S., ABU BAKAR, F. & SALLEH, A. B. 2009. The health risk of formaldehyde to human beings. *American Journal of pharmacology and toxicology,* 4, 98-106.

NOWSHAD, F., ISLAM, M. N. & KHAN, M. S. 2018. Concentration and formation behavior of naturally occurring formaldehyde in foods. *Agriculture & Food Security,* 7, 17.

NYCHAS, G. J. E. & DROSINOS, E. H. 2014. Spoilage of meat. *In:* BATT, C. A. & TORTORELLO, M. L. (eds.) *Encyclopedia of Food Microbiology.* 2nd ed.: Elsevier.

O'GRADY, M. N. & KERRY, J. P. 2008. Smart packaging technologies and their application in conventional meat packaging systems. *In:* TOLDRA, F. (ed.) *Meat Biotechnology.* New York: USA: Springer Science and Business Media.

ODEYEMI, O. A., BURKE, C. M., BOLCH, C. C. J. & STANLEY, R. 2019. Spoilage microbial community profiling by 16S rRNA amplicon sequencing of modified atmosphere packaged live mussels stored at 4^{o}C. *Food Research International,* 121, 568-576.

OKADO, N., NARITA, M. & NARITA, N. 2001. A biogenic amine-synapse mechanism for mental retardation and developmental disabilities. *Brain and Development,* 23, 11-15.

ONYANGO, L. A. & ALRESHIDI, M. M. 2018. Adaptive Metabolism in Staphylococci: Survival and Persistence in Environmental and Clinical Settings. *Journal of Pathogens,* 2018, 1092632.

OSMAN, E. & BOZOGLU, T. F. (eds.) 2016. *Spoilage of Meat and Meat Products*.

OTO, N., OSHITA, S., MAKINO, Y., KAWAGOE, Y., SUGIYAMA, J. & YOSHIMURA, M. 2013. Non-destructive evaluation of ATP content and plate count on pork meat surface by fluorescence spectroscopy. *Meat Science,* 93, 579-585.

OUYANG, Q., WANG, L., ZAREEF, M., CHEN, Q., GUO, Z. & LI, H. 2020. A feasibility of nondestructive rapid detection of total volatile basic nitrogen content in frozen pork based on portable near-infrared spectroscopy. *Microchemical Journal,* 157, 105020.

ØVERLAND, M., RØRVIK, K. A. & SKREDE, A. 1999. Effect of trimethylamine oxide and betaine in swine diets on growth performance, carcass characteristics, nutrient digestibility, and sensory quality of pork. *Journal of Animal Science,* 77, 2143-2153.

PACZKOWSKI, S. & SCHÜTZ, S. 2011. Post-mortem volatiles of vertebrate tissue. *Applied Microbiology*

and Biotechnology, 91, 917-935.

PAPADOPOULOU, O. S., TASSOU, C. C., SCHIAVO, L., NYCHAS, G.-J. E. & PANAGOU, E. Z. 2011. Rapid Assessment of Meat Quality by Means of an Electronic Nose and Support Vector Machines. *Procedia Food Science,* 1, 2003-2006.

PAPUC, C., GORAN, G. V., PREDESCU, C. N. & NICORESCU, V. 2017. Mechanisms of oxidative processes in meat and toxicity induced by postprandial degradation products: a review. *Comprehensive Reviews in Food Science and Food Safety,* 16, 96-123.

PARK, Y. W., KIM, S. M., LEE, J. Y. & JANG, W. 2015. Application of biosensors in smart packaging. *Molecular & Cellular Toxicology,* 11, 277-285.

PARLAPANI, F. F., MICHAILIDOU, S., ANAGNOSTOPOULOS, D. A., KOROMILAS, S., KIOS, K., PASENTSIS, K., PSOMOPOULOS, F., ARGIRIOU, A., HAROUTOUNIAN, S. A. & BOZIARIS, I. S. 2019. Bacterial communities and potential spoilage markers of whole blue crab (Callinectes sapidus) stored under commercial simulated conditions. *Food Microbiology,* 82, 325-333.

PEARSON, D. 1976. *The chemical analysis of foods,* Edinburgh, UK, Churchill Livingstone.

PELLETIER, C. C., CROYAL, M., ENE, L., AGUESSE, A., BILLON-CROSSOUARD, S., KREMPF, M., LEMOINE, S., GUEBRE-EGZIABHER, F., JUILLARD, L. & SOULAGE, C. O. 2019. Elevation of Trimethylamine-N-Oxide in Chronic Kidney Disease: Contribution of Decreased Glomerular Filtration Rate. *Toxins,* 11, 635.

PENG, Y., ZHANG, J., WANG, W., LI, Y., WU, J., HUANG, H., GAO, X. & JIANG, W. 2011. Potential prediction of the microbial spoilage of beef using spatially resolved hyperspectral scattering profiles. *Journal of Food Engineering,* 102, 163-169.

PERALTA, J., BITENCOURT-CERVI, C. M., MACIEL, V. B. V., YOSHIDA, C. M. P. & CARVALHO, R. A. 2019. Aqueous hibiscus extract as a potential natural pH indicator incorporated in natural polymeric films. *Food Packaging and Shelf Life,* 19, 47-55.

PEREIRA, V. A., DE ARRUDA, I. N. Q. & STEFANI, R. 2015. Active chitosan/PVA films with anthocyanins from Brassica oleraceae (Red Cabbage) as Time–Temperature Indicators for application in intelligent food packaging. *Food Hydrocolloids,* 43, 180-188.

PERIASAMY, A. P., CHANG, Y. J. & CHEN, S. M. 2011. Amperometric glucose sensor based on glucose oxidase immobilized on gelatin-multiwalled carbon nanotube modified glassy carbon electrode.

Bioelectrochemistry, 80, 114-120.

PIERDOMINICI-SOTTILE, G., ECHAVE, J. & PALMA, J. 2006. Molecular dynamics study of the active site of methylamine dehydrogenase. *The Journal of Physical Chemistry B,* 110, 11592-11599.

PONNAMPALAM, E. N., HOLMAN, B. W. B. & SCOLLAN, N. D. 2016. Sheep: Meat. *In:* CABALLERO, B., FINGLAS, P. & TOLDRÁ, F. (eds.) *The Encyclopedia of Food and Health.* Oxford: United Kingdom: Academic Press.

PONNAMPALAM, E. N., HOPKINS, D. L., BRUCE, H., LI, D., BALDI, G. & BEKHIT, A. E. D. 2017a. Causes and contributing factors to "dark cutting" meat: Current trends and future directions: A review. *Comprehensive Reviews in Food Science and Food Safety,* 16, 400-430.

PONNAMPALAM, E. N., HOPKINS, D. L., KHAGESWOR, G., JACOBS, J. L., PLOZZA, T., LEWANDOWSKI, P. & BEKHIT, A. 2017b. The use of oxidative stress biomarkers in live animals (in vivo) to predict meat quality deterioration post mortem (in vitro) caused by changes in muscle biochemical components. *Journal of Animal Science,* 95, 3012-3024.

QIAN, S., LI, X., WANG, H., SUN, Z., ZHANG, C., CUAN, W. & BLECKER, C. 2018. Effect of sub-freezing storage (-6, -9 and -12 ^{O}C) on quality and shelf life of beef. *International Journal of Food Science & Technology,* 53, 2129-2140.

QIAO, L., LU, B., DONG, J., LI, B., ZHAO, B. & TANG, X. 2020. Total volatile basic nitrogen content in duck meat of different varieties based on calibration maintenance and transfer by use of a near-infrared spectrometric model. *Spectroscopy Letters,* 53, 44-54.

QIAO, L., TANG, X. & DONG, J. 2017. A feasibility quantification study of total volatile basic nitrogen (TVB-n) content in duck meat for freshness evaluation. *Food Chemistry,* 237, 1179-1185.

QUAN-FU, W., YAN-HUA, H. & PEI-SHENG, Y. 2012. Effect of cold-active protease treatments on Bigeye Tuna (Thunnus obesus) meat during chilled storage. *In:* ZHU, E. & SAMBATH, S. (eds.) *Information Technology and Agricultural Engineering.* Berlin: Springer.

RADER, D. J. 2003. Regulation of reverse cholesterol transport and clinical implications. *The American Journal of Cardiology,* 92, 42-49.

RAHELIC, S., GAWWAD, A. H. & PUAC, S. 1985. Structure of beef longissimus dorsi muscle frozen at various temperatures: Part 2 - Ultrastructure of muscles frozen at -10, -22, -33, -78 and -115°C. *Meat*

Science, 14, 73-81.

RAJAMÄKI, T., ALAKOMI, H. L., RITVANEN, T., SKYTTÄ, E., SMOLANDER, M. & AHVENAINEN, R. 2006. Application of an electronic nose for quality assessment of modified atmosphere packaged poultry meat. *Food Control,* 17, 5-13.

RAN-RESSLER, R. R., KHAILOVA, L., ARGANBRIGHT, K. M., ADKINS-RIECK, C. K., JOUNI, Z. E., KOREN, O., LEY, R. E., BRENNA, J. T. & DVORAK, B. 2011. Branched chain fatty acids reduce the incidence of necrotizing enterocolitis and alter gastrointestinal microbial ecology in a neonatal rat model. *PloS one,* 6, e29032.

RATH, S., RUD, T., PIEPER, D. H. & VITAL, M. 2020. Potential TMA-Producing Bacteria Are Ubiquitously Found in Mammalia. *Frontiers in Microbiology,* 10.

REDDY GANGIDI, R. & PROCTOR, A. 2009. Meat and meat products. *In:* SUN, D. W. (ed.) *Infrared spectroscopy for food quality analysis and control.* San Diego: Academic Press.

REICKS, A. L., BROOKS, J. C., GARMYN, A. J., THOMPSON, L. D., LYFORD, C. L. & MILLER, M. F. 2011. Demographics and beef preferences affect consumer motivation for purchasing fresh beef steaks and roasts. *Meat Science,* 87, 403-411.

RIGAULT, C., MAZUÉ, F., BERNARD, A., DEMARQUOY, J. & LE BORGNE, F. 2008. Changes in L-carnitine content of fish and meat during domestic cooking. *Meat Science,* 78, 331-335.

RODAS-GONZALEZ, A., NARVAEZ-BRAVO, C., BRASHEARS, M. M., ROGERS, H. B., TEDFORD, J. L., CLARK, G. O., BROOKS, J. C., JOHNSON, B. J., RATHMANN, R. J. & MILLER, M. F. 2011. Evaluation of the storage life of vacuum packaged Australian beef. *Meat Science,* 88, 128-138.

RODRÍGUEZ, O., BARROS-VELÁZQUEZ, J., OJEA, A., PINEIRO, C. & AUBOURG, S. P. 2003. Evaluation of sensory and microbiological changes and identification of proteolytic bacteria during the iced storage of farmed turbot (Psetta maxima). *Journal of Food Science,* 68, 2764-2771.

ROGERS, R. W. 2007. Packaging and freezing of beef as related to sensory properties. *In:* SUN, D. W. (ed.) *Handbook of Frozen Food Processing and Packaging.* Boca Raton, USA: Taylor & Francis Publishing.

ROHLFS, R. J. & HILLE, R. 1994. The reaction of trimethylamine dehydrogenase with diethylmethylamine. *Journal of Biological Chemistry,* 269, 30869-30879.

ROSENBERG, E., DELONG, E. F., LORY, S., STACKEBRANDT, E. & THOMPSON, F. 2014. *The Prokaryotes: Actinobacteria*, Springer.

ROUBALOVÁ, R., PROCHÁZKOVÁ, P., PAPEŽOVÁ, H., SMITKA, K., BILEJ, M. & TLASKALOVÁ-HOGENOVÁ, H. 2020. Anorexia nervosa: Gut microbiota-immune-brain interactions. *Clinical Nutrition,* 39, 676-684.

ROWLAND, R. & HUGHES, I. R. 2000. Metabolic activities of the gut microflora in relation to cancer. *Microbial Ecology in Health and Disease,* 12, 179-185.

RUAN, C., ZHANG, Y., SUN, Y., GAO, X., XIONG, G. & LIANG, J. 2019. Effect of sodium alginate and carboxymethyl cellulose edible coating with epigallocatechin gallate on quality and shelf life of fresh pork. *International Journal of Biological Macromolecules,* 141, 178-184.

RUIZ-CAPILLAS, C. & JIMENEZ-COLMENERO, F. 2005. Biogenic amines in meat and meat products. *Critical Reviews in Food Science and Nutrition,* 44, 489-599.

RUKCHON, C., NOPWINYUWONG, A., TREVANICH, S., JINKARN, T. & SUPPAKUL, P. 2014. Development of a food spoilage indicator for monitoring freshness of skinless chicken breast. *Talanta,* 130, 547-554.

SACCANI, G., TANZI, E., PASTORE, P., CAVALLI, S. & REY, M. 2005. Determination of biogenic amines in fresh and processed meat by suppressed ion chromatography-mass spectrometry using a cation-exchange column. *Journal of Chromatography A,* 1082, 43-50.

SAENZ-GARCIA, C. E., CASTANEDA-SERRANO, P., MERCADO SILVA, E. M., ALVARADO, C. Z. & NAVA, G. M. 2020. Insights into the Identification of the Specific Spoilage Organisms in Chicken Meat. *Foods,* 9.

SAHAR, A. & DUFOUR, É. 2014. Use of Fourier transform-infrared spectroscopy to predict spoilage bacteria on aerobically stored chicken breast fillets. *LWT - Food Science and Technology,* 56, 315-320.

SAITO, T. & WATANABE, M. 1998. Characterization of poly(vinylferrocene-co-2-hydroxyethyl methacrylate) for use as electron mediator in enzymatic glucose sensor. *Reactive and Functional Polymers,* 37, 263-269.

SALEEM, F., AMETAJ, B. N., BOUATRA, S., MANDAL, R., ZEBELI, Q., DUNN, S. M. & WISHART, D. S. 2012. A metabolomics approach to uncover the effects of grain diets on rumen health in dairy cows. *Journal of Dairy Science,* 95, 6606-6623.

SALINAS, Y., ROS-LIS, J. V., VIVANCOS, J. L., MARTÍNEZ-MÁÑEZ, R., MARCOS, M. D., AUCEJO, S.,

HERRANZ, N., LORENTE, I. & GARCIA, E. 2014. A novel colorimetric sensor array for monitoring fresh pork sausages spoilage. *Food Control,* 35, 166-176.

SALZANO, A., LICITRA, F., D'ONOFRIO, N., BALESTRIERI, M. L., LIMONE, A., CAMPANILE, G., MICHAEL, J. D. & NEGLIA, G. 2019. Space allocation in intensive Mediterranean buffalo production influences the profile of functional biomolecules in milk and dairy products. *Journal of Dairy Science,* 102, 7717-7722.

SANDHU, S. S. & CHASE JR, T. 1986. Aerobic degradation of choline by Proteus mirabilis: enzymatic requirements and pathway. *Canadian Journal of Microbiology,* 32, 743-750.

SANTOS-YAP, E. E. M. 1996. Fish and seafood. *In:* JEREMIAH, L. E. (ed.) *Freezing effects on food quality.* Boca Raton: CRC Press.

SCHAUDE, C., MEINDL, C., FRÖHLICH, E., ATTARD, J. & MOHR, G. J. 2017. Developing a sensor layer for the optical detection of amines during food spoilage. *Talanta,* 170, 481-487.

SCHUGAR, R. C., SHIH, D. M., WARRIER, M., HELSLEY, R. N., BURROWS, A., FERGUSON, D., BROWN, A. L., GROMOVSKY, A. D., HEINE, M. & CHATTERJEE, A. 2017. The TMAO-producing enzyme flavin-containing monooxygenase 3 regulates obesity and the beiging of white adipose tissue. *Cell Reports,* 19, 2451-2461.

SCHUMANN, B. & SCHMID, M. 2018. Packaging concepts for fresh and processed meat – Recent progresses. *Innovative Food Science & Emerging Technologies,* 47, 88-100.

SCULLY, C. 2014. 23 - Metabolic disorders. *In:* SCULLY, C. (ed.) *Scully's Medical Problems in Dentistry (Seventh Edition).* Oxford: Churchill Livingstone.

SELGAS, D., GARCIA, L., DE FERNANDO, G. G. & ORDÓÑEZ, J. A. 1993. Lipolytic and proteolytic activity of micrococci isolated from dry fermented sausages. *Fleischwirtschaft,* 73, 1164-1166.

SENAPATI, M. & SAHU, P. P. 2020. Meat quality assessment using Au patch electrode Ag-SnO2/SiO2/Si MIS capacitive gas sensor at room temperature. *Food Chemistry,* 324, 126893.

ŞENEL, M. & ABASıYANıK, M. F. 2010. Construction of a novel glucose biosensor based on covalent immobilization of glucose oxidase on poly(glycidyl methacrylate-co-vinylferrocene). *Electroanalysis,* 22, 1765-1771.

ŞENEL, M., NERGIZ, C. & ÇEVIK, E. 2013. Novel reagentless glucose biosensor based on ferrocene cored

asymmetric PAMAM dendrimers. *Sensors and Actuators B: Chemical,* 176, 299-306.

SERVILLO, L., D'ONOFRIO, N., GIOVANE, A., CASALE, R., CAUTELA, D., CASTALDO, D., IANNACCONE, F., NEGLIA, G., CAMPANILE, G. & BALESTRIERI, M. L. 2018a. Ruminant meat and milk contain delta-valerobetaine, another precursor of trimethylamine N-oxide (TMAO) like gamma-butyrobetaine. *Food Chemistry,* 260, 193-199.

SERVILLO, L., D'ONOFRIO, N., NEGLIA, G., CASALE, R., CAUTELA, D., MARRELLI, M., LIMONE, A., CAMPANILE, G. & BALESTRIERI, M. L. 2018b. Carnitine precursors and short-chain acylcarnitines in Water Buffalo milk. *Journal of Agricultural and Food Chemistry,* 66, 8142-8149.

SHANG, X., QIAO, J. & CHEN, Z. 2019. Changes in Lipase and Antioxidant Enzyme Activities during Processing of Cantonese Sausage with D-Sodium Erythorbate. *Journal of Food Quality,* 2019, 1671603.

SHEKELLE, R. B., SHRYOCK, A. M., PAUL, O., LEPPER, M., STAMLER, J., LIU, S. & RAYNOR JR, W. J. 1981. Diet, serum cholesterol, and death from coronary heart disease: the Western Electric Study. *New England Journal of Medicine,* 304, 65-70.

SHENG, X., SHU, D., TANG, X. & ZANG, Y. 2018. Effects of slightly acidic electrolyzed water on the microbial quality and shelf life extension of beef during refrigeration. *Food Science & Nutrition,* 6, 1975-1981.

SHI, C., QIAN, J., HAN, S., FAN, B., YANG, X. & WU, X. 2018a. Developing a machine vision system for simultaneous prediction of freshness indicators based on tilapia (Oreochromis niloticus) pupil and gill color during storage at 4 ^{o}C. *Food Chemistry,* 243, 134-140.

SHI, L., YIN, T., WANG, L., XIONG, G., GAO, R., DING, A., LI, X., WU, W., QIAO, Y., LIAO, L. & JIAO, C. 2020. Effect of pre-chilling time on the physicochemical properties of channel catfish during frozen storage. *International Journal of Refrigeration,* 115, 56-62.

SHI, Y., LI, Z., SHI, J., ZHANG, F., ZHOU, X., LI, Y., HOLMES, M., ZHANG, W. & ZOU, X. 2018b. Titanium dioxide-polyaniline/silk fibroin microfiber sensor for pork freshness evaluation. *Sensors and Actuators, B: Chemical,* 260, 465-474.

SHIMADA, K., SAKUMA, Y., WAKAMATSU, J., FUKUSHIMA, M., SEKIKAWA, M., KUCHIDA, K. & MIKAMI, M. 2004. Species and muscle differences in L-carnitine levels in skeletal muscles based on a new simple assay. *Meat Science,* 68, 357-362.

SIEGMUND, B. & PFANNHAUSER, W. 1999. Changes of the volatile fraction of cooked chicken meat during chill storing: results obtained by the electronic nose in comparison to GC-MS and GC olfactometry. *Zeitschrift Für Lebensmitteluntersuchung Und-Forschung A,* 208, 336-341.

SIKORSKI, Z. E. & KOLAKOWSKA, A. 1994. Changes in proteins in frozen stored fish. *In:* SIKORSKI, Z. E., PAN, B. S. & SHAHIDI, F. (eds.) *Seafood proteins.* New York: Springer.

SILVA, C. M. G. & GLORIA, M. B. A. 2002. Bioactive amines in chicken breast and thigh after slaughter and during storage at 4±1 ºC and in chicken-based meat products. *Food Chemistry,* 78, 241-248.

SINELLI, N., BARZAGHI, S., GIARDINA, C. & CATTANEO, T. M. P. 2005. A preliminary study using Fourier transform near infrared spectroscopy to monitor the shelf-life of packed industrial ricotta cheese. *Journal of Near Infrared Spectroscopy,* 13, 293-300.

SINTERMANN, J., SCHALLHART, S., KAJOS, M., JOCHER, M., BRACHER, A., MÜNGER, A., JOHNSON, D., NEFTEL, A. & RUUSKANEN, T. 2014. Trimethylamine emissions in animal husbandry. *Biogeosciences,* 11, 5073-5085.

SMITS, E., SCHRAM, J., NAGELKERKE, M., KUSTERS, R., VAN HECK, G., VAN ACHT, V., KOETSE, M., VAN DEN BRAND, J. & GERLINCK, G. Development of printed RFID sensor tags for smart food packaging. 14th International Meeting on Chemical Sensors, 2012 Nuremberg, GER. 20-23.

SMOLANDER, M., HURME, E., LATVA-KALA, K., LUOMA, T., ALAKOMI, H.-L. & AHVENAINEN, R. 2002. Myoglobin-based indicators for the evaluation of freshness of unmarinated broiler cuts. *Innovative Food Science & Emerging Technologies,* 3, 279-288.

SOLADOYE, O. P., JUÁREZ, M. L., AALHUS, J. L., SHAND, P. & ESTÉVEZ, M. 2015. Protein oxidation in processed meat: Mechanisms and potential implications on human health. *Comprehensive Reviews in Food Science and Food Safety,* 14, 106-122.

SOSNIKI, A. 2016. Pork quality. *In:* PRZYBYLSKI, W. & HOPKINS, D. L. (eds.) *Meat Quality: Genetic and Environmental Factors.* New Jersey, USA: CRC Press (Taylor & Fracis Group).

STEPHAN, R., STIERLI, F. & UNTERMANN, F. 1997. Chemical attributes characterizing sticky post-mortem ageing in beef. *Meat Science,* 47, 331-335.

STERZEL, R. B., LUFT, F. C., GAO, Y., SCHNERMANN, J., BRIGGS, J. P., GANTEN, D., WALDHERR, R., SCHNABEL, E. & KRIZ, W. 1988. Renal disease and the development of hypertension in salt-sensitive

Dahl rats. *Kidney International,* 33, 1119-1129.

SU, W. H., HE, H. J. & SUN, D. W. 2017. Non-destructive and rapid evaluation of staple foods quality by using spectroscopic techniques: A review. *Critical Reviews in Food Science and Nutrition,* 57, 1039-1051.

SUBRAMANIAM, S. & FLETCHER, C. 2018. Trimethylamine N-oxide: Breathe new life. *British Journal of Pharmacology,* 175, 1344-1353.

SUN, G., YIN, Z., LIU, N., BIAN, X., YU, R., SU, X., ZHANG, B. & WANG, Y. 2017. Gut microbial metabolite TMAO contributes to renal dysfunction in a mouse model of diet-induced obesity. *Biochemical and Biophysical Research Communications,* 493, 964-970.

SUN, W., LIU, Y., JIA, L., SALDAÑA, M. D. A., DONG, T., JIN, Y. & SUN, W. 2020. A Smart Nanofiber Sensor Based on Anthocyanin/Poly-L-Lactic Acid for Mutton Freshness Monitoring. *International Journal of Food Science & Technology,* In Press.

SUN, Y., FU, M., LI, Z. & PENG, X. 2018. Evaluation of freshness in determination of volatile organic compounds released from pork by HS-SPME-GC-MS. *Food Analytical Methods,* 11, 1321-1329.

SZYMECZKO, R., BURLIKOWSKA, K., IBEN, C., PIOTROWSKA, A. & BOGUSŁAWSKA-TRYK, M. 2007. Ileal absorption of L-carnitine from diets used in reproductive polar fox (Alopex lagopus L.) nutrition. *Acta Agriculturae Scand Section A,* 57, 142-147.

TAHERI-GARAVAND, A., FATAHI, S., OMID, M. & MAKINO, Y. 2019. Meat quality evaluation based on computer vision technique: A review. *Meat Science,* 156, 183-195.

TAN, Z., HUANG, Z., LV, Y., LI, Y. & CHEN, D. 2019. A gas Fourier transform infrared spectroscopy methodology for the rapid and accurate discrimination of chicken spoilage through volatiles analysis. *Flavour and Fragrance Journal,* 34, 271-279.

TANG, W. H. W., WANG, Z., LEVISON, B. S., KOETH, R. A., BRITT, E. B., FU, X., WU, Y. & HAZEN, S. L. 2013. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *New England Journal of Medicine,* 368, 1575-1584.

TANTRATIAN, S. & KAEPHEN, K. 2020. Shelf-life of shucked oyster in epigallocatechin-3-gallate with slightly acidic electrolyzed water washing under refrigeration temperature. *LWT - Food Science and Technology,* 118, 108733.

TAO, F. & PENG, Y. 2014. A method for nondestructive prediction of pork meat quality and safety attributes by hyperspectral imaging technique. *Journal of Food Engineering,* 126, 98-106.

TEJADA, M. & HUIDOBRO, A. 2002. Quality of farmed gilthead seabream (*Sparus aurata*) during ice storage related to slaughter method and gutting. *European Food Research and Technology,* 215, 1-7.

TOLDRA, F. 1998. Proteolysis and lipolysis in flavour development of dry-cured meat products. *Meat Science,* 49, 101-110.

TOLDRÁ, F. 2008. Biotechnology of Flavor Generation in Fermented Meats. *In:* TOLDRÁ, F. (ed.) *Meat Biotechnology.* New York, NY: Springer New York.

TONG, Y. Y., CHEN, D. H., SHI, Z. B. & TONG, J. 2010. A mathematical model of relationship of mechanics parameter and its freshness of chicken. *Journal of Agricultural Mechanization Research,* 32, 174-176.

TRÉZL, L., CSIBA, A., JUHASZ, S., SZENTGYÖRGYI, M., LOMBAI, G., HULLÁN, L. & JUHÁSZ, A. 1997. Endogenous formaldehyde level of foods and its biological significance. *Zeitschrift für Lebensmitteluntersuchung und-Forschung A,* 205, 300-304.

TRIPATHI, M. K. & KARIM, S. A. 2011. Effect of yeast cultures supplementation on live weight change, rumen fermentation, ciliate protozoa population, microbial hydrolytic enzymes status and slaughtering performance of growing lamb. *Livestock Science,* 135, 17-25.

TRIPOLT, N. J., LEBER, B., TRIEBL, A., KÖFELER, H., STADLBAUER, V. & SOURIJ, H. 2015. Effect of Lactobacillus casei Shirota supplementation on trimethylamine-N-oxide levels in patients with metabolic syndrome: An open-label, randomized study. *Atherosclerosis,* 242, 141-144.

TSOY, O., RAVCHEEV, D. & MUSHEGIAN, A. 2009. Comparative genomics of ethanolamine utilization. *Journal of Bacteriology,* 191, 7157-7164.

UFNAL, M., ZADLO, A. & OSTASZEWSKI, R. 2015. TMAO: A small molecule of great expectations. *Nutrition,* 31, 1317-1323.

UMUHUMUZA, L. C. & SUN, X. 2011. Rapid detection of pork meat freshness by using L-cysteine-modified gold electrode. *European Food Research and Technology,* 232, 425-431.

VAZ, F. M. & WANDERS, R. J. A. 2002. Carnitine biosynthesis in mammals. *Biochemical Journal,* 361, 417-429.

VECIANA-NOGUES, M. T., MARINE-FONT, A. & VIDAL-CAROU, M. C. 1997. Biogenic amines as hygienic quality indicators of tuna. Relationships with microbial counts, ATP-related compounds, volatile amines, and organoleptic changes. *Journal of Agricultural and Food Chemistry,* 45, 2036-2041.

VELASQUEZ, M. T., RAMEZANI, A., MANAL, A. & RAJ, D. S. 2016. Trimethylamine N-oxide: the good, the bad and the unknown. *Toxins,* 8, 326.

WANG, L., DING, L., YU, Z., ZHANG, T., MA, S. & LIU, J. 2016. Intracellular ROS scavenging and antioxidant enzyme regulating capacities of corn gluten meal-derived antioxidant peptides in HepG2 cells. *Food Research International,* 90, 33-41.

WANG, Q., LONERGAN, S. M. & YU, C. 2012. Rapid determination of pork sensory quality using Raman spectroscopy. *Meat Science,* 91, 232-239.

WANG, Q., XIE, Y. F., ZHAO, W. J., LI, P., QIAN, H. & WANG, X. Z. 2014a. Rapid microchip-based FAIMS determination of trimethylamine, an indicator of pork deterioration. *Analytical Methods,* 6, 2965-2972.

WANG, X., ZHAO, M., JU, R., SONG, Q., HUA, D., WANG, C. & CHEN, T. 2013. Visualizing quantitatively the freshness of intact fresh pork using acousto-optical tunable filter-based visible/near-infrared spectral imagery. *Computers and Electronics in Agriculture,* 99, 41-53.

WANG, Y. & DUNCAN, T. V. 2017. Nanoscale sensor for assuring the safety of food products. *Current Opinion in Biotechnology,* 44, 74-86.

WANG, Y., LI, F., ZHUANG, H., LI, L., CHEN, X. & ZHANG, J. 2015. Effects of plant polyphenols and α-tocopherol on lipid oxidation, microbiological characteristics, and biogenic amines formation in dry-cured bacons. *Journal of Food Science,* 80, C547-C555.

WANG, Z., BERGERON, N., LEVISON, B. S., LI, X. S., CHIU, S., JIA, X., KOETH, R. A., LI, L., WU, Y. & TANG, W. H. W. 2019. Impact of chronic dietary red meat, white meat, or non-meat protein on trimethylamine N-oxide metabolism and renal excretion in healthy men and women. *European Heart Journal,* 40, 583-594.

WANG, Z., HE, Z., ZHANG, D., LI, H. & WANG, Z. 2020. Using oxidation kinetic models to predict the quality indices of rabbit meat under different storage temperatures. *Meat Science,* 162, 108042.

WANG, Z., KLIPFELL, E., BENNETT, B. J., KOETH, R., LEVISON, B. S., DUGAR, B., FELDSTEIN, A. E., BRITT,

E. B., FU, X. & CHUNG, Y. M. 2011. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature,* 472, 57-63.

WANG, Z., TANG, W. H. W., BUFFA, J. A., FU, X., BRITT, E. B., KOETH, R. A., LEVISON, B. S., FAN, Y., WU, Y. & HAZEN, S. L. 2014b. Prognostic value of choline and betaine depends on intestinal microbiota-generated metabolite trimethylamine-N-oxide. *European Heart Journal,* 35, 904-910.

WELLS, N., YUSUFU, D. & MILLS, A. 2019. Colourimetric plastic film indicator for the detection of the volatile basic nitrogen compounds associated with fish spoilage. *Talanta,* 194, 830-836.

WILSON, A., TEFT, W. A., MORSE, B. L., CHOI, Y. H., WOOLSEY, S., DEGORTER, M. K., HEGELE, R. A., TIRONA, R. G. & KIM, R. B. 2015. Trimethylamine-N-oxide: a novel biomarker for the identification of inflammatory bowel disease. *Digestive Diseases and Sciences,* 60, 3620-3630.

WINTHER, S. A., ØLLGAARD, J. C., TOFTE, N., TARNOW, L., WANG, Z., AHLUWALIA, T. S., JORSAL, A., THEILADE, S., PARVING, H. H. & HANSEN, T. W. 2019. Utility of plasma concentration of trimethylamine n-oxide in predicting cardiovascular and renal complications in individuals with type 1 diabetes. *Diabetes Care,* 42, 1512-1520.

WOJNOWSKI, W., NAMIEŚNIK, J. & PŁOTKA-WASYLKA, J. 2019. Dispersive liquid-liquid microextraction combined with gas chromatography–mass spectrometry for in situ determination of biogenic amines in meat: Estimation of meat's freshness. *Microchemical Journal,* 145, 130-138.

XIA, Z., ZHAI, X., LIU, B. & MO, Y. 2016. Conductometric titration to determine total volatile basic nitrogen (TVB-N) for post-mortem interval (PMI). *Journal of Forensic and Legal Medicine,* 44, 133-137.

XIAO-WEI, H., ZHI-HUA, L., XIAO-BO, Z., JI-YONG, S., HAN-PING, M., JIE-WEN, Z., LI-MIN, H. & HOLMES, M. 2016. Detection of meat-borne trimethylamine based on nanoporous colorimetric sensor arrays. *Food Chemistry,* 197, 930-936.

XIAO, K., GAO, G. & SHOU, L. 2014a. An improved method of detecting pork freshness based on computer vision in on-line system. *Sensors & Transducers,* 169, 42-48.

XIAO, Y., JIAOJIAO, J., GUOHUA, H., FANGYUAN, Y., MINMIN, W., JIE, H., XIAOGUO, Y. & SHANGGUI, D. 2014b. Determination of the Freshness of Beef Strip Loins (M. longissimus lumborum) Using Electronic Nose. *Food Analytical Methods,* 7, 1612-1618.

XIONG, X., HE, B., JIANG, D., DONG, X., YU, C. & QI, H. 2020. Postmortem biochemical and textural

changes in the sea cucumber *Stichopus japonicus* body wall (SJBW) during iced storage. *LWT - Food Science and Technology,* 118, 108705.

XIONG, Y. L. 1997. Protein denaturation and functionality losses. *In:* ERICKSON, M. C. & HUNG, Y. C. (eds.) *Quality in frozen food.* Boston: Springer.

XIONG, Z., SUN, D. W., PU, H., GAO, W. & DAI, Q. 2017. Applications of emerging imaging techniques for meat quality and safety detection and evaluation: A review. *Critical Reviews in Food Science and Nutrition,* 57, 755-768.

XIONG, Z., SUN, D. W., PU, H., XIE, A., HAN, Z. & LUO, M. 2015. Non-destructive prediction of thiobarbituric acid reactive substances (TBARS) value for freshness evaluation of chicken meat using hyperspectral imaging. *Food Chemistry,* 179, 175-181.

XIONG, Z., SUN, D. W., ZENG, X. A. & XIE, A. 2014. Recent developments of hyperspectral imaging systems and their applications in detecting quality attributes of red meats: A review. *Journal of Food Engineering,* 132, 1-13.

XU, F., CAO, S., WANG, C., WANG, K., WEI, Y., SHAO, X. & WANG, H. 2019. Antimicrobial activity of flavonoids from Sedum aizoon L. against Aeromonas in culture medium and in frozen pork. *Food Science & Nutrition,* 7, 3224-3232.

XU, R., WANG, Q. Q. & LI, L. 2015. A genome-wide systems analysis reveals strong link between colorectal cancer and trimethylamine N-oxide (TMAO), a gut microbial metabolite of dietary meat and fat. *BMC Genomics,* 16, 4.

XU, Y., CHEN, Q., LIU, Y., SUN, X., HUANG, Q., OUYANG, Q. & ZHAO, J. 2018. A novel hyperspectral microscopic imaging system for evaluating fresh degree of pork. *Korean Journal for Food Science of Animal Resources,* 38, 362-375.

YANCEY, P. H. & SIEBENALLER, J. F. 1999. Trimethylamine oxide stabilizes teleost and mammalian lactate dehydrogenases against inactivation by hydrostatic pressure and trypsinolysis. *Journal of Experimental Biology,* 202, 3597-3603.

YANG, C., QI, Y., ZHENG, J., FAN, X., LIANG, P. & SONG, C. 2018. Efficacy of Various Preservatives on Extending Shelf Life of Vacuum-Packaged Raw Pork during 4°C Storage. *Journal of Food Protection,* 81, 636-645.

YAO, C. K., MUIR, J. G. & GIBSON, P. R. 2016. Insights into colonic protein fermentation, its modulation and potential health implications. *Alimentary Pharmacology & Therapeutics,* 43, 181-196.

YAO, M. E., LIAO, P. D., ZHAO, X. J. & WANG, L. 2020. Trimethylamine-N-oxide has prognostic value in coronary heart disease: a meta-analysis and dose-response analysis. *BMC Cardiovascular Disorders,* 20, 7.

YOST, C. K. & NATTRESS, F. M. 2000. The use of multiplex PCR reactions to characterize populations of lactic acid bacteria associated with meat spoilage. *Letters in Applied Microbiology,* 31, 129-133.

YOUSEFI, M., FARSHIDI, M. & EHSANI, A. 2018. Effects of lactoperoxidase system-alginate coating on chemical, microbial, and sensory properties of chicken breast fillets during cold storage. *Journal of Food Safety,* 38, 12449.

YU, Y. J., AMORIM, M., MARQUES, C., CALHAU, C. & PINTADO, M. 2016. Effects of whey peptide extract on the growth of probiotics and gut microbiota. *Journal of Functional Foods,* 21, 507-516.

ZARAGOZÁ, P., FUENTES, A., FERNÁNDEZ-SEGOVIA, I., VIVANCOS, J. L., RIZO, A., ROS-LIS, J. V., BARAT, J. M. & MARTÍNEZ-MÁÑEZ, R. 2013. Evaluation of sea bream (Sparus aurata) shelf life using an optoelectronic nose. *Food Chemistry,* 138, 1374-1380.

ZEISEL, S. H. 1990. Choline deficiency. *The Journal of Nutritional Biochemistry,* 1, 332-349.

ZEISEL, S. H., WISHNOK, J. S. & BLUSZTAJN, J. K. 1983. Formation of methylamines from ingested choline and lecithin. *Journal of Pharmacology and Experimental Therapeutics,* 225, 320-324.

ZEQUAN, X., ZIRONG, W., JIANKUN, L., XIN, M., HOPKINS, D. L., HOLMAN, B. W. B. & BEKHIT, A. E. A. 2019. The effect of freezing time on the quality of normal and pale, soft and exudative (PSE)-like pork. *Meat Science,* 152, 1-7.

ZHAI, X., LI, Z., SHI, J., HUANG, X., SUN, Z., ZHANG, D., ZOU, X., SUN, Y., ZHANG, J., HOLMES, M., GONG, Y., POVEY, M. & WANG, S. 2019. A colorimetric hydrogen sulfide sensor based on gellan gum-silver nanoparticles bionanocomposite for monitoring of meat spoilage in intelligent packaging. *Food Chemistry,* 290, 135-143.

ZHAI, X., SHI, J., ZOU, X., WANG, S., JIANG, C., ZHANG, J., HUANG, X., ZHANG, W. & HOLMES, M. 2017. Novel colorimetric films based on starch/polyvinyl alcohol incorporated with roselle anthocyanins for fish freshness monitoring. *Food Hydrocolloids,* 69, 308-317.

ZHAI, X., ZOU, X., SHI, J., HUANG, X., SUN, Z., LI, Z., SUN, Y., LI, Y., WANG, X., HOLMES, M., GONG, Y., POVEY, M. & XIAO, J. 2020. Amine-responsive bilayer films with improved illumination stability and electrochemical writing property for visual monitoring of meat spoilage. *Sensors and Actuators B: Chemical,* 302, 127130.

ZHANG, J., WANG, Y., PAN, D. D., CAO, J. X., SHAO, X. F., CHEN, Y. J., SUN, Y. Y. & OU, C. R. 2016. Effect of black pepper essential oil on the quality of fresh pork during storage. *Meat Science,* 117, 130-136.

ZHANG, J., ZOU, X., ZHAI, X., HUANG, X., JIANG, C. & HOLMES, M. 2019a. Preparation of an intelligent pH film based on biodegradable polymers and roselle anthocyanins for monitoring pork freshness. *Food Chemistry,* 272, 306-312.

ZHANG, W., CHEN, S., LIAO, Y., WANG, D., DING, J., WANG, Y., RAN, X., LU, D. & ZHU, H. 2013a. Expression, purification, and characterization of formaldehyde dehydrogenase from Pseudomonas aeruginosa. *Protein Expression and Purification,* 92, 208-213.

ZHANG, W., XIAO, S. & AHN, D. U. 2013b. Protein oxidation: Basic principles and implications for meat quality. *Critical Reviews in Food Science and Nutrition,* 53, 1191-1201.

ZHANG, Y. & LIM, L.-T. 2018. Colorimetric array indicator for NH3 and CO2 detection. *Sensors and Actuators B: Chemical,* 255, 3216-3226.

ZHANG, Y., ZHANG, Y., ZHOU, Y., LI, G. H., YANG, W. Z. & FENG, X. S. 2019b. A review of pretreatment and analytical methods of biogenic amines in food and biological samples since 2010. *Journal of Chromatography A,* 1605, 360361.

ZHANG, Y., ZHU, L., DONG, P., LIANG, R., MAO, Y., QIU, S. & LUO, X. 2018. Bio-protective potential of lactic acid bacteria: Effect of Lactobacillus sakei and Lactobacillus curvatus on changes of the microbial community in vacuum-packaged chilled beef. *Asian-Australasian Journal of Animal Sciences,* 31, 585-594.

ZHAO, F., ZHOU, G., YE, K., WANG, S., XU, X. & LI, C. 2015. Microbial changes in vacuum-packed chilled pork during storage. *Meat Science,* 100, 145-149.

ZHAO, H. T., FENG, Y. Z., CHEN, W. & JIA, G. F. 2019a. Application of invasive weed optimization and least square support vector machine for prediction of beef adulteration with spoiled beef based on visible near-infrared (Vis-NIR) hyperspectral imaging. *Meat Science,* 151, 75-81.

ZHAO, J., ZHANG, T., LIU, Y., WANG, X., ZHANG, L., KU, T. & QUEK, S. Y. 2018. Qualitative and quantitative assessment of DNA quality of frozen beef based on DNA yield, gel electrophoresis and PCR amplication and their correlations to beef quality. *Food Chemistry,* 260, 160-165.

ZHAO, S., LI, N., LI, Z., HE, H., ZHAO, Y., ZHU, M., WANG, Z., KANG, Z. & MA, H. 2019b. Shelf life of fresh chilled pork as affected by antimicrobial intervention with nisin, tea polyphenols, chitosan, and their combination. *International Journal of Food Properties,* 22, 1047-1063.

ZHAO, T., SU, G., WANG, S., ZHANG, Q., ZHANG, J., ZHENG, L., SUN, B. & ZHAO, M. 2017. Neuroprotective effects of acetylcholinesterase inhibitory peptides from Anchovy (Coilia mystus) against glutamate-induced toxicity in PC12 cells. *Journal of Agricultural and Food Chemistry,* 65, 11192-11201.

ZHENG, L., ZHENG, J., XIE, Y., LI, Z., GUO, X., SUN, G., SUN, Z., XING, F. & SUN, Y. 2019. Serum gut microbe-dependent trimethylamine N-oxide improves the prediction of future cardiovascular disease in a community-based general population. *Atherosclerosis,* 280, 126-131.

ZHU, P., DU, J., XU, B. & LU, M. 2017. Modified unsupervised discriminant projection with an electronic nose for the rapid determination of Chinese mitten crab freshness. *Analytical Methods,* 9, 1806-1815.