School of Molecular and Life Sciences

Development of Novel Edible Coatings to Limit the Effect of Moisture Loss on the Shelf Life of Beef

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Declaration

To the best of my knowledge and belief, this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Shamika Thushara Gorokgaha Gedarawatte

01/04/2021

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I just stared into my computer screen without blinking for a few minutes, thinking from where to start and where to end. Many people have helped me achieve this moment.

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List of Publications

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Statement of Attribution

The following tables describe the contribution made by each author towards the research outputs from this thesis and the co-authors were listed in no particular order.

Paper 1: located in Chapter 3

Gedarawatte, S. T., Ravensdale, J. T., Johns, M. L., Azizi, A., Al-Salami, H., Dykes, G. A., & Coorey, R. (2021). Effectiveness of gelatine and chitosan spray coating for extending shelf life of vacuum-packaged beef. *International Journal of Food Science and Technology* (in early view). DOI: https://doi.org/10.1111/ijfs.15025

	Conception and Design	Acquisition of Data and Method	Data Conditioning and Manipulation	Analysis and Statistical Method	Interpretation and Discussion	Reviews and Feedback	Final Approval
Shamika T. G. Gedarawatte	65%	90%	90%	100%	100%	0%	20%
Co-Author 1 Acknowledgement: I, as a Co-Author, endorse that this level of contribution by the candidate indicated above is appropriate Signed: March 2021							
Ranil Coorey	10%	0%	0%	0%	0%	30%	30%
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Abbreviations

a^*	Redness
A_{21} (%)	Population area ratio of T_{21}
A_{22} (%)	Population area ratio of T_{22}
ANOVA	Analysis of variance
b^*	Yellowness
BC	Bacterial cellulose
BCL	Blast chilling
BCN	Coated with BCNs and vacuum-packaged
BCNs	Bacterial cellulose nanocrystals
BPW	Buffered peptone water
BCW	wrapped with BC-sheets and vacuum-packaged
CC	Conventional air chilling
CHC	Coated with 1% chitosan and vacuum-packaged (CHC)
CI	Crystallinity index
CPMG	Carr–Purcell–Meiboom–Gill
DBP	Dehydrated beef protein
EMB	Eosin-Methylene Blue
FC	Fast chilling
FESEM	Field emission scanning electron microscope
GEC	Coated with 10% gelatine and vacuum-packaged
GRAS	Generally Recognized As Safe
HPP	High pressure processing
HS	Hestrin and Schramm
L^*	Lightness
LAB	Lactic acid bacteria
LTL	*Longissimus thoracis et lumborum*
MAP	Modified atmospheric packaging
MDA	Malonaldehyde
MRS	Man, Rogosa and Sharpe
NBCN	Coated with NBCNs and vacuum-packaged
NBCNs	Nisin-loaded bacterial cellulose nanocrystals
NMR	Nuclear magnetic resonance
OCT	Optimal cutting temperature
PEF	Pulsed electric field

PBS	Phosphate-buffered saline
pm	Post-mortem
P_I	Isoelectric point
preb	Colour evaluation at pre-blooming conditions
postb	Colour evaluation at post-blooming conditions
RC	Rapid chilling
RC®	Rinse and Chill®
SC	Spray chilling
SEM	Scanning electron microscopy
STAA	Streptomycin-Thallous Acetate-Actidione
T_2	Transverse relaxation time
T_{21}	T_{21} relaxation time
T_{22}	T_{22} relaxation time
T_{2lm}	Overall transverse relaxation time
TBARS	2-thiobarbituric acid reactive substances
UNC	Uncoated and vacuum-packaged
UNW	Unwrapped and vacuum-packaged
VP	Vacuum Packaging
VP-FESEM	Variable pressure field emission scanning electron microscope
VSP	Vacuum skin packaging
WHC	Water-holding capacity
W_{dry}	Dry weight of BC-sheets
W_{wet}	Wet weight of swollen BC-sheets
XRD	X-ray diffraction

Abstract

Beef muscle consists of approximately 75% water and one of the key challenges faced by the red meat industry is reducing moisture loss during storage. Moisture loss is associated with key financial losses due to the reduction in product saleable weight, rejection of export orders and consumer dissatisfaction due to loss of sensory quality. It can cause unattractive product appearance and may jeopardise food safety by providing a growth substrate for spoilage and pathogenic microorganisms. Vacuum packaging causes the highest moisture loss compared to all other packaging techniques due to the physical compression applied during the vacuuming operation. Purge and drip formation are the two key mechanisms responsible for moisture loss in meat and purge formation is the major contributing factor for the moisture loss associated with vacuum-packaged storage of beef. Methods that can be employed to reduce purge loss in vacuum-packaged beef are limited, as the majority of research has focused on reducing weight loss in aerobically packaged meat. This thesis describes different coating systems and wrapping applications undertaken to control purge loss in vacuum-packaged beef and the interaction of these systems with the structure of the beef muscle.

The impact of applying gelatine and chitosan spray coatings on controlling moisture loss and other quality losses were investigated and compared against uncoated beef. Both edible coatings were not effective in reducing purge loss as storage time increased. Chitosan coating showed a significant reduction in lipid oxidation and microbial spoilage compared to uncoated and gelatine coated beef. Beef coated with chitosan exhibited significantly higher sensorial acceptance compared to gelatine coated meat and did not negatively affect the other physicochemical parameters of meat. Chitosan spray coating would be a promising application that could extend the shelf life of vacuum-packaged beef.

The effectiveness of applying bacterial cellulose as a wrap in controlling moisture accumulation and other quality degradations of vacuum-packaged beef was studied and compared against unwrapped beef. The bacterial cellulose wrap was able to achieve a significant reduction in moisture accumulation and increase in meat redness and yellowness compared to unwrapped beef. Scanning electron microscopic images revealed that reduction in purge accumulation could be either due to the absorption of

purge by bacterial cellulose nanofibers or by trapping purge in the gaps of bacterial cellulose porous network. As per XRD results, the mechanical strength of bacterial cellulose films remained quite stable with the increase of vacuum-packaged storage period. The wrapping treatment increased the growth of beef spoilage bacteria by acting as a substrate for their attachment. Results showed that bacterial cellulose could be used as a purge absorbent in meat vacuum packages.

Bacterial cellulose was identified as the most suitable material to produce nanocrystal spray coating solution due to its ability in absorbing purge. Bacterial cellulose nanocrystals were formed by acid hydrolysis and they were loaded with nisin peptide to create antimicrobial active nanocrystals as bacterial cellulose in its innate form enhances the growth of spoilage microorganisms. Bacterial cellulose nanocrystals showed stable zeta-potential (- 43 mV) and 5 mg/ml bacterial cellulose nanocrystal suspension loaded with nisin had zeta-potential values ≥ - 30 mV. Encapsulation efficiency significantly increased with the increase of nisin concentration. The antimicrobial activity of nanocrystals was assessed against two Gram-positive vacuum-packaged beef spoilage bacteria known as *Lactobacillus rhamnosus* and *Leuconostoc mesenteroides*. Nanocrystals loaded with 2 and 2.5 mg/ml nisin were effective in inactivating both microorganisms. Nisin loading into bacterial cellulose nanocrystals was further confirmed by SEM, TEM and FTIR. Nisin-loaded bacterial cellulose nanocrystals could be used as an antimicrobial and reinforcing agent in active food packaging applications.

Beef sections were spray coated with unloaded and nisin-loaded bacterial cellulose nanocrystal solutions, stored under chilled storage conditions (- 1°C ± 1.0 ºC) and their impact on meat shelf life was evaluated over a one month storage period. Both nanocoating systems were not able to control purge loss as the storage time increased. Poor electrochemical interactions between purge channels and the nanocrystals could have led to reduced water-holding capacity as shown by the Kelvin probe force microscopy and zeta-potential measurements. Nisin-loaded nanocrystals were effective in controlling microbial spoilage compared to unloaded bacterial cellulose nanocrystals. Assessment of electrochemical properties of muscle matrix with the use of advanced analytical techniques could aid in designing food nanocoating systems that would be effective in reducing moisture and other quality losses.

The findings of this thesis provided insight into how techniques common in food science and material science can give a comprehensive understanding of how effective different coating materials can be at preserving vacuum-packaged meat. New knowledge was also presented that may help to design novel nanocoating systems that will be effective in controlling food quality losses. This study suggests that advanced microscopy techniques could help in determining the mechanisms related to food quality degradation over time.

Chapter 1. Aims and overview of the thesis

This project aimed to investigate novel methods to extend the shelf life of vacuum-packaged beef by reducing the moisture loss associated with purge and drip formation. This thesis investigated three main types of meat coating applications to reduce moisture accumulation in vacuum-packaged beef; i) traditional edible spray coating treatment, ii) edible wrapping treatment and iii) nanocrystal spray coating treatment. The sub-objectives of this project were as follows;

a) To determine the effectiveness of traditional spray coating (gelatine and chitosan) on moisture loss of vacuum-packaged beef during chilled storage conditions

b) To determine the impact of edible wrapping treatment (bacterial cellulose) on moisture loss and accumulation inside vacuum-packaged beef cuts during chilled storage conditions

c) To produce the nanocrystals from the most effective coating or wrapping material and to assess their physicochemical properties and antimicrobial activity

d) To determine the effectiveness of nanocrystal spray coating on controlling moisture loss from beef

This thesis consists of six chapters written in the format of one review paper, four research papers and finally, a general conclusion and suggestions for future work.

Chapter 2 provides a comprehensive review of the impact of current and emerging post-slaughter treatments on the moisture loss of red meat. This chapter also briefly discusses the mechanisms related to purge and drip formation and the issues faced by the red meat industry due to these losses.

Chapter 3 describes the impact of applying gelatine (10%) and chitosan (1%) as spray coatings to extend the shelf life of vacuum-packaged beef and compares the effectiveness of these coatings against uncoated and vacuum-packaged beef. The impact of gelatine and chitosan coatings on purge loss and drip loss were assessed together with low frequency nuclear magnetic resonance (LF-NMR) analysis. The

effect of these coatings on the physicochemical, microbiological and sensorial characteristics of meat was investigated. Gelatine and chitosan were not effective in controlling purge loss as the storage time progressed.

Chapter 4 investigates the effect of applying bacterial cellulose (BC) as a wrapping material in controlling purge accumulation in vacuum-packaged beef. The effect of BC was compared against unwrapped meat in controlling purge loss, drip loss along with LF-NMR, physicochemical, microbiological and sensorial evaluations. BC was effective in controlling purge accumulation and therefore, morphological, crystallinity and purge absorptivity measurements were carried out to understand its mechanism in controlling purge accumulation. Results indicated that BC enhanced the microbial growth by acting as a substrate for microbial attachment that could be mitigated by loading BC with a food-grade antimicrobial agent.

Bacterial cellulose was chosen as the most effective material in controlling purge loss based on the results of chapter 3 and 4. Hence, in chapter 5, bacterial cellulose nanocrystals (BCNs) and a series of nisin-loaded BCNs (NBCNs) were produced using the complexation method in order to test their applicability as nanoparticle spray coatings. All nanocrystals were assessed for their size, zeta-potential, nisin encapsulation efficiency, storage stability and antimicrobial activity. The most effective NBCNs were selected and they were further characterized for their morphology, crystallinity and functionality.

Based on the results of chapter 5, the most effective concentrations of BCNs and NBCNs were chosen to develop spray coating systems. Chapter 6 investigates the effectiveness of applying BCNs and NBCNs as spray coating before the beef was vacuum packaged. The shelf life study was carried out and compared against vacuum-packaged beef only. The nanocrystals were not effective in controlling purge loss and Kelvin probe force microscopy and zeta-potential analysis was carried out to determine the possible justifications for their ineffectiveness in controlling purge loss.

Chapter 7 outlines the conclusions derived based on the results of chapters 3 to 6 and the future outlook for developing a more efficient system for controlling moisture loss from vacuum-packaged beef.

Chapter 2. Impact of Current and Emerging Post-slaughter Treatments on Moisture Loss by Red meat: A Review

Abstract

Moisture loss is associated with a reduction in meat quality which can in turn reduce profits for red meat processors. Financial losses may be specifically due to a reduction of saleable product yield, damage to export markets and consumer dissatisfaction because of poor tenderness, juiciness and unattractive product appearance. A number of post-slaughter treatments which are applied during red meat processing and packaging can affect purge and drip formation in raw red meat products. The key post-slaughter factors which affect purge loss and drip loss are chilling, freezing and thawing, aging, injection of non-meat ingredients and packaging. Furthermore, novel techniques are being tested in red meat processing lines to improve the quality of the meat cuts. These include edible coating applications and meat tenderization techniques such as SmartStretch™, Pi-Vac® Elasto-Pack system, pulsed electric field (PEF), high pressure processing (HPP) and ultrasound treatments. This review provides an overview of recent research on both current and emerging post-slaughter treatments that affect the moisture loss of beef, lamb, mutton, deer, buffalo and goat raw meat products. It also characterizes purge and drip formation mechanisms associated with each treatment and emphasizes better processing and packaging practices, which will help reduce the moisture loss.

2.1 Introduction

Lean meat is made up of approximately 75% water (Brewer, 2014). A lean muscle consists of muscle bundles that are composed of muscle fibres. Each muscle fibre is composed of myofibrils. The repeating contractile unit of a myofibril is the sarcomere which consists of two types of myofilaments longitudinally oriented and ordered in a regular array (Den Hertog-Meischke, Van Laack, & Smulders, 1997). Thick filaments predominantly consist of myosin protein, while thin filaments mainly consist of actin protein. About 85% of myowater is held in the intra-myofibrillar network which is the space between thick and thin filaments. The remaining myowater is located in the extra-myofibrillar network which is either in between myofibrils or muscle fibres (Pearce, Rosenvold, Andersen, & Hopkins, 2011).

The ability of the post-mortem muscle to maintain its water content even under the application of external pressure is referred to as water-holding capacity (WHC) (Huff-Lonergan, 2006). One of the key challenges faced by the meat industry is preventing the loss of moisture from lean meat tissue during storage. When a muscle is cut, a red aqueous solution of proteins oozes from the cut surfaces and accumulates in the package. This moisture loss is known as the purge or weep loss. If the meat is hung in a closed container for a specific period at refrigeration temperature, the resulting moisture loss is known as drip loss (Honikel, 1998). The openings of channels through which purge could be lost and excessive purge accumulation in vacuum-packaged beef are illustrated in Figure 2.1. Purge or drip mainly consists of sarcoplasmic proteins, with glycolytic enzymes and myoglobin which gives meat its red colour (Offer & Cousins, 1992; Offer et al., 1989).

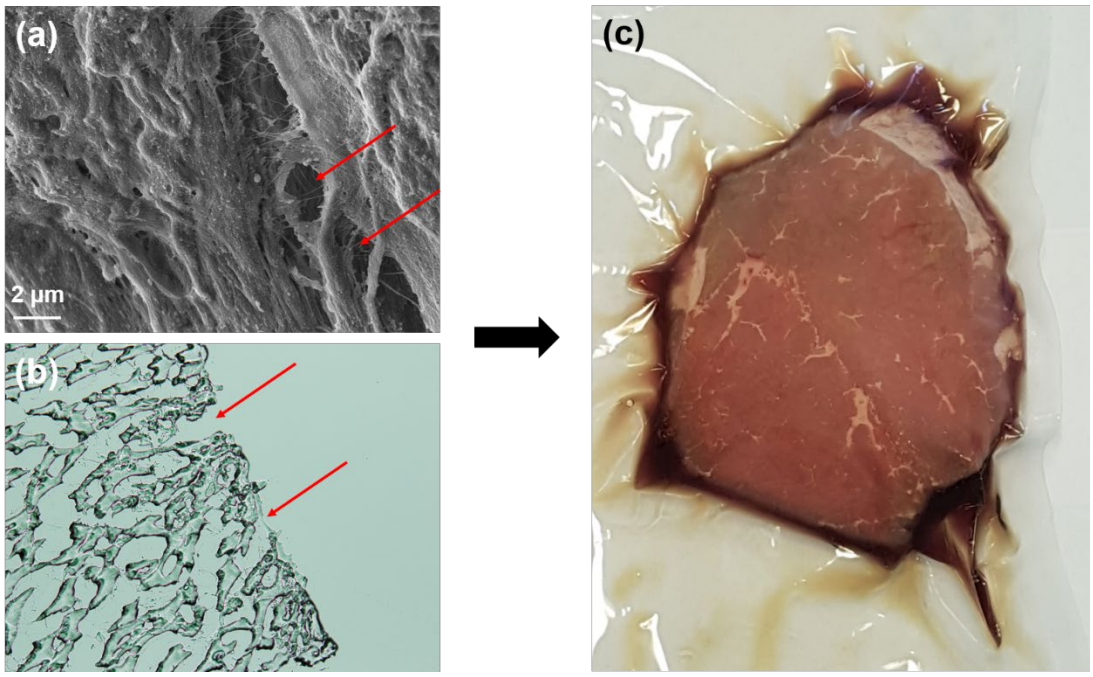

Figure 2.1 SEM image (a) and bright filled light microscope image (mag. x 100) (b) of the transverse section of bovine eye round showing channel openings through which purge could be lost and excessive purge in vacuum-packaged beef eye round steak aged for 5 weeks (c). Red arrows point to channel opening.

Purge formation occurs due to three key events: net charge effect, steric effect, and post-mortem proteolysis or ageing. The net charge effect involves muscle conversion to meat. During conversion, oxygen supply ceases, leaving the cells to rely on

anaerobic glycolysis to generate energy and maintain cellular integrity. The conversion of glycogen to lactic acid leads to a drop in muscle pH from approximately 7.0 –7.2 to 5.0 which equals the isoelectric point (P_I) of key proteins such as myosin and actin (Offer et al., 1989). At P_I, the positive and negative charges of proteins are equal which means the proteins are zwitterionic. Neutralizing protein charge causes a loss in their WHC and reduces the inter-myofibrillar space. This leads to expulsion of water into the extra-myofibrillar spaces, where it is finally lost from the muscle cell (Den Hertog-Meischke et al., 1997; Huff-Lonergan, 2009; Huff-Lonergan & Lonergan, 2005). Steric effects involve the rigour mortis process. At the onset of rigour mortis, actomyosin cross-bridges form between myofilaments, leaving less space for water located in the extra-myofibrillar space (Den Hertog-Meischke et al., 1997; Huff-Lonergan & Lonergan, 2005; Offer et al., 1989). Additionally, with rigour development, sarcomeres can shorten, resulting in water expulsion within the myofibril due to less space available.

Ageing is the process of storing meat post-rigour at chilling temperatures, either to improve meat quality or to supply distant markets (Farouk, Mustafa, Wu, & Krsinic, 2012). The endogenous enzymes known as ‘calpains’ degrade two types of proteins during the ageing process (Honikel, 2014). The first type of proteins that form the intermediate filaments is desmin, titin, nebulin, and vinculin. The second type, known as costameres, link the adjacent myofibrils or myofibrils with cell membranes. Early post-mortem degradation of these proteins releases the constraints within the cell. It increases the space available for water, which in turn improves WHC (Huff-Lonergan & Lonergan, 2005; Pearce et al., 2011). When the proteins remain intact, myofibril shrinkage will affect the whole cell and cause gaps between both muscle cells and bundles. This mechanism forms the drip channels, allowing purging of the drip from the meat (Kristensen & Purslow, 2001; Offer & Cousins, 1992). The degradation of another type of protein, known as integrin, contributes to the drip channel formation resulting in more significant drip loss (Lawson, 2004; Straadt, Rasmussen, Young, & Bertram, 2008; Zhang, Lonergan, Gardner, & Huff-Lonergan, 2006). Integrin proteins form the connections between the extracellular matrix and the cell cytoskeleton.

Excess purge results in financial losses in various ways, including reducing saleable weight, loss of export markets, and dissatisfied consumers. Certain export markets

such as Egypt and Saudi Arabia have rejected Australian meat in the past, claiming that the product is no longer wholesome due to the presence of purge (Barlow, McMillan, & Stark, 2016). Purge imparts a greater negative impact on package appearance (Cheng & Sun, 2008; Offer & Cousins, 1992), product juiciness and tenderness (Warner, 2017), thus reducing consumer acceptability and consequent sale value (Cheng & Sun, 2008; Rooyen, Allen, Kelly-Rees, & O'Connor, 2018). In addition, purge creates an excellent growth substrate for both spoilage and pathogenic microorganisms as it contains sarcoplasmic proteins, glycolytic enzymes and myoglobin (Offer et al., 1989). This may jeopardize consumer safety and product shelf life (Lagerstedt, Lundstrom, & Lindahl, 2011).

The generic processes that take place at abattoirs such as stunning, evisceration, electrical stimulation, carcass suspension and boning have not been captured under this review as these processes have been extensively reviewed previously. This review only focuses on post-slaughter treatments that are carried out at meat processing and packaging facilities to manufacture raw red meat products. Chilling, freezing, ageing, injecting non-meat ingredients and packaging are the key post-slaughter processes that impact purge and drip formation during processing and packaging of raw red meat cuts. Meat tenderization techniques and edible coating applications can be considered as emerging post-slaughter treatments which are currently being tested in red meat processing and packaging lines to improve the quality of the meat. Novel meat tenderization techniques include SmartStretch™, Pi-Vac® Elasto-Pack system, pulsed electric field (PEF), high pressure processing (HPP) and ultrasound treatments. These techniques also impact the purge and drip formation and a proper understanding of these processes will be vital in improving meat quality without causing any detrimental effects.

A comprehensive understanding of the factors which can impact moisture loss during meat processing and packaging is critical in preventing excess fluid loss and subsequent negative impacts on the red meat industry. This review aims to critically evaluate the impact of both current and emerging post-slaughter treatments carried out at meat processing and packaging facilities on purge and drip formation and propose control measures that can reduce moisture loss of red meat cuts, lamb, mutton, buffalo, goat and deer.

2.2 Current post-slaughter treatments

The first factor which influences consumers to purchase a meat cut is its physical appearance. Excess purge in the meat packaging makes the product unattractive to consumers (Hope-Jones, Strydom, Frylinck, & Webb, 2012). Various post-slaughter processes applied during meat processing and packaging such as chilling, freezing and thawing, ageing, injecting non-meat ingredients, and packaging techniques (Figure 2.2) affect the WHC of meat which leads to purge and drip losses.

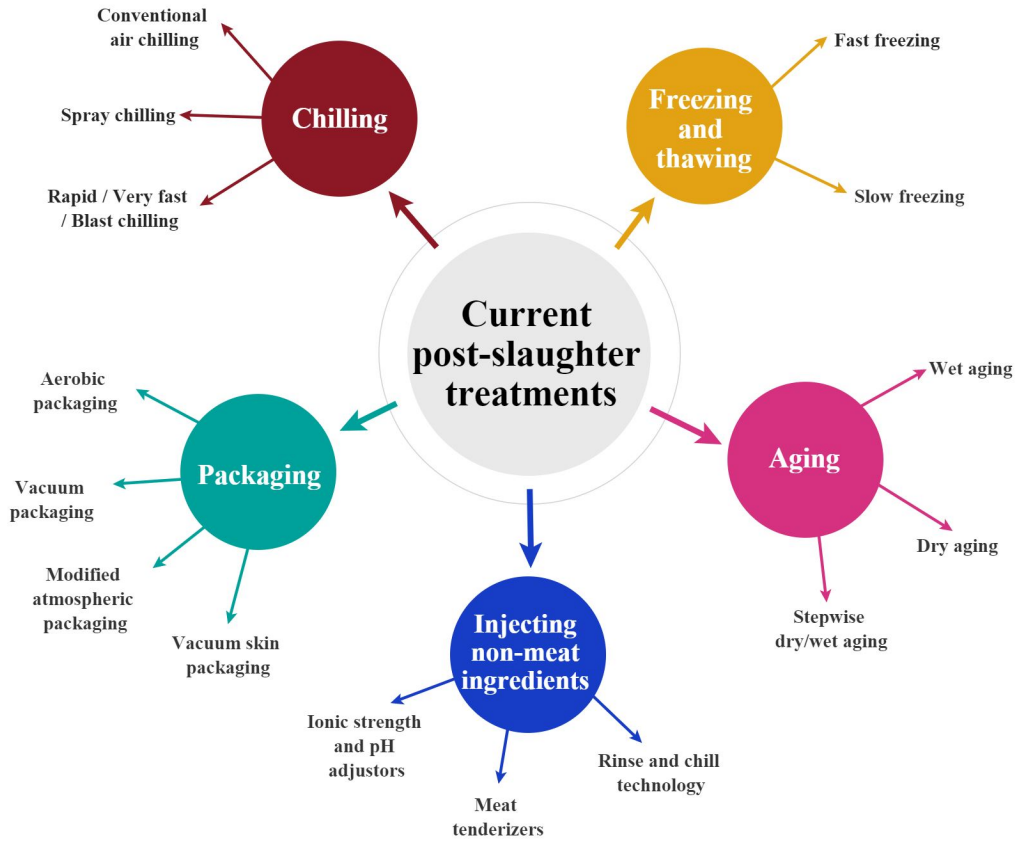

Figure 2.2 Current post-slaughter treatments

2.2.1 Chilling

Post-mortem chilling of red meat carcasses is primarily employed to ensure food safety and maximize shelf life. According to Savell, Mueller, and Baird (2005), employing chilling parameters that minimize cold shortening of beef and lamb is essential and

can be addressed by ensuring that muscle temperature is not below 10°C before the pH reaches 6.2. Conventional air chilling (CC) involves subjecting the carcasses to a temperature of 0 ± 1°C, relative humidity of 85 – 90% and an air velocity of 1-4 m/s for a duration of 18 to 24 h (Mesquita et al., 2003). Conventional air chilling also controls the factors which can promote or inhibit cold shortening. There are additional chilling techniques such as spray chilling, rapid chilling and delay chilling which can alter the impact of cold-induced shortening on meat carcasses. Chilling is responsible for moisture loss due to both evaporative loss and purge loss. Table 2.1 presents information regarding the previous work which has been conducted to evaluate the effect of different chilling treatments on moisture loss of red meat.

Spray chilling (SC) is the process of intermittent spraying of cold water onto carcasses during the first 3 to 8 h post-slaughter (Hippe, Field, Ray, & Russell, 1991). The effectiveness of SC in reducing moisture loss during chilling is well established by studies comparing SC versus CC in beef (Kinsella et al., 2006; Mesquita et al., 2003; Prado & de Felicio, 2010; Unruh, Montgomery, Garcia, & Brown, 2003), in lamb (Brown, Chourouzidis, & Gigiel, 1993) and venison (Wiklund, Kemp, & Wu, 2010). All these studies reported that spray chilled sides lost significantly less weight during chilling than conventionally chilled sides. Therefore, the effect of SC on evaporative loss is irrespective of the red meat type and muscle type. According to Wiklund et al. (2010), air chilling removes muscle surface moisture by evaporation, while SC prevents evaporation of muscle surface moisture by allowing moisture to evaporate from the wet surface created by water spraying. Therefore, in air chilling, evaporated surface moisture is replaced by internal muscle moisture. In SC, internal moisture is preserved and 'natural' moisture content is stabilized compared to air chilling. However, some differences in evaporative loss exist between studies which could be related to different parameters utilized by SC systems such as rate of spray cycle, cycle duration and spray volume. Strydom and Buys (1995) had significantly higher saving in evaporative loss (1.10%) compared to the saving in evaporative loss (0.19%) reported by Kinsella et al. (2006). The significant differences in evaporative loss savings could be due to major differences in spray volumes used by two studies in which Kinsella et al. (2006) and Strydom and Buys (1995) used spray volumes of 1.33 l/h and 40.2 l/h, respectively. Only a few studies have reported the spray volume used in their studies. It is important to mention spray volume in future studies since it will

help red meat processors set their SC parameters to achieve a minimal evaporative loss and maximize process efficiencies.

Evaluation of water losses that may occur following the SC process, when the meat has already been packaged, is also important to see whether SC causes the same effect with the ageing of meat. Strydom and Buys (1995), Greer and Jones (1997) and Unruh et al. (2003) in beef, Wiklund et al. (2010) in venison and Brown et al. (1993) in lamb found no significant differences in moisture losses after different ageing periods as mentioned in Table 2.1. In contrast, Prado and de Felicio (2010) observed significantly ($P < 0.05$) higher purge loss in sprayed sides compared to non-sprayed sides of beef striploin. No significant differences in other physiological factors (such as pH and sarcomere length) were reported between sides subjected to spray and air chilling in this study. Based on the results of different red meat studies, it is clear that SC can reduce evaporative loss and has no influence on purge loss.

Mechanisms used to rapidly cool carcasses are termed as “rapid”, “very fast”, and “blast” chilling systems (Savell et al., 2005). There is no consistent definition used by authors in defining these systems. In this review, the same terminology is used rather than standardizing it.

Drip loss during vacuum storage has been reported to decrease after rapid chilling (RC) of bull meat (Li et al., 2006), fast chilling (FC) of meat of Chinese yellow cattle (Liu et al., 2015) and beef (Sikes, Jacob, D'Arcy, & Warner, 2017) in comparison to conventionally chilled control samples. Sikes et al. (2017) stated that this difference is mainly due to the slower pH decline rate and higher ultimate pH of the very fast chilled samples compared to the control. An increase in intracellular osmotic pressure could also account for this increased WHC of chilled samples. Raised osmotic pressure may be caused by the bond splitting between myofibrillar protein aggregates and the breakdown of sarcoplasmic proteins to amino acids (Sikes et al., 2017). These findings are in agreement with the study conducted on beef muscles by Aalhus, Janz, Tong, Jones, and Robertson (2001), who reported that drip loss in the *longissimus lumborum* was significantly lower ($P < 0.01$) in blast chilled carcasses when compared with conventionally chilled carcasses. However, in the same study, no difference in drip loss was observed for the *semimembranosus* muscle. This finding is similar to the findings of Hildrum, Solvang, Nilsen, Frøystein, and Berg (1999) and Jacob and

Thomson (2012), who found there is no effect of RC on the drip loss of bull meat and FC on the drip loss of lamb meat. Jacob and Thomson (2012) also reported that there was an effect of muscle type ($P < 0.01$) on drip loss. Fast chilled *semitendinosus, semimembranosus* and *longissimus dorsi* muscles have shown an increasing order in drip loss. This may have been influenced by the decreasing order of sarcomere lengths shown by these three muscles. These results suggest that FC treatments may affect the different muscle structures differently and cause differences in drip loss measurements.

In contrast, very fast chilled beef (chilled at -21°C until the core temperature reached 0°C; Li et al., 2012) has had significantly ($P<0.05$) high purge loss at 1 d post-mortem compared to the samples chilled at 14°C for 10 h. This increase in purge loss may be caused by the calcium-induced shrinkage of myofibrils and the extent of shrinkage during rigour development (Marsh, Cassens, Kauffman, & Briskey, 1972). This resulted in cold-shortened muscles exhibiting increased drip loss in early post-mortem stages (Li et al., 2012; Sikes et al., 2017). Similar results were reported by Aalhus, Robertson, Dugan, and Best (2002) who studied blast chilling (BCL) treatment at different temperatures (-20°C, and -35°C) for different durations (3, 5, 7 or 10 h). They reported that BCL displayed increased drip loss after 10 h of BCL at -20°C, and after 7 h and 10 h of BCL at -35°C compared to the control chilled sample. Blast chilled carcasses stored for up to 7 h at -20°C and up to 5 h at -35°C resulted in no significant difference in drip loss compared to the control samples. The authors concluded that extreme chilling regimes negatively influence drip loss, while moderate chilling regimes cause no significant impact on drip loss. Moderate BCL conditions only cause surface freezing of the fat layer of the carcass thereby blocking the moisture loss. Conversely, extreme BCL conditions may extend into the muscle and cause mechanical damage to the membranes by forming ice crystals. In addition, these conditions may alter the stability of myofibrillar proteins and altogether reduce the ability to hold water in the muscle (Aalhus et al., 2002).

Application of rapid chilling systems to carcasses may result in cold-induced toughening and compromised meat quality (Aalhus, Tong, Robertson, & Jones, 1991; Koohmaraie, Doumit, & Wheeler, 1996; Li et al., 2006). Many authors (Aalhus et al., 2001; Janz, Aalhus, & Price, 2001; Li et al., 2006) have suggested using these systems

in conjunction with ES because of its ability to accelerate the onset of rigour. A few studies have also suggested that RC (Li et al., 2006) and BCL (Aalhus et al., 2001) were able to reduce the increased drip loss associated with ES.

Table 2.1 Summary of research to examine the effect of chilling on the moisture loss of red meat

Meat	Muscle type	Measurement	Chilling treatments	Ageing period	Main findings	Reference
Lamb	*LD* [a]	Drip loss %	Conventional chilling versus two spray chilling treatments (Double spray, and multiple spray)	5 d	No significant effect of chilling treatment on drip loss	(Brown et al., 1993)
Beef	*LT* [b]	Mass loss	Conventional chilling versus spray chilling. Spray chilling for different durations (10, 14, and 17 h)	3, and 7 d	No significant effect of chilling treatment on mass loss	(Strydom & Buys, 1995)
Steer	*LT* [b]	Weight loss	Conventional air chilling versus spray chilling. Spray chilling for different durations (4, 8, 12, and 16 h)	2, 16, 30, and 44 d	No significant effect of chilling treatment on weight loss	(Greer & Jones, 1997)
Bull	*LD* [a]	Drip loss %	Conventional chilling versus rapid chilling	6 d	No significant effect of chilling treatment on drip loss	(Hildrum et al., 1999)
Heifers	*LL* [c] *and SM* [d]	Drip loss (mg/g)	Conventional chilling, blast chilling, ES and blast chilling	6 d	Blast chilling caused reduced drip loss for *LL*, but no significant effect on *SM*	(Aalhus et al., 2001)
Beef	NA[1]	Drip loss (g/kg)	Control chilling versus blast chilling at different temperatures, and for different durations (at -20°C, and -35°C for 3,5, 7, and 10 h)	4 d	Blast chilling caused higher drip losses for the cuts chilled at -20°C for 10 h and at -35°C for 7 and 10 h. But no significant effect of chilling rate on drip losses for the cuts chilled at -20°C for 7 h and at -35°C for 5 h	(Aalhus et al., 2002)

Meat	Muscle type	Measurement	Chilling treatments	Ageing period	Main findings	Reference
Beef	NA[1]	Weight loss %	Conventional chilling versus spray chilling	1 d	Spray chilled cuts had significantly lower evaporative loss than air-chilled cuts	(Mesquita et al., 2003)
Heifers	NA[1]	Purge loss %	Conventional chilling versus spray chilling	7 d	No significant effect of spray chilling on purge loss	(Unruh et al., 2003)
Bull	*LM*[e]	Purge loss %	No ES and conventional chilling, no ES and rapid chilling, ES and conventional chilling, ES and rapid chilling	1 d	Rapidly chilled cuts had significantly lower purge loss than conventionally chilled cuts	(Li et al., 2006)
Beef	NA[1]	Weight loss %	Conventional chilling versus spray chilling	1 d	Spray chilled cuts had significantly lower evaporative loss than air-chilled cuts	(Kinsella et al., 2006)
Beef	*LL*[c]	Purge loss %	Conventional air chilling, conventional spray chilling, slow air chilling and slow spray chilling	7, 14, 30 and 60 d	Spray chilled cuts had significantly higher purge loss than air-chilled cuts	(Prado & de Felicio, 2010)
Red deer	*LD*[a]	Purge loss %	Control air chilling versus spray chilling	21, and 63 d	No significant effect of chilling treatment on purge loss	(Wiklund et al. 2010)
Lamb	*LD*[a], *SM*[d] and ST[f]	Drip loss %	Control air chilling versus fast chilling (0°C in 5 h)	1 d	No significant effect of chilling treatment on drip loss	(Jacob & Thomson, 2012)
Chinese yellow cattle	*LL*[c]	Purge loss %	Very fast chilling (-21°C) and chilling at 0°C, 7°C, and 14°C	1, 7, 14, and 21 d	Very fast chilled cuts had significantly higher purge loss than conventionally chilled cuts at 1 d pm	(Li et al., 2012)

Meat	Muscle type	Measurement	Chilling treatments	Ageing period	Main findings	Reference
Chinese yellow cattle	*LL* [c]	Purge loss %	Stepwise chilling (Fast chilling for 2 h followed by chilling at 1±1°C for 48 h) versus conventional chilling	1, 7, and 14 d	Stepwise chilled cuts had significantly lower purge loss than conventionally chilled cuts	(Liu et al., 2015)
Beef	NA[1]	Drip loss %	Conventional chilling, delayed chilling, immediate chilling (Very fast chilling)	2, 5, and 14 d	Immediate chilled cuts had lower purge loss (but not significant) than conventionally chilled cuts	(Sikes et al., 2017)

[a] *longissimus dorsi;* [b] *longissimus thoracis;* [c] *longissimus lumborum;* [d] *semimembranosus;* [e] *longissimus;* [f] *semitendinosus.* [1] Data was not shown.

2.2.2 Freezing and thawing

Freezing is one of the most effective methods used in the red meat industry to prolong the shelf life of meat products. Freezing of meat allows wholesalers and retailers to achieve longer storage periods, allowing greater flexibility in inventory handling than chilling of meat (Setyabrata & Kim, 2019). Freezing negatively affects meat WHC and tenderness due to the damage it causes to the cell membrane by ice crystal formation (Lagerstedt, Enfalt, Johansson, & Lundstrom, 2008). Vieira, Diaz, Martinez, and Garcia-Cachan (2009) and Aroeira et al. (2016) studied the impact of frozen conditions on vacuum-packaged beef stored for 90 d and 40 d, respectively. Both studies reported that freezing and subsequent thawing increased the exudate loss from meat. These results support the findings of Kandeepan and Biswas (2007) who reported an increase in drip loss in buffalo meat stored for 75 d at -10 ºC. Similar results were reported in a study conducted to evaluate the impact of extended frozen storage for 21 months on lamb meat (Muela, Monge, Sanudo, Campo, & Beltran, 2015; Muela, Sanudo, Campo, Medel, & Beltran, 2010). Muela et al. (2010) found lamb *Longissimus thoracis et lumborum* (*LTL*) muscle stored at -18ºC for 1 month showed lower thawing loss values than meat exposed to the same conditions for 3 or 6 months. Research on the same muscle type stored at similar freezing conditions for 21 months further confirmed that thawing losses significantly increased with frozen storage duration (Muela et al., 2015). Similar to the above findings, Daszkiewicz et al. (2018) reported that frozen/thawed lamb meat from the *LTL* muscle was characterized by significantly high drip loss compared to the fresh meat. They have frozen meat at -26ºC for 6 or 12 months.

Freezing causes ice crystal formation which disrupts the integrity of the cell membrane resulting in the release of myowater exudates from the intracellular to the extracellular region (Aroeira et al., 2016). With the long term frozen storage, the size of the ice crystals increases, increasing the cellular damage which might be the reason for the marked increase in thaw loss with the extended storage time (Muela et al., 2015). Shortening of the sarcomere, increased enzyme activity (Kandeepan & Biswas, 2007), protein oxidation and a shift in the P_I of key muscle proteins (Muela et al., 2015) might be the other reasons for the noticeable increase in moisture loss during longer frozen storage.

The impact of freezing on moisture loss of red meat may vary with the different methods and rates used for freezing. Muela et al. (2010) studied the effect of different freezing methods such as blast air freezing, tunnel freezing and liquid nitrogen freezing on the quality of lamb meat and found no relationship between thawing loss and the freezing technique as well as the quick freezing rate. Kim, Liesse, Kemp, and Balan (2015) reported that fast freezing resulted in lower purge loss values of beef loins than slowly frozen counterparts. The lower purge loss could be due to less physical damage to the fibre cells and chemical changes to the proteins caused by small ice crystal formation through fast freezing (Mateo-Oyague & Perez-Chabela, 2004). In contrast, slow freezing disrupts muscle fibres and cause shrinkage of cells through large ice crystal formation (Kim et al., 2015), resulting in more significant purge loss.

Though freezing extends the shelf life of meat, it also causes considerable quality degradation and water loss upon thawing due to extracellular cryo-damage. Many studies reported that water loss could be minimized by ageing meat before freezing (Coombs, Holman, Collins, Friend, & Hopkins, 2017; Farouk, Wiklund, Stuart, & Dobbie, 2009; Kim et al., 2015; Wiklund et al., 2009). Farouk et al. (2009) determined that the longer the beef *semimembranosus* muscle was aged at -1.5 ºC before being frozen and thawed, the lower the amount of water lost from the meat as purge. This observation was supported by drip loss findings in lamb *longissimus lumborum* muscle samples that were aged at -1.5 ºC for different durations (2 and 3 weeks) before freezing at -18 ºC (Kim, Luc, & Rosenvold, 2013). Wiklund et al. (2009) reported that purge loss percentage values were lower in aged-then-frozen lamb loin samples as compared to aged-never-frozen samples. This might be due to the lower level of muscle structural protein disintegration caused by ageing before freezing. This disintegration may neutralize the negative impact of freezing on water retention (Farouk et al., 2009; Wiklund et al., 2009). Initial ageing creates a buffering effect against the physical damage caused by freezing through the degradation of costamere linkages and subsequent swelling of muscle fibres (Kim, Kim, Seo, Setyabrata, & Kim, 2018). Choe, Stuart, and Kim (2016) and Holman, Coombs, Morris, Kerr, and Hopkins (2017) reported there was no significant difference in purge loss between aged-then-frozen and aged only samples of lamb and beef loins, respectively. This may be due to the differences in water retention capacities of muscle structural proteins rather than

the fibre or cell damage caused by ice crystal formation (Grayson, King, Shackelford, Koohmaraie, & Wheeler, 2014; Lagerstedt et al., 2008).

Setyabrata and Kim (2019) conducted a study recently to determine the impact of ageing/freezing sequence on WHC of beef *longissimus lumborum* and *semitendinosus* muscles. They subjected the meat to 3 different treatments (ageing only, ageing then freezing and freezing then ageing). Samples subjected to freezing then ageing has had the highest purge loss followed by the samples subjected to ageing then freezing and ageing only treatments. Significantly higher purge loss reported by frozen-then-aged samples might be due to the expansion of drip channels by freezing at early post-mortem, as evidenced by the histology results. Most importantly, these results have been shown in the samples from both muscle tissues. This study indicates the importance of ageing before freezing in retaining WHC of frozen red meat cuts.

Current studies have mainly examined the impact of freezing then thawing and ageing before freezing on the moisture loss of beef and lamb. Future research should focus on investigating these approaches on the moisture loss of goat and deer meat. Little research has been carried out to determine how moisture loss varies with different freezing methods and freezing rates, and further trials should be conducted to assess this. It is hypothesized that the rate of thawing may also impact the degree of moisture loss. Leygonie and Hoffman (2020) found out that the thawing rate had no effect on the drip loss of the ostrich moon steaks. However, as this study was performed using ostrich meat, it is unclear if these results are an accurate comparison of the effects of thawing rates in red meat. None of the studies has evaluated the impact of the rate of thawing on moisture loss of red meat. Future studies should focus on this gap and the findings will help red meat processors to optimize the way they carry out their thawing operation.

2.2.3 Ageing (post-mortem proteolysis)

The ageing of red meat has become an essential practice in the red meat industry to satisfy the consumers' high expectations (Laster et al., 2008). Ageing involves storing meat at refrigerated temperatures for sufficient time to improve meat palatability characteristics such as flavour, tenderness, and juiciness (Campbell, Hunt, Levis, & Chambers, 2001). The most common ageing methods are known as dry ageing and

wet ageing. Wet ageing is the most commonly practised method in the meat industry and refers to storing meat cuts in vacuum packages at refrigerated temperature (Laster et al., 2008). In contrast, dry ageing refers to storing unpackaged meat under controlled temperature and humidity conditions (Smith et al., 2008).

Previous studies evaluated the impact of wet ageing (Irurueta, Cadoppi, Langman, Grigioni, & Carduza, 2008) and dry ageing (de Huidobro, Miguel, Onega, & Blázquez, 2003) on bovine muscles and concluded that WHC significantly increased during the respective ageing periods. Marino et al. (2014) investigated wet ageing of meat from three cattle breeds and found that extended ageing resulted in lower drip loss values in meat aged for 21 d compared with meat aged for 1 d ($P < 0.01$). A recent study conducted by Crivelli, Tirloni, Bernardi, Rossi, and Stella (2019) also reported improved WHC in short-term wet-aged beef muscles. The significant increase in pH over the ageing period may have increased the water-binding capacity of muscle. Ageing induced increase in pH can occur due to the proteolytic enzymes which cause cellular membrane leakage and increased ion migration, resulting in increased net protein charge (Boakye & Mittal, 1993). In addition, during the post-mortem ageing structure of the muscle loosen due to the degradation of intramuscular collagen (Purslow, 2005), myofibrillar and cytoskeletal proteins (Huff-Lonergan & Lonergan, 2005). This increases the accessibility of water to capillary spaces with higher availability of hydrophilic sites, thus improving meat WHC due to ageing.

Farouk et al. (2012) reported that the WHC of *semimembranosus* meat samples of bovine increased with the progress of the wet ageing period. This was evidenced by a decline in drip loss of bovine *M. semimembranosus* muscles stored at -1.5 ºC measured centrifugally, gravimetrically, and applying pressure to meat (Farouk et al., 2012). The explanation for this phenomenon given by Farouk et al. (2012) is quite different compared to the explanation for drip loss given by Huff-Lonergan and Lonergan (2005). This explanation is known as the “sponge effect” hypothesis and is based on the idea that when proteolysis occurs, muscle structural proteins are broken and the drip channels are disrupted. This creates a “sponge effect” due to the hindrance caused by debris falling into the drip channels. The water is physically entrapped and water loss by gravity is reduced. The formation of a more viscous drip due to the increased amount of dissolved solids and soluble proteins enhances the “sponge effect” by

slowing down the drip flow within the drip channels. Results of previous studies conducted by Farouk et al. (2007) and Farouk et al. (2009) showed that ageing for longer periods resulted in improved WHC of venison meat.

In contrast, several studies have reported increased drip loss of wet-aged beef stored at 2 - 4 °C for 14 to 21 d (Florek, Litwinczuk, Skalecki, & Ryszkowska-Siwko, 2007; Kim & Lee, 2003; Li et al., 2012; Waritthitham, Lambertz, Langholz, Wicke, & Gauly, 2010). Garssen, Geesink, Hoving-Bolink, and Verplanke (1995) and Ludwiczak, Stanisz, Bykowska, Składanowska, and Ślósarz (2017) reported increased drip loss with wet ageing of *longissimus lumborum* sections of veal calves and decreased WHC with wet ageing of *semimembranosus* muscles of fallow deer, respectively. Several recent studies also reported significantly increased purge losses over long term wet ageing of different beef muscles (Colle et al., 2015; Holman, Bailes, Kerr, & Hopkins, 2019) as well as game meat (Needham, Laubser, Kotrba, Bureš, & Hoffman, 2020). Needham et al. (2020) have also observed an increase in drip loss over ageing. In addition, Utama et al. (2020) reported significantly decreased WHC in dry-aged beef muscles over a 50 d ageing period.

The increase of drip loss and purge loss with ageing could be due to the susceptibility of meat to develop protein oxidation and denaturation (Kim et al., 2018). Ageing induced protein oxidation causes the formation of carbonyls and sulfhydryl groups, leading to loss of functional groups and results in protein disulphide cross-linking (Kim et al., 2018). This diminishes protein functionality and negatively affects muscle WHC. Besides, denaturation of muscle proteins occurs with meat ageing which reduces their ability to hold water molecules (Wiklund, Stevenson-Barry, Duncan, & Littlejohn, 2001). The loss of protein tertiary structure exposes the previously folded residuals of hydrophobic amino acids and negatively influences the muscle WHC. However, none of the above studies has shown the statistical correlation between decreased WHC and protein oxidation or denaturation. This is important to consider in future red meat ageing studies to provide a comprehensive explanation of the mechanism behind decreased WHC with ageing.

A few studies have evaluated the impact of dry ageing versus wet ageing on weight loss. Lepper-Blilie, Berg, Buchanan, and Berg (2016) have reported wet-aged beef loins lost significantly ($P < 0.001$) less weight compared to dry-aged loins which were

stored for 49 d post-mortem. In this study, weight loss has been interpreted as a combination of both purge and trimming loss. Another study established a more significant initial weight loss in dry-aged beef loins than their wet-aged counterparts (Kim, Kemp, & Samuelsson, 2016). Hastie et al. (2019) recently conducted a study to determine the impact of dry ageing versus wet ageing of two different mutton primal cuts and found significantly lower weight loss in wet-aged cuts compared to dry-aged cuts. Furthermore, Li, Babol, Wallby, and Lundstrom (2013) and Prieto et al. (2017) have found significantly low weight loss in wet-aged beef muscles compared to bag dry-aged counterparts. These dry ageing bags have a high water transmission rate that may simulate traditional dry ageing and allow the red meat processors to dry age meat without adapting the plant's temperature and relative humidity conditions (Prieto et al., 2017). Dry ageing causes gradual dehydration of muscles due to hanging in the open air, resulting in increased weight loss due to both dehydration and trimming. On contrarily, wet ageing results in significantly lower weight loss than dry ageing due to the absence of dehydration and trimming losses.

Stepwise dry/wet ageing is a modified dry ageing method in which the carcass is first dry-aged followed by wet ageing of sub-primals after the separation process (Kim et al., 2018). Kim, Meyers, Kim, Liceaga, and Lemenager (2017) evaluated dry ageing against stepwise dry/wet ageing. In stepwise dry/wet ageing, carcasses were initially dry-aged for 10 d followed by wet ageing for another 7 d. They reported that stepwise dry/wet-aged beef loins exhibited a lower amount of water loss than dry-aged loin samples. Zhang, Yoo, Mungure, Bekhit, and Farouk (2018) also reported similar results in their study which assessed bag dry ageing against stepwise bag dry/wet ageing of beef muscles. They also evaluated the impact of different air velocities (0.5, 1.5 and 2.5 m/s) in quality parameters of samples subjected to stepwise ageing treatments and reported significantly increased weight loss with the increase of air velocities. This could have been due to the accelerated surface drying of muscles due to increased air velocities. Another study conducted by de Faria Vilella et al. (2019) also reported supporting results to the above studies. They have found the lowest total moisture loss in wet-aged beef cuts, followed by dry/wet, then wet/dry and dry-aged alone ($P < 0.05$). Dry ageing associates with cooler shrink and trimming and causes lower total product yield during the fabrication process. In addition, the absence of packaging in dry ageing may cause more protein oxidation compared to the wet-aged

meat samples, thus resulting in higher weight loss in dry-aged samples (Kim et al., 2017).

Therefore, a novel approach of combining dry ageing with wet ageing may help to reduce the loss of saleable product weight while maintaining the positive eating quality attributes associated with meat dry ageing. However, further research is required to identify the optimal processing combinations of stepwise ageing treatments to capitalize on the positive aspects of this treatment. The current research is limited to evaluating stepwise dry/wet ageing treatment on beef muscles. Future research should be conducted to determine whether this will generate similar results in other red meat cuts. The usage of natural polymers such as chitosan, plant cellulose, bacterial cellulose and alginate in developing dry ageing bags should be considered in future studies. These materials will help imitate traditional dry ageing by providing high water transmission. Also, it can be assumed that they will significantly reduce the losses associated with microbial growth and protein oxidation by acting as antimicrobial agents and oxygen barrier materials, respectively.

2.2.4 Injecting non-meat ingredients

Red meat processors use several non-meat ingredients to improve the quality of meat. They have been used mainly to increase the tenderness and WHC of the raw meat based on their functional properties. The key additives used in red meat processing are sodium chloride/phosphate, calcium chloride, calcium lactate, sodium carbonate and weak organic acids such as lactic acid and acetic acid. These additives act as ionic strength and pH adjustors of the meat products. The WHC of the meat is minimal when the meat pH is very close to the P_I of key muscle proteins. These additives are used to shift the meat pH just above or below the P_I of the muscle proteins to prevent muscle proteins from reaching their zwitterionic point. Therefore, more protein charges will be available to attract more water molecules, which helps achieve minimal moisture loss conditions. In this review, only the most commonly used ingredients which affect the moisture loss of raw red meat products are discussed. Table 2.2 shows the information regarding previous studies conducted to assess the impact of applying non-meat ingredients on purge loss and drip loss of different red meat cuts.

Table 2.2 Summary of research to examine the effect of different non-meat ingredients on the moisture loss of red meat

Type of chemical treatment	Meat	Muscle type	The composition of the solution	Target weight gain	Measurement	Ageing period	Main findings	Reference
Phosphate	Beef	NA[1]	- 3.6% NaCl + 4.5% phosphate + 1% Herbalox seasoning Vs. - 3.6% NaCl + acid solubilized protein + 1% Herbalox seasoning	10%	Purge loss %	11 d	Phosphate injected meat had significantly lower purge loss than acid solubilized protein injected meat	(Vann & Dewitt, 2007)
	Beef	NA[1]	- 3.6% NaCl + 4.5% sodium phosphate + 1% Herbalox seasoning Vs. - 3.6% NaCl + 5% dehydrated beef protein + 1% Herbalox seasoning	10%	Purge loss %	6 d	Phosphate injected meat had significantly higher purge loss than dehydrated beef protein injected meat	(Lowder et al., 2011)
	Beef	*SM* [a]	- 0.5% NaCl + 0.25% sodium tripolyphosphate and enzyme injection	15%	Purge loss %	2 d	Injected meat had significantly lower drip loss than non-injected meat	(Pietrasik & Shand, 2011)

Type of chemical treatment	Meat	Muscle type	The composition of the solution	Target weight gain	Measurement	Ageing period	Main findings	Reference
	Beef	*BF*[f] *and PP*[i]	- 2.0% NaCl + 0.3% phosphate + 0.2% dextrose + 0.05% sodium ascorbate + 0.015% sodium nitrite at chilled (2 - 4°C) and elevated (15 - 17°C) temperatures	15%	Yield %	1 d	No significant effect of brine temperature on yield percentage	(Keenan et al., 2016)
Phosphate and calcium lactate	Beef	*ST*[b] *and LM*[c]	- 0.2 M calcium lactate - 8.4% phosphate and 4.2% NaCl - Calcium lactate followed by phosphate and NaCl	5.5%	Weight loss %	7 d	Injected *ST* muscle had significantly higher weight loss than non-injected *ST* muscle. *LM* muscle injected with phosphate had significantly lower weight loss than *LM* muscle injected with only calcium lactate	(Lawrence et al., 2003b)
	Beef	NA[1]	- 2.2% NaCl + 4.4% sodium phosphate + 1% or 2% beef broth + natural flavouring or 1% or 2% kappa carrageenan - 2.4% Calcium lactate + 1% or 2% beef broth + natural	11.5%	Purge loss %	5 d	Calcium lactate injected meat had significantly higher purge loss than NaCl and phosphate injected meat	(Lawrence et al., 2004)

Type of chemical treatment	Meat	Muscle type	The composition of the solution		Target weight gain	Measurement	Ageing period	Main findings	Reference
				flavouring or 1% or 2% kappa carrageenan					
Calcium Chloride	Beef	*SM* [a]	-	0.3 M $CaCl_2$ injection at 1, 12 and 24 h pm	10%	Drip loss %	10 d	Injected meat had significantly higher drip loss than non-injected meat	(Boleman et al., 1995)
	Beef	*LL* [d] *and SM* [a]	-	200 mM $CaCl_2$	10%	Purge loss %	7 d	Injected meat had significantly higher purge loss than non-injected meat	(Lansdell et al., 1995)
	Beef	*LD* [e]	-	0.2, 0.3, 0.4 M $CaCl_2$ injection at 45 mins or 24 h pm	10%	Drip loss %	7 d	Injected meat had significantly higher drip loss than non-injected meat	(Jaturasitha et al., 2004)
	Beef	*LD* [e]	-	250 mM $CaCl_2$	10%	Drip loss %	5 d	Injected meat had significantly higher drip loss than non-injected meat	(Bunmee et al., 2014)
	Beef	*LL* [d] *and SM* [a]	-	2.2% $CaCl_2$	5%	Purge loss %	14 d	Injected meat had higher purge loss than non-injected meat	(Colle et al., 2018)
Plant-based protease extracts	Buffalo	*BF* [f]	-	2.0% cucumis extract	NA[1]	WHC	2 d	Ginger, papain treated samples and control samples had higher WHC than cucumis treated samples	(Naveena et al., 2004)
			-	5.0% ginger extract					
			-	0.2% papain					

Type of chemical treatment	Meat	Muscle type	The composition of the solution	Target weight gain	Measurement	Ageing period	Main findings	Reference
	Beef	*PP*[i]	- 50 ppm bromelain - 50 ppm bromelain + 20 ppm papain - 5.0% ginger extract	10%	Drip loss %	2 d	No significant effect of injection treatments on drip loss	(Moon, 2018)
Bicarbonate	Beef	*BF*[f]	- 1.2 M NaCl + 0.25 M bicarbonate + 0.1% ascorbic acid	20%	Drip loss %	5 d	Injected meat had significantly lower drip loss than non-injected meat	(Sultana et al., 2008)
Lactic acid and acetic acid	Beef	NA[1]	- 2% lactic acid + 2% acetic acid (v/v)	NA[1]	Purge loss %	112 d	Injected meat had significantly lower purge loss than non-injected meat	(Goddard et al., 1996)
Rinse and Chill®	Beef	*LL*[d], *ST*[b] *and QF*[g]	- 98.52% water; balance: saccharides, NaCl, phosphate blend Vs. - 0.3 M $CaCl_2$	10%	Purge loss %	14 d	No significant effect of RC® treatment on purge loss	(Dikeman et al., 2003)
	Bison	*LL*[d] *and TB*[h]	- 98.5% water; balance: glucose, polyphosphates, glycerine, maltose	NA[1]	Purge loss %	2 d	RC® treated meat had significantly higher purge loss than non-treated meat	(Mickelson & Claus, 2016)
	Lamb	*LL*[d]	- 98.5% water; balance: glucose, polyphosphates, maltose	10%	Purge loss %	7 d	No significant effect of RC® treatment on purge loss	(Fowler et al., 2017)

[a] *semimembranosus;* [b] *semitendinosus;* [c] *longissimus;* [d] *longissimus lumborum;* [e] *longissimus dorsi;* [f] *biceps femoris;* [g] *quadriceps femoris;* [h] *triceps brachii;* [i] *pectoralis profundus.*

[1] Data was not shown.

Injecting meat with a brine solution that consists of NaCl and sodium phosphate is a common practice in the red meat industry. These two ingredients act synergistically to modify the myofibrillar protein to improve the WHC of the meat cuts (Offer et al., 1989). Red meat processors use these additives to compensate for the purge loss which occurs during the packaging, transportation and retail display of the meat products (Baublits, Pohlman, Brown Jr, & Johnson, 2005). Pietrasik and Shand (2011) found that beef *semimembranosus* muscles injected with 0.5% NaCl and 0.25% phosphate solution in combination with other treatments had significantly ($P < 0.05$) lower purge loss values compared to control samples. They used a brine solution to achieve 15% enhancement by weight of the samples which were vacuum-packaged and stored at 4°C for 48 h. When the meat pH is shifted away from the P_I of key muscle proteins the ability of meat proteins to bind water molecules increases. Salt incorporation into a solution increases the ionic strength which results in increasing the number of hydrophilic protein interactions. This helps the meat proteins to bind more free water molecules. Lawrence, Dikeman, Hunt, Kastner, and Johnson (2003b), on the other hand, reported *semitendinosus* beef samples injected with 0.2 M calcium lactate followed by 8.4% phosphate and 4.2% NaCl solution lost more ($P < 0.05$) weight during retail display than the non-injected samples. In the same study, they reported *longissimus* steaks injected with only phosphate solution retained more ($P < 0.05$) water than those injected with only calcium lactate solution. In another study, they found higher ($P < 0.05$) purge loss values for steaks after calcium lactate treatment than from phosphate treatment (Lawrence, Dikeman, Hunt, Kastner, & Johnson, 2004).

Beef strip loin was injected with either a control brine solution or brine containing dehydrated beef protein (DBP) up to 110% of their initial weight (Lowder, Goad, Lou, & Dewitt, 2011). Control brine solution consisted of 3.6% NaCl and 4.5% sodium phosphate and the DBP solution consisted of 3.6% NaCl and 5% DBP. Steaks injected with the control brine solution had greater ($P < 0.0001$) total purge values than steaks injected with the DBP solution. Conversely, significantly ($P < 0.05$) low purge loss values have been reported for beef striploin steaks injected with a control brine solution containing 3.6% NaCl and 4.5% phosphate compared to the steaks injected with acid solubilized protein solution (Vann & Dewitt, 2007). In both studies, meat sample weights were increased up to 110% from their initial weight after injection,

modified atmosphere packaging and storage at 4°C. Lowder et al. (2011) suggested that the lower purge loss values of steaks injected with protein solution might be due to the increased hydrophilic interactions and thickening effect of collagen proteins at low temperatures. Vann and Dewitt (2007) indicated that the higher purge loss values of samples injected with protein solution could be associated with poor binding of the protein with meat due to insufficient amounts of protein entering the solution to form a good matrix for binding.

A recent study conducted by Keenan, Hayes, Kenny, and Kerry (2016) evaluated the impact of injecting brine solutions at chilled (2-4 °C) and elevated (15-17 °C) temperatures to beef samples from two muscles. Muscles were injected up to 115% of their initial weight, and the brine solution consisted of 2.0 % NaCl, 0.3% sodium phosphate and other ingredients, as mentioned in Table 2.2. No impact of brine temperature has been observed in yield percentage values in both muscles. This study shows that the brine solution's temperature may not impact the WHC of red meat. However, further studies with the injection of different brine formulations at different temperatures in different red meat cuts other than beef should be carried out to establish a clear conclusion.

In addition to improving WHC, meat processors also use different ingredients to enhance other meat quality characteristics, which may affect the WHC as a side effect. One such example is the addition of calcium chloride ($CaCl_2$) to improve meat tenderness (Jaturasitha, Thirawong, Leangwunta, & Kreuzer, 2004; Lawrence, Dikeman, Hunt, Kastner, & Johnson, 2003a). The activity of the calpain system depends on calcium ion concentration (Goll, Thompson, Li, Wei, & Cong, 2003). An infusion of $CaCl_2$ solution facilitates enzymatic myofibrillar proteolysis and improve meat tenderness. On the other hand, extra chloride may reduce WHC (Jaturasitha et al., 2004) and negatively affect meat's purge loss. This is in agreement with the findings of Boleman, Boleman, Bidner, McMillin, and Monlezun (1995) and Lansdell, Miller, Wheeler, Koohmaraie, and Ramsey (1995) who found that drip loss was higher in $CaCl_2$ injected beef steaks than that of the non-injected control steaks. Boleman et al. (1995) used a 0.3 M $CaCl_2$ solution and Lansdell et al. (1995) used a 0.2 M $CaCl_2$ solution with beef steak samples vacuum-packaged and stored at 2 ºC for 10 d and 7 d. Similarly, more recent studies reported that drip loss was greater ($P<0.05$) in $CaCl_2$

treated beef samples compared to non-treated meat (Bunmee, Jaturasitha, Kreuzer, & Wicke, 2014; Colle et al., 2018; Jaturasitha et al., 2004).

Jaturasitha et al. (2004) examined the impact of $CaCl_2$ injection time on drip loss by injecting $CaCl_2$ solutions with varying concentrations (0, 0.2, 0.3 and 0.4 M) up to 110% of the initial meat weight at either 45 mins or 24 h post-mortem. They determined that the average increase in drip was about 100% ($P<0.05$) with early injection (45 mins post-mortem) compared to late injection (24 h post-mortem). Boleman et al. (1995) who suggested a linear trend between the drip loss and the time of injection have reported contradictory results. These authors injected a 0.3 M $CaCl_2$ solution at 1 h, 12 h, and 24 h post-mortem to obtain a 10% weight gain in beef *semimembranosus* muscles. In general, all the above studies reported that $CaCl_2$ injected samples had higher drip loss values than control samples. Bunmee et al. (2014) suggested this might be due to three reasons. First, this might be due to the extra volume of solution injected in proportion to meat weight. Second, the presence of Ca^{2+} ions in meat may cause an interruption to the water absorption. Third, the increased proteolytic enzyme activity due to the presence of Ca^{2+} ions may cause an extra release of myowater by freeing myofibre cell content.

Except for $CaCl_2$, Naveena, Mendiratta, and Anjaneyulu (2004) and Moon (2018) have tested different plant-based proteolytic enzymes as meat tenderizers and have evaluated their impact on meat WHC. Naveena et al. (2004) have marinated buffalo meat with the proteolytic enzyme extract of *Cucumis trigonus Roxb* (Kachri), *Zingiber officinale roscoe* (Ginger rhizome) or papain. Samples treated with Cucumis have had a significant reduction in WHC compared to other samples, and no significant change in WHC of control samples (distilled water) and samples treated with ginger and papain extracts have been observed. This could be due to the marked reduction in pH of Cucumis treated samples compared to all other samples. Cucumis extract has shown significantly low pH values (4.8-5.0) values compared to ginger and papain extracts (6.5 and 6.25 respectively). Likewise, Moon (2018) has tested the tenderizing effect of bromelain, a mix of bromelain and papain and ginger extract in beef brisket samples. They also found no significant difference in drip loss between any of the treatments and the control sample injected with distilled water. Most interestingly, they have also observed no change in meat pH between injected samples and control samples.

Therefore, it can be assumed that if the protease extract does not change the meat pH, it may improve the tenderness of red meat without having any detrimental impacts on meat water retention.

Other chemicals widely used to treat red meat carcasses are bicarbonate and weak organic acids. Only very few studies have evaluated the impact of these chemicals on the moisture loss of red meat samples. Significantly lower drip loss values were reported for beef muscles injected to a target of 120% of initial weight with a solution consisting of 1.2 M NaCl, 0.25 M sodium carbonate and 0.1% ascorbic acid compared to control samples (Sultana et al., 2008). The lower drip loss could be due to the brine solution's high buffering capacity and ionic strength. In addition, Cl^- ions cause swelling of myofibrils and increase water retention within the protein network. Goddard, Mikel, Conner, and Jones (1996) analyzed the effect of spraying a mixture of 2% lactic and 2% acetic acid (v/v) on the purge loss of beef strip loins. They found significantly lower percentage purge values for acid sprayed striploin samples as compared to control strip loins. The increase in water-binding capacity might be caused by the difference in the P_I of the muscle compared with that of the organic acid applied. .

Meat processors often use Rinse and Chill® (RC®) technology which entails vascular rinsing off the residual blood with a chilled isotonic substrate solution to improve meat tenderness and WHC (Mickelson & Claus, 2016). A few studies have recently been conducted to determine the effect of RC® technology on the purge loss of red meat carcasses. Mickelson and Claus (2016) reported that bison carcasses subjected to RC® processes had a greater ($P < 0.05$) purge than the control samples. No difference in purge loss due to the RC® process has been reported in the studies of Dikeman et al. (2003) and Fowler, Claus, and Hopkins (2017) who infused at 10% of live weight to steer and lamb carcasses, respectively. These studies have not provided a potential reason behind these contradicting results.

Overall, the above studies are limited by the lack of information on the impact of chemical treatments on the moisture loss of a range of alternative red meat cuts such as deer, goat and lamb. Most of the studies have focused on evaluating solutions containing phosphate and $CaCl_2$ on purge loss of beef. Few studies have considered the impact of other types of chemicals such as carbonate, weak organic acids and newer

technologies, such as the RC® process on the moisture loss of retail and wholesale red meat cuts. Further work is recommended to close these gaps in the current literature and provide red meat processors with valuable input on the post-slaughter process to minimize the occurrence of moisture loss with no detrimental impact on quality. Also, it is important to evaluate the possibility of replacing synthetic ingredients with natural compounds such as plant extracts of proteolytic enzymes in future red meat studies. The addition of plant extracts as bioactive compounds in fresh meat is becoming an attractive strategy to improve meat quality (Munekata et al., 2020), as this may help red meat processors satisfy the emerging consumer trend of having chemical-free products.

2.2.5 Packaging techniques

The packaging of fresh meat is carried out to delay microbial spoilage, prevent contamination, reduce weight loss, and extend the product's shelf life. Different types of meat packaging techniques exist, each with a specific purpose and technological attributes. These packaging techniques include overwrapping of meat for short term chilled storage under retail display conditions, modified atmospheric packaging (MAP) for long term chilled storage or display, and vacuum packaging (VP) for long term chilled or frozen transport to distant or export markets (Kerry, O'Grady, and Hogan 2006). Meat quality attributes, including purge loss and drip loss, may vary significantly depending on the packaging technique used by different processors. Previous studies conducted to evaluate the impact of different packaging techniques on the moisture loss of red meat are summarized in Table 2.3.

One of the leading packaging techniques used by the current case-ready red meat market is MAP which provides prolonged shelf life compared to aerobic packaging. It also offers a more attractive “cherry red” colour of meat due to oxymyoglobin formation. It does, however, increase the rate of lipid oxidation (Kim, Huff-Lonergan, Sebranek, & Lonergan, 2010; Lund, Heinonen, Baron, & Estevez, 2011) and the growth of aerobic spoilage microorganisms compared to vacuum storage (Arvanitoyannis & Stratakos, 2012). The main gases used in MAP of meat cuts are oxygen, carbon dioxide and nitrogen, at different concentrations. Several studies have analyzed the effect of different gas concentrations used in MAP on purge loss of red meat. Recent studies conducted by Lopacka, Poltorak, and Wierzbicka (2017) and

Yang et al. (2016) on normal beef steaks and beef steaks with high marbling, respectively, did not report significant differences in purge loss among packages with different gas compositions. These results are in agreement with Clausen, Jakobsen, Ertbjerg, and Madsen (2009) and Vergara, Gallego, García, and Landete-Castillejos (2003) who determined there was an absence of differences in the drip loss of various MAP treatments on beef and western red deer (*Cervus elaphus*) steaks, respectively. A few studies have reported significant differences in drip loss between MAP red meat samples (Ercolini, Russo, Torrieri, Masi, & Villani, 2006; Smulders, Hiesberger, Hofbauer, Dogl, & Dransfield, 2006; Vergara & Gallego, 2001). Vergara and Gallego (2001) and Smulders et al. (2006) found that a high CO_2 content resulted in less water loss compared to the samples packed with low concentrations of CO_2 in packs of lamb *longissimus dorsi*, and bull striploin. Ercolini et al. (2006) reported the lowest weight loss for beef steaks packaged with no CO_2 compared to those packaged with high CO_2 concentrations. Sivertsvik, Rosnes, and Jeksrud (2004) suggested that CO_2 dissolution into the tissue fluid leads to the formation of carbonic acid and this results in acidification of the meat, leading to protein denaturation and decreased WHC. In addition, CO_2 may bind with meat proteins and decrease the ability to bind water (Lopacka, Poltorak, & Wierzbicka, 2016). This results in the WHC of the meat decreasing and meat packaged under high CO_2 concentrations having high moisture loss compared to the samples packaged under low CO_2 concentrations.

Vacuum packaging extends the shelf life of red meat by generating anaerobic conditions. Vacuum packaging can result in an unattractive dark, purplish-red colour of the meat due to the absence of O_2 and cause purge formation. Payne, Durham, Scott, and Devine (1998) studied the components of VP systems affecting drip loss in chilled beef and they determined that packaging samples with rigid support under the standard VP conditions extensively reduced drip formation compared to the samples without rigid support. This is because of the physical compression of meat caused by the VP process (Payne et al., 1998). They also showed that the level of vacuum application did not have a significant effect on drip loss. Payne et al. (1998), and Aspé, Roeckel, Martí, and Jiménez (2008) studied beef meat vacuum-packaged in heat shrink bags and compared the results with the steaks vacuum-packaged in normal vacuum bags. In both studies, samples in the heat shrink bags lost the lowest amount of drip compared to samples in the standard vacuum bags. Heat shrink bags caused less physical

squeezing of meat due to their softer and more pliable nature (Payne et al., 1998). The thermal contractibility of heat shrink bags also reduces the vacuum void volume and reduces the fluid flow inside the package (Aspé et al., 2008).

Vacuum skin packaging (VSP) is a relatively new VP technique that involves heating of the upper film of the packaging, resulting in it sticking close to the meat (Clausen et al., 2009). This technique shows distinct advantages: reduced purge loss and high WHC over the traditional VP technique (Arvanitoyannis & Stratakos, 2012). Clausen et al. (2009) and Strydom and Hope-Jones (2014) compared vacuum skin packaged beef with beef packaged under vacuum thermoforming conditions and vacuum packaging in heat shrink bags, respectively. In both studies, vacuum skin packaged samples showed significantly lower purge loss than the other samples at 20 and 21 d, respectively. The firmer wrap of VSP leaves fewer cavities for purge to collect than conventional heat shrink bags (Strydom & Hope-Jones, 2014).

Several studies have been conducted to assess how beef quality parameters are affected by MAP as compared to VP. These studies have reported that vacuum-packaged steaks had a higher drip loss compared to the steaks packaged under MAP conditions (Hur, Jin, Park, Jung, & Lyu, 2013; Lindahl, Lagerstedt, Ertbjerg, Sampels, & Lundstrom, 2010; Yang et al., 2016). Similar results were observed in a study conducted by Sekar, Dushyanthan, Radhakrishnan, and Babu (2006) on buffalo meat (*Babulus bubalis*) packed under three different packaging conditions. These authors observed the highest drip losses in vacuum-packaged samples in comparison to the aerobically packed and modified atmospherically packed samples. High drip loss is caused by the squeezing of the meat due to the drastic changes in pressure applied during the vacuum packaging process (Sekar et al., 2006). The water loss was lower in MAP samples than the aerobically packed samples in two studies which compared aerobic, VP and MAP techniques (Hur et al., 2013; Sekar et al., 2006). These authors suggest this might be attributed to the headspace gas pressure maintained in the MAP packages. In contrast, several other studies reported that MAP beef steaks had higher drip loss values than VP steaks (Bağdatli & Kayaardi, 2014; Smulders et al., 2006; Zakrys-Waliwander, O'Sullivan, O'Neill, & Kerry, 2012). Zakrys-Waliwander et al. (2012) investigated the effect of high O_2 MAP in comparison to the VP of beef *longissimus dorsi* steaks. They suggest that increased drip loss values in high O_2 MAP steaks may be linked to

protein oxidation and disulphide bond formation due to the high O_2 atmosphere maintained inside the packages.

Studies that compared the impact of MAP, VP and VSP on drip loss of red meat cuts are limited. Only one study evaluated how quality parameters of bull *longissimus dorsi* steaks are affected by high O_2 MAP compared to VP and VSP techniques (Lagerstedt, Ahnstrom, & Lundstrom, 2011). These authors established that VSP steaks had the lowest amount of purge loss and VP steaks had the highest after 14 d storage at 4 °C. Conversely, Lopacka et al. (2016) determined no difference in drip loss between beef *longissimus lumborum* steaks packed as VSP, high O_2 MAP and their combination. Most importantly, both studies (Lagerstedt, Ahnstrom, et al., 2011; Lopacka et al., 2016) have used the same gas concentration of 80% O_2 and 20% CO_2 for MAP. The authors of these studies provided no potential rationale for drip loss results. However, the contradictory results could be due to the usage of different types of muscles used in the studies.

The incorporation of carbon monoxide (CO) in fresh red meat packages has been proposed recently. Low concentrations of CO has been effective in increasing oxidative stability (Stahlke et al., 2019) and producing a more stable cherry red colour due to the formation of carboxymyoglobin (Rooyen, Allen, Kelly-Rees, et al., 2018) in red meat. Also, the use of CO at levels lower than 1% has been allowed in several countries, including the United States, Australia and New Zealand since the low concentrations are entirely safe for consumers (Stahlke et al., 2019).

A few studies have assessed the impact of CO in purge loss of beef steaks packaged in both VP and MAP. Stetzer et al. (2007) reported that CO had no effect on purge loss of beef steaks stored in 0.4% CO incorporated MAP compared to high oxygen MAP. Likewise, Aspé et al. (2008), Rooyen, Allen, Gallagher, and O'Connor (2018) and Rooyen, Allen, Kelly-Rees, et al. (2018) reported that CO pretreatments did not affect purge loss of vacuum-packaged beef steaks. In their study, Rooyen, Allen, Kelly-Rees, et al. (2018) had exposed meat to a range of different CO concentrations and exposure times before vacuum packaging. Most importantly, all these studies have not observed a significant impact on meat pH due to the CO pretreatment. No effect on purge loss due to CO pretreatment might have resulted from this. However, all these studies have evaluated the CO pretreatment at very low percentages, maximum been reported as

5% (Please refer to Table 2.3). Lyu, Shen, Ding, and Ma (2016) have reported significantly low purge loss in beef samples pretreated with a mixture of low ozone (O_3; 2 to 10%) and high CO gas percentages (90 to 100 %) compared to unpretreated samples before vacuum packaging. This has been mainly due to significantly low protein denaturation and microbial growth reported by pretreated samples compared to unpretreated samples. However, the feasibility of usage O_3 and high percentages of CO in the red meat industry is very limited because of the high cost associated with the usage of O_3, and the maximum allowable limit of CO is reported as less than 1% (Stahlke et al., 2019).

Several important limitations need to be considered concerning packaging and moisture loss. Most of the studies mentioned above have not assessed the cumulative impact of packaging with other immediate post aging treatments such as tenderization, the temperature of post-rigour storage and injecting non-meat ingredients. Studies have typically been carried out to evaluate the effect of different packaging treatments on moisture loss of beef. Only a few studies have evaluated the impact of packaging on purge in lamb and deer meat, and no study has been conducted on goat meat. Different packaging treatments on different meat cuts have also not been studied. If these gaps in our understanding of packaging and moisture loss can be addressed, this could help the industry determine whether the packaging should be varied based on muscle tissue type. Also, key limitations in comparing packaging techniques are changes in gas compositions used for MAP and quality parameters of packaging materials. A limited amount of studies has been conducted to compare the effect of all three packaging treatments of MAP, VP and VSP on moisture loss of red meat cuts. Future studies that consider these limitations should be undertaken to provide a “best practice” framework for packaging red meat.

Table 2.3 Summary of research to examine the effect of different packaging techniques on the moisture loss of red meat

Type of packaging treatment	Meat	Muscle type	The gas composition or other treatment conditions	Measurement	Ageing period	Main findings	Reference
MAP [2]	Lamb	*LD* [a]	- 20% CO_2/10% O_2 /70% N_2 - 40% CO_2 /60% N_2 - 80% CO_2/20% O_2 - 80% CO_2/20% N_2	WHC	16 d	Meat aged in 20% CO_2 MAP lost more water than meat aged in other MAP conditions	(Vergara & Gallego, 2001)
	Red deer	NA[1]	- 40% CO_2 /60% N_2 - 80% CO_2/20% O_2 - 80% CO_2/20% N_2	Drip loss %	23 d	No significant effect of different MAP conditions on drip loss	(Vergara et al., 2003)
	Beef	*LD* [a]	- 60% O_2/40% CO_2 - 20% O_2/40% CO_2/40% N_2	Weight loss %	14 d	Meat aged in MAP had significantly higher weight loss than meat aged under aerobic conditions at the end of 7 d of shelf life	(Ercolini et al., 2006)
	Beef	NA[1]	- 0.4% CO/30% CO_2 /69.6% N_2 - 80% O_2/20% CO_2	Purge loss %	26 d	No significant effect of CO incorporation on purge loss	(Stetzer et al., 2007)
	Beef	*LL* [b] *and* *GM* [c]	- 50% O_2/20% CO_2 /30% N_2 - 65% O_2/20% CO_2/15% N_2 - 80% O_2/20% CO_2	Drip loss %	12 d	No significant effect of different O_2 concentrations on drip loss	(Lopacka et al., 2017)

Type of packaging treatment	Meat	Muscle type	The gas composition or other treatment conditions	Measurement	Ageing period	Main findings	Reference
VP [3]	Beef	NA[1]	- Fast vacuum vs. slow vacuum and extent of vacuum - Heat shrink bags vs. standard vacuum bags	Drip loss % Drip loss %	28 d 42 d	No significant effect of the rate of vacuum application on drip loss Meat aged in heat shrink bags had lower drip loss than meat aged in standard vacuum bags	(Payne et al., 1998) (Payne et al., 1998)
	Beef	*LD* [a]	- Heat contractile bags vs. non-heat contractile bags - - Pre-treatment of CO (95% N_2/5% CO) vs. normal VP	Drip loss %	77 d	Meat aged in heat contractile bags had significantly lower drip loss than meat aged in non-heat contractile bags No significant effect of CO pre-treatment on drip loss	(Aspé et al., 2008)
	Beef	*LL* [b] *and PM* [d]	- Pre-treatment of O_3 and CO (CO varied from 90 to 100%/O_3 varied from 2 to 10%) vs. normal VP	Purge loss %	46 d	Meat pre-treated with O_3 and CO had significantly lower purge loss than meat packaged in normal VP	(Lyu et al., 2016)
	Beef	*LTL*[e]	- Pre-treatment of CO (0.4 to 5%) for 5, 7 or 24 h vs. normal VP	Purge loss %	28 d	No significant effect of CO pre-treatment on purge loss	(Rooyen, Allen, Kelly-Rees, et al., 2018)

Type of packaging treatment	Meat	Muscle type	The gas composition or other treatment conditions	Measurement	Ageing period	Main findings	Reference
	Beef	*LTL*[e]	- Pre-treatment of CO (5% CO/60% CO_2 /35% N_2) vs. pre-treatment of 60% CO_2/40% N_2 for 3, 5 or 7 h	Purge loss %	28 d	No significant effect of CO pre-treatment on purge loss	(Rooyen, Allen, Gallagher, et al., 2018)
VP vs. VSP [4]	Beef	*LD*[a]	- VP in thermoforming bags vs. VSP	Weight loss %	20 d	Steaks aged in VSP had significantly lower weight loss than steaks aged in vacuum thermoforming bags	(Clausen et al., 2009)
	Beef	NA[1]	- VP in heat shrink bags vs. VSP	Purge loss %	21 d	Meat aged in VSP had significantly lower purge loss than meat aged in VP	(Strydom & Hope-Jones, 2014)
MAP vs. VP	Buffalo	NA[1]	- High O_2 MAP (80% O_2/20% CO_2) vs. VP	Drip loss %	21 d	Meat aged in MAP had significantly lower drip loss than meat aged in VP	(Sekar et al., 2006)
	Bull	NA[1]	- MAP (70% N_2/30% CO_2, 100% CO_2) vs. VP	Drip loss %	23 d	Meat aged in MAP had significantly higher drip loss than meat aged in VP	(Smulders et al., 2006)
	Beef	*LD*[a]	- High O_2 MAP (80% O_2/20% CO_2) vs. VP and combination of MAP and VP	Purge loss %	25 d	Meat solely aged in MAP had lower purge loss than meat aged in combination of MAP and VP at the end of 10 d of shelf life	(Lindahl et al., 2010)

Type of packaging treatment	Meat	Muscle type	The gas composition or other treatment conditions	Measurement	Ageing period	Main findings	Reference
	Beef	*LD* [a]	- MAP (80% O_2/20% CO_2) vs. VP	Drip loss %	14 d	Meat aged in MAP had significantly higher drip loss than meat aged in VP	(Zakrys-Waliwander et al., 2012)
	Beef	*LD* [a]	- MAP (30% CO_2/70% N_2) vs. VP	WHC	21 d	Meat aged in MAP had significantly lower WHC than meat aged in VP at the end of 14 d of shelf life	(Hur et al., 2013)
	Beef	*LD* [a]	- MAP (60% O_2/40% CO_2, 60% O_2/20% CO_2/20% N_2) vs. VP	Weight loss %	35 d	Meat aged in MAP had higher weight loss than meat aged in VP	(Bağdatli & Kayaardi, 2014)
	Beef	*LL* [b]	- MAP (80% O_2/20% CO_2, 50% O_2/30% CO_2/20% N_2, 0.4% CO/30% CO_2/69.6% N_2) vs. VP	Purge loss %	12 d	Meat aged in all types MAP conditions had significantly lower purge loss than meat aged in VP. But no significant difference in purge loss observed among MAP conditions	(Yang et al., 2016)
MAP vs. VSP	Beef	*LL* [b]	- MAP (80% O_2/20% CO_2) vs. VSP and combination of MAP and VSP	Drip loss %	12 d	No significant effect of packaging treatment on drip loss	(Lopacka et al., 2016)
MAP vs. VP vs. VSP	Beef	*LD* [a]	- MAP (80% O_2/20% CO_2) vs. VP vs. VSP	Purge loss %	14 d	Meat aged in VSP had the lowest purge loss, and meat aged in VP had the highest purge loss during the storage	(Lagerstedt et al., 2011)

[a] *longissimus dorsi;* [b] *longissimus lumborum;* [c] *gluteus medius*; [d] *psoas major;* [e] *Longissimus thoracis et lumborum.* [1] Data was not shown; [2] Modified atmospheric packaging; [3] Vacuum packaging; [4] Vacuum skin packaging.

2.3 Emerging post-slaughter treatments

Novel techniques are emerging in the red meat industry which can consistently provide high-quality products to consumers. SmartStretch™, Pi-Vac® Elasto-Pack system, PEF, HPP and ultrasound treatments are emerging meat tenderization techniques that can be applied to either pre-rigour or post-rigour red meat cuts (Warner et al., 2017). The use of edible coatings is also being investigated in the meat industry due to their ability to prevent purge, improve product presentation and eliminate the need for having absorbent pads inside the meat packages. Tenderization techniques and edible coatings may alter the meat WHC and the impact of each technique on moisture loss of red meat will be discussed in the following section. Table 2.4 summarizes the findings from studies carried out to determine the impact of PEF, HPP and ultrasound treatments on the moisture loss of different types of red meat cuts.

Table 2.4 Summary of research to examine the effect of different tenderization techniques on the moisture loss of red meat

Type of tenderization treatment	Meat	Muscle type	Treatment parameters and conditions		Measurement	Ageing period	Main findings	Reference
PEF [2]	Beef	*ST* [a]	-	1.1-2.8 kV/cm, 12.7-226 kJ/kg, 5-200 Hz	Weight loss %	NA[1]	PEF treated meat had more weight loss compared to meat treated with a water bath to achieve a temperature difference of 5 and 22 °C	(O'Dowd et al., 2013)
	Beef	*LL* [b] *and SM* [c]	-	5 and 10 kV, 20, 50 and 90 Hz	Purge loss %	21 d	Purge loss increased with the increase in applied voltage and frequency	(Bekhit et al., 2014)

Type of tenderization treatment	Meat	Muscle type	Treatment parameters and conditions	Measurement	Ageing period	Main findings	Reference
	Beef	*LTL* [d]	- 1.4 kV/cm, 20 µs, 10 Hz, 300 and 600 pulses	Weight loss %	26 d	No significant impact of PEF treatment on weight loss	(Arroyo et al., 2015)
	Beef	*ST* [a]	- 1.4 kV/cm, 250 kJ/kg, 50 Hz, 20 µs	Purge loss %	7 d	PEF treated meat had significantly higher purge loss compared to meat not treated with PEF	(Faridnia et al., 2015)
	Beef	*LL* [b]	- 10 kV (0.58-0.73 kV/cm), 20 µs, 90 Hz	Purge loss %	7 d	No significant impact of PEF treatment on purge loss	(Suwandy et al., 2015b)
	Beef	*LL* [b] *and SM* [c]	- 5 and 10 kV (0.28-0.51 kV/cm and 0.31-0.56 kV/cm), 20, 50 and 90 Hz	Purge loss %	21 d	No significant impact of PEF treatment on purge loss of *LL* muscle PEF treated *SM* muscle had significantly higher purge loss compared to non-treated samples	(Suwandy et al., 2015a)
	Beef	*LL* [b] *and SM* [c]	- 10 kV (0.50-0.58 kV/cm), 20 µs, 90 Hz. Repeats of 1X, 2X, 3X	Purge loss %	21 d	No significant impact of PEF treatment on purge loss of *LL* muscle PEF treated *SM* muscle had significantly higher purge loss, regardless of the number of PEF repetitions	(Suwandy et al., 2015c)
	Beef	*LL* [b] *and SM* [c]	- 10 kV (0.44-0.48 kV/cm), 20 µs, 90	Purge loss %	21 d	Purge loss increased with every extra application of PEF and the highest	(Bekhit et al., 2016)

Type of tenderization treatment	Meat	Muscle type	Treatment parameters and conditions	Measurement	Ageing period	Main findings	Reference
			Hz. Repeats of 1X, 2X, 3X			purge loss resulted in the most intensive PEF treatment (3X)	
	Beef	*BF*[f]	- 1.7 – 2.0 kV/cm, 185 kJ/kg, 50 Hz, 20 µs	Purge loss %	21 d	PEF treated meat had significantly higher purge loss after 3 or 7 d of ageing compared to meat not treated with PEF	(Faridnia et al., 2016)
	Beef	*LL*[b]	- Low PEF (2.5 kV (0.23 kV/cm), 20 µs, 200 Hz) - High PEF (10 kV (0.68 kV/cm), 20 µs, 200 Hz)	Purge loss %	14 d	Meat treated with high PEF had higher purge loss compared to meat treated with low PEF and non-treated control samples	(Khan et al., 2017)
	Beef	*PE*[g]	- 0.7 or 1.5 kV/cm, 90-100 kJ/kg, 50 Hz, 20 µs	Purge loss %	14 d	PEF treated meat had significantly higher purge loss after 3 or 7 d of ageing compared to meat not treated with PEF	(Alahakoon et al., 2019b)
	Beef	*SM*[c]	- 5 kV (0.36 kV/cm), 20 µs, 90 Hz - 10 kV (0.60 kV/cm), 20 µs, 20 Hz	Purge loss %	14 d	No significant impact of PEF treatment on purge loss	(Bhat et al., 2019a)

Type of tenderization treatment	Meat	Muscle type	Treatment parameters and conditions	Measurement	Ageing period	Main findings	Reference
	Beef	*BF*[f]	- 5 kV (0.38 kV/cm), 20 µs, 90 Hz - 10 kV (0.61 kV/cm), 20 µs, 20 Hz	Purge loss %	14 d	No significant impact of PEF treatment on purge loss	(Bhat et al., 2019b)
HPP [3]	Beef	*ST*[a]	- 100, 200, 300, 400 AND 500 MPa for 5 mins at 15 ± 3 ˚C	WHC	NA[1]	Meat subjected to pressures above 100 MPa showed a significant reduction in WHC	(Kim et al., 2007)
	Beef	*LD*[e]	- 650 MPa at 20˚C or -35˚C for 10 mins, 433 MPa/min	Drip loss %	14 d	Meat subjected to HPP had the highest drip loss compared to meat not subjected to HPP	(Fernandez et al., 2007)
	Beef	*LD*[e]	- 200, 400 and 600 MPa at 10, 20˚C or 30˚C for 20 mins	Expressible moisture %	NA[1]	No significant impact of HPP at 200 MPa and increased expressible moisture % at 400 and 600 MPa	(Marcos et al., 2010)
	Beef	*LTL*[d]	- 200, 400 and 600 MPa at 20˚C for 20 mins	Expressible moisture %	NA[1]	No significant impact of HPP at 200 MPa and increased expressible moisture % at 400 and 600 MPa	(Marcos & Mullen, 2014)
	Beef	*TB*[h]	- 450 and 600 MPa for 2 s, 3, 6, 10 and 15 mins	Expressible moisture %	NA[1]	Pressurized samples had significantly decreased expressible moisture % compared to non-treated samples	(Sun et al., 2017)

Type of tenderization treatment	Meat	Muscle type	Treatment parameters and conditions	Measurement	Ageing period	Main findings	Reference
	Yak	*Thigh muscle*	- 0 – 450 MPa for 15 mins or 250 MPa for 0 – 30 mins	WHC	NA[1]	The WHC of meat first increased and then decreased with increased time and pressure	(Ma et al., 2019)
Ultrasound	Beef	*LTL*[d] *and ST*[a]	- 24 kHz, 12 W/cm^2 for 4 mins	Drip loss %	8.5 d	No significant impact of US treatment on drip loss	(Jayasooriya et al., 2007)
	Beef	*ST*[a]	- 40 kHz, 1500 W for 10, 20, 30, 40, 50 or 60 mins	WHC	0 d	US treated meat had significantly lower WHC compared to meat not treated with US	(Chang et al., 2015)
	Beef	*LD*[e]	- 37 kHz, 7 W/cm^2 for 60 mins	WHC	7 d	US treated meat had significantly lower WHC immediately after sonication and higher WHC after ageing compared to control samples	(Carrillo-Lopez et al., 2019)
	Beef	*LD*[e]	- 40 kHz, 11 W/cm^2 for 60 mins	WHC	14 d	No significant impact of US treatment on WHC with ageing	(Peña-Gonzalez et al., 2019)
	Beef	*BF*[f]	- 20 kHz, 26-84 W/cm^2 at 18-28 ˚C for 15 mins	WHC	0 d	Meat subjected to variable US intensities and temperatures with a fixed sonication time had lower WHC compared to unsonicated samples	(Fallavena et al., 2020)
			- 20 kHz, 22-84 W/cm^2 at 10 ˚C for 15 mins			Meat subjected to only variable US intensities with a fixed sonication time and temperature had no significant changes in WHC compared to unsonicated samples	

Type of tenderization treatment	Meat	Muscle type	Treatment parameters and conditions	Measurement	Ageing period	Main findings	Reference
	Beef	*LL* [b], *CC* [i] and *IP* [j]	- 40 kHz, 11 W/cm^2 for 40, 60 or 80 mins	WHC	14 d	US treated meat had significantly higher WHC compared to control samples with ageing	(Gonzalez-Gonzalez et al., 2020)

[a] *semitendinosus;* [b] *longissimus lumborum;* [c] *semimembranosus;* [d] *Longissimus thoracis et lumborum;* [e] *longissimus dorsi;*[f] *Biceps femoris;* [g] *Pectoral;* [h] *triceps brachii;* [i] *cleidooccipitalis;* [j] *infraspinatus*.

[1] Data was not shown; [2] Pulsed electric field; [3] High-pressure processing.

2.3.1 Meat tenderization techniques

2.3.1.1 SmartStretch™

SmartStretch™ can improve the tenderness of hot-boned primals by stretching muscles pre-rigour or by reducing the contraction at rigour (Taylor, Toohey, van de Ven, & Hopkins, 2013). This technique uses air pressure to eject meat into a tubular polythene packaging to constrict the muscle and consequently prevent any further contraction (Toohey, van de Ven, Thompson, Geesink, & Hopkins, 2012a).

SmartStretch™ treated hot-boned sheep whole meat cuts resulted in more significant ($P < 0.05$) purge loss than the non-stretched controls (Taylor, van de Ven, & Hopkins, 2011; Toohey et al., 2012a; Toohey, van de Ven, Thompson, Geesink, & Hopkins, 2013). In contrast, many studies have reported that there was no significant difference in purge loss between the controls and smart stretched samples of hot-boned beef (Taylor, Toohey, van de Ven, & Hopkins, 2012; Toohey, van de Ven, Thompson, Geesink, & Hopkins, 2012b). In all these studies, they have evaluated the impact of stretching on purge loss in different muscles. Except for one study (Taylor et al., 2013), all the above studies determined that stretching increased sarcomere length significantly in sheep and beef cuts. Huff-Lonergan and Lonergan (2005) determined that shorter sarcomeres resulted in greater purge losses, and this appears to agree with the studies evaluating purge loss in sheep tissue after stretching. However, increased sarcomeres have not caused any adverse effect on purge loss in different beef muscles studied after stretching. In all the studies, stretching has not caused any significant effect on either pH or temperature parameters. The contrasting results between beef and sheep meat purge loss may be due to the compositional difference between the two meat types. Despite previous groups' work, the relationship between stretching and purge loss is still poorly understood, and further research needs to examine the links between these parameters to evaluate the effectiveness of commercial products like SmartStretch™ as meat packaging tools.

2.3.1.2 Pi-Vac®

Pi-Vac® Elasto-Pack system is another innovative meat tenderization technique applied to hot-boned muscles during the pre-rigour stage (Hopkins, 2014). This system

uses a partial vacuum to insert the meat into flexible elastic packaging to prevent any muscle contraction. The resulting product is called tenderbound meat (Troy, 2006).

O'Sullivan, Korzeniowska, White, and Troy (2003) determined that drip loss was significantly ($P<0.05$) lower for tenderbound beef compared to both artificially restrained muscle (by suspending a 4 kg weight from a hanging muscle) and the control (no restraint applied to the muscle). Another trial was conducted to compare the impact of Pi-Vac® system and ES on the tenderness of fast chilled hot-boned beef *longissimus dorsi* muscle (Troy, 2006). This study's findings revealed that tenderbound meat had the lowest drip loss compared to the electrically stimulated product and the control. Further research is needed on beef and other red meat cuts to determine the exact mechanism behind low drip formation by Pi-Vac® system. The findings of future studies will encourage the red meat processors to adopt this technology in their processing and packaging facilities and obtain red meat cuts with low moisture losses.

2.3.1.3 Pulsed electric field

The pulsed electric field is a non-thermal technology that induces an electric field (kV/cm) on food placed between two electrodes (Barbosa-Cánovas & Sepúlveda, 2005). In PEF, energy is delivered into the meat as short wave pulses which can form temporary or permanent pores and result in loss of cell viability by a mechanism called electroporation (Zimmermann, 1986). This mechanism could alter the physical characteristics of meat.

O'Dowd, Arimi, Noci, Cronin, and Lyng (2013) and Faridnia et al. (2015) established that PEF treated beef *semitendinosus* muscles lost more weight as purge than non-treated control samples. Faridnia, Bremer, Burritt, and Oey (2016) and Alahakoon, Oey, Bremer, and Silcock (2019b) further confirmed these results by the studies conducted on beef *Biceps femoris* and brisket (*pectoral*) muscle samples, respectively. Both studies have observed significantly higher purge losses in PEF treated samples after 3 or 7 d of ageing compared to non-PEF treated samples. This may be due to the formation of pores in the cell membrane by electroporation and the increased flow of free water out of meat by electric field enhancement (Alahakoon, Oey, Bremer, & Silcock, 2019a; O'Dowd et al., 2013). Faridnia et al. (2016) observed greater myofibril ruptures along the Z line, and degraded myofibril organization in PEF treated samples

compared to untreated samples. This could have further decreased the muscle's ability to retain its moisture.

Studies on cold-boned beef muscles have demonstrated that purge loss percentage increased linearly with an increase in applied voltage (Bekhit, van de Ven, Suwandy, Fahri, & Hopkins, 2014; Khan et al., 2017). Bekhit, Suwandy, Carne, van de Ven, and Hopkins (2016) found similar results on hot-boned beef loins and topsides in which purge loss percentage increased by 1.38% for every extra application of PEF. An increase in voltage or repetition in PEF treatment resulted in high water loss either because of protein denaturation caused by high-temperature generation (Bekhit et al., 2014; Khan et al., 2017) or physical damage caused by super-contraction of sarcomeres (Suwandy, Carne, van de Ven, Bekhit, & Hopkins, 2015a). However, only in one study Suwandy, Carne, van de Ven, Bekhit, and Hopkins (2015c) have reported that the purge loss of cold-boned beef loin and topside samples were not increased proportionately with repeated PEF treatments though the change in conductivity and temperature difference increased with the repetition of PEF treatments. The variation of results is not fully clear but may be due to the use of different sample sizes and PEF chambers.

On the contrary, Suwandy, Carne, van de Ven, Bekhit, and Hopkins (2015b), Suwandy et al. (2015a), and Arroyo et al. (2015) reported that PEF treatment did not influence the purge loss in cold-boned beef loins, hot-boned beef loins and beef *LTL* muscle respectively. These findings could have resulted due to the prevention of severe protein denaturation by the relatively low-temperature change observed during the treatment (Bekhit et al., 2016). Recent research by Bhat, Morton, Mason, and Bekhit (2019a) and Bhat, Morton, Mason, and Bekhit (2019b) on beef *Semimembranosus* and *Biceps femoris* samples respectively, further supports these findings. No effect of PEF applications was observed on the purge loss during the whole ageing period. Also, these recent two studies reported that PEF treatment did not significantly impact the myofibrillar fragmentation index (MFI) of muscles (Bhat et al., 2019a, 2019b). This might also be attributed to the water retention of muscles since PEF did not enhance the generation of pores. Although the use of PEF has been extensively studied in the laboratory for tenderising fresh red meat, its application in the industry is very limited. This could be due to the need of optimization of PEF parameters for product-specific

applications (Gómez et al., 2019) (e.g. different meat cuts from the same animal or different types of red meat). Therefore, it is vital to analyze the correlation between purge loss and other parameters such as a change in conductivity, temperature, and MFI in future research to understand better the mechanisms associated with purge loss of muscles.

2.3.1.4 High-pressure processing

High-pressure processing is another novel meat tenderization technique in which the meat is statically pressurized by a liquid transmitter (Simonin, Duranton, & De Lamballerie, 2012). This technique involves applying high hydrostatic pressure (100-800 MPa) at room temperature for a few minutes (Pinton et al., 2020) and can be applied to both pre-rigour and post-rigour meat. Although many studies have been conducted to determine the effect of HPP on meat tenderization and colour, only a few studies have evaluated the impact of HPP on moisture loss from meat. A previous study by Kim, Lee, Lee, Kim, and Yamamoto (2007) reported a significant reduction in WHC of beef *Semitendinosus* muscle treated at 200 – 400 MPa compared to the muscle treated at 100 MPa and non-treated samples. This finding was supported by Fernandez et al. (2007) who determined that raw beef pressurized at 650 MPa for 10 min presented the highest drip loss compared to other samples. Pressure above 200 – 400 MPa can induce myofibrillar protein aggregation and denaturation (Ma & Ledward, 2004) and result in high water loss from the muscle tissues.

Recent studies that evaluated the impact of HPP on the WHC of different beef muscles have reported similar findings. Marcos, Kerry, and Mullen (2010) and Marcos and Mullen (2014) found no impact of HPP at 200 MPa of beef *LTL* and *longissimus dorsi* samples, respectively, while samples pressurized at 400 and 600 MPa showed decreased WHC compared to non-pressurized control samples. The authors reported a negative correlation ($P < 0.01$) between WHC and sarcoplasmic protein solubility in both studies. This confirmed that the reduction in WHC could be due to the pressure-induced denaturation of sarcoplasmic proteins. Ma et al. (2019) also reported increased WHC of yak meat samples subjected to different pressure levels until 250 MPa but significantly decreased WHC of samples subjected to 350 and 450 MPa levels compared to untreated samples. In the same study, they have studied the impact of different HPP application time (250 MPa for 0 – 30 mins) on beef samples and have

determined improved WHC up to 20 mins processing and decreased WHC at longer processing times. This may be due to the application time induced sarcoplasmic protein denaturation.

The results of all the above studies are in contrast with the findings of Sun, Sullivan, Stratton, Bower, and Cavender (2017) who reported that beef *triceps brachii* samples subjected to 450 and 600 MPa showed improved WHC compared to non-pressurized samples. They have not studied the impact of HPP on protein solubility and even no shift in pH had been observed with the application of HPP treatments. Therefore, it is difficult to hypothesize a proper mechanism behind these findings. It is important to analyze the correlation between protein solubility and HPP application in future studies. This will provide a better understanding of the impact of HPP on water retention in meat. Also, most of the above studies have not assessed the impact of HPP on meat ageing and how this will impact the moisture loss of other types of red meat. Because all the studies have only evaluated the effect of HPP on different beef meat cuts, future research should focus on these factors since other red meat processors will not be able to achieve high-quality meat cuts without ensuring less purge inside the meat packages.

2.3.1.5 Ultrasound treatment

Another novel approach for meat tenderisation is the use of ultrasound (US) which can cause muscle fibre disruption through cavitation related mechanisms such as high temperature, pressure, shear and generation of free radicals (Jayasooriya, Torley, D'Arcy, & Bhandari, 2007). A limited number of studies have evaluated the impact of US treatment on WHC of red meat tissues.

Most of the authors (Carrillo-Lopez, Luna-Rodriguez, Alarcon-Rojo, & Huerta-Jimenez, 2019; Chang, Wang, Tang, & Zhou, 2015; Fallavena, Marczak, & Mercali, 2020) have reported decreased WHC in sonicated beef samples compared to control samples just after the completion of US treatment without ageing. Chang et al. (2015) subjected the beef samples for different sonication times; their findings indicated decreased WHCs with increased sonication times. Fallavena et al. (2020) studied the impact of variable US intensities and temperatures on the WHC of beef samples and determined that these parameters had no significant impact on muscle water loss.

Decreased WHC could have resulted due to the disruption of muscle cellular structure caused by US treatment. Ultrasound treatment affects membrane permeability and causes detrimental impacts on interfilamental spacing due to the changes in osmotic pressure and ionic strength (Fallavena et al., 2020).

A few studies have investigated the impact of US treatment on WHC of different beef muscles with ageing. Carrillo-Lopez et al. (2019) and Gonzalez-Gonzalez et al. (2020) reported improved WHC in sonicated samples with increased ageing. Both studies determined increased WHC values with ageing compared to unsonicated samples. The pH of the samples subjected to ultrasonication was not as low as the samples which were not subjected to ultrasonication (Carrillo-Lopez et al., 2019). The combined effect of time and power of the US may have led to increasing pH of myofibrillar proteins; hence the WHC was improved by increasing muscle pH (Amiri, Sharifian, & Soltanizadeh, 2018). Ultrasonication causes polymerization of myosin due to moderate oxidation which could also lead to an improvement in WHC with ageing (Peña-Gonzalez, Alarcon-Rojo, Garcia-Galicia, Carrillo-Lopez, & Huerta-Jimenez, 2019). Ultrasound treatment may affect different muscles differently. Gonzalez-Gonzalez et al. (2020) reported low WHC in *longissimus lumborum* samples compared to *infraspinatus* and *cleidooccipitalis* samples subjected to the same sonication treatments and aged for the same period. This could be attributed to the lowest pH reported in *lumborum* samples compared to the other two samples and the higher impact of the US on the chemical structure of the myofibrillar proteins of *lumborum* samples.

In contrast, Jayasooriya et al. (2007) reported no significant effect of US treatment on ageing-induced drip loss of beef muscles. This is in agreement with the recent findings by Peña-Gonzalez et al. (2019) who found that the US treatment did not affect WHC of aged beef samples. Jayasooriya et al. (2007) initially treated samples with US followed by ageing, but Peña-Gonzalez et al. (2019) allowed the samples to age and then treated them with US. Therefore, it can be assumed that regardless of whether US is applied pre or post ageing, it does not influence the muscle WHC.

Based on the above findings, in general, US treatment reduces WHC of muscles just after they were subjected to the sonication treatment due to the fibre disruption through cavitation related mechanisms. However, it does not negatively influence the ageing-

induced WHC due to the protective effect against the ageing-induced decline of pH. Further research will help determine the proper mechanisms associated with US treatment and WHC, mainly focusing on the correlation between US parameters and meat tissue parameters associated with WHC, such as pH and protein solubility. It is essential to conduct studies to evaluate the impact of US treatment and its varying parameters on WHC of other red meat types as well because all the studies mentioned above have only evaluated the influence of the US on different beef tissues. The determination of WHC by other methods such as purge and drip loss should also be considered in future studies since all the studies except for one (Jayasooriya et al., 2007) have evaluated WHC via the filter paper press method. This will help to determine whether US treatment affects the WHC by centrifugal and gravitational methods differently or not.

2.3.2 Edible coatings

Any type of thin layer material used for coating of food to extend its shelf life which can be consumed together with the food is defined as an edible coating (Dehghani, Hosseini, & Regenstein, 2018; Hassan, Chatha, Hussain, Zia, & Akhtar, 2018). Edible coatings can reduce water loss and minimise the loss of saleable meat weight. Table 2.5 lists previous studies that have examined the effect of edible coatings on the moisture loss of red meat cuts.

Table 2.5 Summary of research to examine the effect of edible coating application on the moisture loss of red meat

Type of coating application	Meat	Type of the muscle or the meat cut	Coating formulations and packaging conditions	Measurement	Ageing period	Main findings	Reference
Gelatine	Beef	Tenderloins	- 20% bovine gelatine - MAP (80% O_2/20% CO_2)	Purge	14 d	Coated meat had significantly lower purge values compared to uncoated meat	(Antoniewski et al., 2007)
	Beef	Eye round	- 3% and 6% gelatine, 0.5% and 1.0% chitosan and 6% glycerol - No packaging	Weight loss %	5 d	Coated meat had significantly lower weight loss compared to uncoated meat. Gelatine was the agent mainly contributed to the reduction in weight loss	(Cardoso et al., 2016)
	Beef	*LD* [a]	- Gelatine solution enriched with henna aqueous extract - No packaging	Weight loss %	8 d	Coated meat had significantly lower purge values compared to uncoated meat	(Jridi et al., 2018)
Chitosan	Beef	*LD* [a]	- Electrospun chitosan nanofiber mats - No packaging	Weight loss %	21 d	Meat wrapped with fibre mats had significantly lower weight loss compared to unwrapped meat	(Gudjonsdottir et al., 2015)

[a] *longissimus dorsi*

Few studies have evaluated the impact of edible coatings on the weight loss of beef. Antoniewski, Barringer, Knipe, and Zerby (2007) and Jridi et al. (2018) coated beef steaks with 20% bovine gelatine and gelatine enriched with henna *(L. inermis)* extract, respectively, and found that the weight loss was significantly lower for coated samples compared to uncoated samples at the end of the storage period. This is also in agreement with the findings of Cardoso et al. (2016) who studied the effect of gelatine, chitosan and glycerol blends on the weight loss of beef eye round cuts at retail display conditions. Low weight loss values associated with coated samples have been achieved mainly due to the low water permeability of the coatings and gelatine's ability to act as a moisture-sacrificing agent (Cardoso et al., 2016) by attracting moisture via hydrophobic amino acids (Jridi et al., 2018). Though the studies mentioned above have achieved a reduction in moisture loss, edible coating treatments have not yet been implemented at the industrial level. Four major constraints for preventing the application of the edible coating in the meat industry include;

- The negative implication of edible coatings on other important meat quality parameters such as colour and odour which can lead to consumer dissatisfaction.

- The suitability of edible coatings combined with packaging treatments such as MAP and VP has not yet been evaluated. Most of the previous studies have evaluated the effectiveness of edible coating application on retail display conditions without any packaging treatment.

- The impact of edible coatings on consumer safety has not yet been extensively studied.

- The industrial applicability of proposed edible coating treatments is limited.

Nanotechnology applications are also slowly being realized in the food industry. Gudjónsdóttir et al. (2015) were able to achieve significantly lower weight loss values for beef steaks wrapped with electrospun chitosan nanofiber mats. Given that raw red meat products are highly perishable, the application of edible coatings and edible coatings formulated with nanoparticles to address this issue should be investigated in-depth. These studies should mainly focus on their ability to reduce moisture loss via either constriction of cut ends of meat capillaries or blocking the openings of drip

channels which could be effective in all types of meat cuts and versatile applicability through all types of red meat processing and packaging facilities so that the industry can implement these processers. In addition to edible coatings, biodegradable polymers such as cellulose, acacia gum, chitosan and alginate could be used to develop absorption pads and wraps which can be used in red meat packaging. These materials have high water absorption capacities and will help satisfy the consumers interested in consuming eco-friendly products.

2.4 Conclusion

Moisture loss affects important quality parameters of red meat such as juiciness, tenderness, and product appearance, affecting saleable weight and profitability. A detailed understanding of post-slaughter treatments carried out at meat processing, and packaging facilities that influence the WHC is essential to achieve a product with less purge accumulation. The impact of each post-slaughter treatment on purge or drip loss depends on the method used and the parameters associated with each technique, the animal species and the type of muscle itself. Novel methods such as meat tenderization techniques and edible coating applications are also being tested in red meat processing and packaging lines to evaluate their impact on moisture loss. Based on the current literature, future research should focus on evaluating the cumulative impact on moisture loss from different post-slaughter treatments and how moisture loss varies with changes in the method and the parameters linked with each technique. In addition, while current research has mainly focused on beef, lamb and deer meat, further work on other red meat species such as goat and buffalo should also be conducted, particularly those techniques that can be easily adopted by the industry relatively easily. There are no published studies up to now that confirm a method that can be applied to all red meat cuts to prevent moisture loss, while maintaining or enhancing other quality parameters, shelf-life and safety that is industrially applicable. Therefore, it is important to research on the application of edible coating which can physically stop moisture loss in all types of meat cuts as this technique can be designed to maintain or enhance all quality parameters and safety.

Chapter 3. Effectiveness of gelatine and chitosan spray coating for extending shelf life of vacuum-packaged beef

Information contained in this chapter has been published and been modified at thesis examination as follows:

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Abstract

The effects of applying gelatine (10%) and chitosan (1%) as spray coatings to extend the shelf life of vacuum-packaged beef was studied and compared against uncoated vacuum-packaged beef for up to 21 d. The impact of edible coatings on the water-holding properties of vacuum-packaged beef was investigated by LF-NMR together with physicochemical, microbiological and sensorial assessments. Chitosan coating significantly reduced the lipid oxidation of meat by ~30 - 36% and inhibited the growth of lactic acid bacteria in purge by ~ 1 - 2 log cfu compared to purge collected from uncoated and gelatine coated samples. It did not negatively affect the meat pH, tenderness and colour, and exhibited significantly higher sensorial acceptance compared to beef coated with gelatine. Both coatings were not effective in reducing purge loss in vacuum-packaged meat as the storage time increased. This study suggests that chitosan spray coating could be easily adapted to industrial plant settings as an antioxidant and antimicrobial application before carrying out the meat vacuum packaging operation.

3.1 Introduction

Vacuum packaging of fresh beef is commonly used to delay microbial and physicochemical degradation which facilitates the transportation of meat to distant markets. The shelf life of vacuum-packaged meat can be further extended by super-chilled storage (-1.5 °C) compared to frozen (-18 °C) and conventional chilled storage (2 °C) due to its ability to minimize structural damages, protein denaturation and delay bacterial growth (Chen et al., 2020). However, vacuum packaging causes greater purge

loss in beef compared to aerobic packaging (Sekar, Dushyanthan, Radhakrishnan, & Babu, 2006) and modified atmospheric packaging (Yang et al., 2016) and accumulated purge may increase bacterial spoilage by acting as a growth substrate (Lagerstedt, Ahnstrom, & Lundstrom, 2011; Rooyen, Allen, Kelly-Rees, & O'Connor, 2018). Gedarawatte et al. (2020) reported that purge from vacuum-packaged beef had significantly higher lactic acid bacteria (LAB) and *Brochothrix thermosphacta* counts compared to the meat surface.

Few studies have evaluated the impact of combined treatment of edible coatings and vacuum packaging on beef quality parameters. Antoniewski, Barringer, Knipe, and Zerby (2007) and Duran and Kahve (2020) evaluated the impact of gelatine and chitosan spray coatings on quality parameters of vacuum-packaged beef, respectively. Antoniewski et al. (2007) reported there was no significant change in the weight loss of gelatine coated and vacuum-packaged meat during storage for 14 d. Duran and Kahve (2020) found a significant reduction in oxidative and proteolytic degradation of chitosan coated and vacuum-packaged meat compared to vacuum-packaged meat only. Antoniewski et al. (2007) only assessed the impact of gelatine coating on weight loss, colour and lipid oxidation of meat while Duran and Kahve (2020) only evaluated the impact of chitosan coating on lipid oxidation, proteolytic degradation and microbiological safety of meat. Both these studies lacked a detailed investigation on the impact of edible coating on water-holding properties, sensorial and overall physicochemical properties of vacuum-packaged meat.

Gelatine is one of the most common edible coating materials used in meat products due to its film forming ability, high water-binding capacity, low cost and high availability and has been evaluated in many other studies for its effectiveness in controlling purge loss in aerobic packaging conditions (Antoniewski et al., 2007; Cardoso et al., 2019; Cardoso et al., 2016; Jridi et al., 2018). Chitosan is a natural polysaccharide that is formed by deacetylation of chitin and has been used as an edible coating for beef during aerobic storage (Abdallah, Mohmaed, Mohamed, & Emara, 2017; Cardoso et al., 2019; Cardoso et al., 2016; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018) and modified atmosphere storage (Langroodi et al., 2018). Although chitosan and gelatine have been assessed as potential edible coatings for controlling purge loss under retail display conditions (aerobic storage), their use as edible coatings

for vacuum-packaged meat (anaerobic storage) has not yet been fully investigated. In all the studies above, meat has been stored at traditional refrigeration conditions (4 °C) and the impact of coatings on meat stored at super-chilled storage (-1.5 °C) conditions has not been assessed. In addition, coatings have been applied as films by a dipping technique and not by a spraying technique. A spray technique is more effective than dipping when adapting the coating application for industrial settings. Given that vacuum packaging causes the highest purge loss among all meat packaging applications, controlling purge loss and an associated reduction of microbial growth in purge will help red meat processors to further extend the shelf life of meat. These materials would be suitable substrates for controlling purge loss in vacuum-packaged beef as they have been effective in controlling purge loss under retail display conditions by acting as water barriers (Cardoso et al., 2019; Cardoso et al., 2016). This study investigated the potential of chitosan and gelatine coatings to control purge loss, bacterial numbers in purge, and other physicochemical parameters of beef stored at super-chilled storage conditions.

3.2 Materials and methods

3.2.1 Experimental setup

3.2.1.1 Preparation of gelatine and chitosan spray coating solutions

A series of different concentrations of gelatine and chitosan solutions were tested in a preliminary study to determine the optimum concentration of each solution which could be easily applied as a spray and had minimal impact on the organoleptic properties of the meat. The gelatine solution was prepared according to Antoniewski et al. (2007) with some modifications. A 10% (w/v) gelatine (type B) solution was prepared by dissolving 20 g of gelatine (Thermo Fisher, Victoria, Australia) in 200 ml of distilled water at 70 ºC and stirred for 30 mins until completely dissolved. Type B gelatine is likely to cause minimal alteration to the structure of the beef because of its bovine origin and closeness of its isoelectric point (pH ~ 4-5) (Ramos, Valdes, Beltran, & Garrigós, 2016) to the isoelectric point of beef (pH ~ 5) (Offer et al., 1989). Fish gelatine is less stable compared to the gelatine from the mammalian origin (Ramos et al., 2016) which further justified the selection of bovine gelatine.

The chitosan solution was prepared as previously described by Abdallah et al. (2017) with slight modifications. A 1% (w/v) chitosan solution was prepared by dissolving 2 g chitosan (low molecular weight (50-190 kDa), Sigma-Aldrich, St. Louis, USA) in 200 ml of 1% acetic acid (Chem-Supply, Gillman, Australia) followed by stirring at 60 ºC for 1 h. The use of acetic acid as a food additive is widely accepted and considered as Generally Recognized As Safe (GRAS) for consumption by the US Food and Drug Administration (FDA, 2020), Food Standards Australia New Zealand (FSANZ, 2019), and Food Standards Agency UK (FSA, 2020). When the chitosan was fully dissolved, 10 ml of glycerol (Chem-Supply, Gillman, Australia) and 0.4 ml of Tween-80 (Chem-Supply, Gillman, Australia) were added as a plasticizer and emulsifying agent, respectively, and the mixture was stirred at 60 ºC for a further 30 mins.

3.2.1.2 Steak preparation and coating treatments

Beef eye round (*Musculus semitendinosus*) primal cuts were sourced from a local butcher (Perth metropolitan area, Western Australia, Australia) within 24 h post-slaughter. All visible fat and connective tissues were trimmed off under aseptic conditions and a total of 84 steaks (10 cm x 8 cm x 1.5 cm) were prepared. Three groups of 28 steaks each, with steaks randomly assigned to each group, were treated as follows;

Group A - Uncoated and vacuum-packaged (UNC)

Group B - Coated with 10% gelatine and vacuum-packaged (GEC)

Group C - Coated with 1% chitosan and vacuum-packaged (CHC)

The GEC and CHC samples were spray coated by using a spray gun (W590 Flexio, Wagner GmbH, Markdorf, Germany) at 0.18 litre/min flow rate with the horizontal flat jet nozzle (1.8 mm nozzle opening) position. The spray gun was positioned about 45 cm directly vertical to the steak samples and the samples were flipped manually to coat both sides. The spraying of each solution was conducted for about 2 mins to ensure the complete coverage of all steaks. The steaks subjected to GEC treatment were allowed to fully dry under a laminar airflow (HH 48, Holten LaminAir, Thermo Fisher Scientific, Bath, UK) at an air velocity of 0.40 – 0.45 m/s for 30 mins. The

steaks subjected to CHC treatment were placed in a refrigerator at 4 ºC for 30 mins in order to remove the excess coating solution. After completion of drying, the samples were vacuum-packaged (easyPACK-mk2, Webomatic, Bochum, Germany) using vacuum bags (Vital Packaging, Perth, Australia) which had a thickness of 65 µm, oxygen transmission rate of < 40 cc/m^2/24 h at 25 ºC and moisture vapour transmission rate of < 7 g/m^2/24 h at 38 ºC. The control samples (UNC) were uncoated and directly vacuum-packaged (vacuum level maintained at level 10 which is the maximum) as described above. All the samples were stored at - 1 ºC ± 1.0 ºC for 3 weeks and seven steaks were randomly sampled from each treatment at each sampling point (day 1, 7, 14 and 21) and subjected to the analysis as described in section 3.2.2.

3.2.2 Analytical methods

3.2.2.1 Purge loss and drip loss analysis

Purge loss was measured as per Cardoso et al. (2016) with slight modifications. The samples were removed from the vacuum bags, gently blotted with filter paper to remove excess purge on the surface and weighed. The results were expressed as percentage purge loss relative to the initial weight (day 0). Drip loss was measured as per Honikel (1998) with some modifications. The samples were removed from vacuum bags; they were weighed and placed in containers on the supporting mesh and the containers were closed tightly to ensure no airflow over the samples. The containers were stored at 4 ºC ± 1.0 ºC for 18 h. After 18 h, the samples were reweighed and the results were expressed as drip loss relative to the weights measured after opening the vacuum bags. The analysis of cooking loss was beyond the scope of this study as the key focus of this project was to determine the moisture loss of the vacuum packaged beef during storage at sub-zero chilling temperature conditions.

3.2.2.2 Low-field nuclear magnetic resonance

Transverse relaxation time (T_2) analysis was performed using a benchtop nuclear magnetic resonance analyser (12 MHz; GeoSpec, Oxford Instruments, Abingdon, UK) to assess the water distribution in beef tissues. The T_2 analysis was carried out according to the method described by Gedarawatte et al. (2020) without any modifications. Approximately 3 g of sample was cut from each treatment, placed in a

sample tube (10 mm) and analyzed with a Carr–Purcell–Meiboom–Gill pulse sequence which had a τ value of 100 µs. In each scan, 5500 echoes were collected with 8 repeated scans for each. The recycling delay was 6 s and the collected data was analyzed to produce T_2 probability distributions using in-house regularization techniques (REGEDS software) as per Fridjonsson, Hasan, Fourie, and Johns (2013).

3.2.2.3 Meat pH determination

Meat pH was measured by directly inserting a calibrated pH probe (standard buffers of pH 4.0 and 7.0, TPS Pty. Ltd., Brisbane, Australia) into the sample as mentioned by Kim, Kemp, and Samuelsson (2016).

3.2.2.4 Meat colour analysis

Meat colour was analysed soon after removing from the vacuum bag (pre-blooming conditions) and after letting the meat bloom at refrigeration temperatures (4 ºC ± 1.0 ºC) for 1 h (post-blooming conditions). Colour measurements (L^* (lightness), a^* (redness) and b^* (yellowness)) from three random locations of each sample was taken by a BYK colourimeter (BYK-Gardner GmbH, Geretsried, Germany). The instrument had a D 65 light source and a 10° observer with an 11 mm aperture and it was calibrated as described in Gedarawatte et al. (2020) before taking any measurements.

3.2.2.5 Tenderness

Meat blocks with the dimensions of 6 × 6 × 3 cm were cut from each treatment, cooked and cooled according to Li et al. (2012) without any modifications. Six cubes (approximately 1 × 1 x 1 cm) were obtained from each meat block and sheared perpendicular to the grain of the muscle fibres with a V-shaped Warner–Bratzler shear blade (p-WBT) attached to a Perten texture analyser (TVT 6700, Perten instruments, Hägersten, Sweden). The blade was maintained at a crosshead speed of 4 mm/s. The average peak force values were expressed as load in Newtons (N).

3.2.2.6 Lipid oxidation

The lipid oxidation of meat was evaluated by measuring 2-thiobarbituric acid reactive substances (TBARS) as per Cardoso et al. (2016) without any modifications. The

samples were removed from each treatment every 7 d, immediately frozen at -80 ºC and analysed within 3 d. The absorbance measurements, TBARS values reporting and standard curve preparation were carried out as mentioned by Gedarawatte et al. (2020) without any modifications.

3.2.2.7 Microbial analysis

Meat (at day 1, 7, 14 and 21) and purge (at day 7, 14 and 21) samples were collected aseptically and tested for LAB and *Brochothrix thermosphacta.* The presence of *E. coli* was also assessed in meat samples on day 1 and 21 and in purge samples on day 7 and 21.

Briefly, a 10 g sample of meat trimmings was placed in 90 ml of buffered peptone water (BPW; Oxoid Ltd., Hants, UK) and homogenized for 2 mins using a homogenizer (PRO250, PRO Scientific Inc., Oxford, USA). A 1:10 dilution of purge was prepared using BPW and homogenized as described above. Decimal dilution series of both meat and purge samples were prepared and spread plated onto de Man, Rogosa and Sharpe (MRS; Oxoid Ltd., Hampshire, England) agar for LAB, Streptomycin-Thallous Acetate-Actidione (STAA; Oxoid Ltd., Hampshire, England) agar for *B. thermosphacta* and Eosin-Methylene Blue (EMB; Oxoid Ltd., Hants, UK) agar for *E. coli*. Plates were incubated at 30 °C ± 1°C for MRS (120 ± 3 h), 22 °C ± 1°C for STAA (48 ± 3 h) and 37 °C ± 1°C for EMB (24 ± 3 h) and counts were reported as $\log_{10}$CFU/g for meat samples and $\log_{10}$CFU/ml for purge samples.

3.2.2.8 Sensory analysis

The sensory analysis of raw beef was carried out by a semi-trained panel on day 1, 10 and 21. The panellists were selected, trained and sensory analysis was conducted according to the methods suggested by AS 2542.1.3:2014 (Standards Australia, 2014) and Lawless and Heymann (2010). The sensory panel consisted of 4 males and 5 females between the ages of 25 to 55 years. The panellists were trained on beef colour standards, odour, texture and firmness standards and descriptive tests in two training sessions. Ethics approval was obtained from the Curtin University Human Research Ethics Committee (Approval number: HRE2019-0038) and informed consent from each panellist was obtained before conducting the sensory assessment. Panellists were

screened based on whether they purchase and consume beef fortnightly. Beef samples were allowed to bloom at 4 ºC for 1 h before they were presented to the panellists. The purpose was to mimic retail display conditions and the panellists were asked to assess the samples for their colour, odour, firmness and overall acceptability using the general Labeled Magnitude Scale as described by Kalva, Sims, Puentes, Snyder, and Bartoshuk (2014). The panellists were instructed that the scale covered hedonic experiences from the strongest disliked experience imaginable to the strongest liked experience imaginable. They were asked to mark the scale which was a structured line of 15 cm (Appendix D; separate scale for each parameter) with the sample number assigned for each treatment based on their preferences. The score for each sample was defined by measuring the length from the bottom of the scale to the tic mark indicated by the panellists.

3.2.3 Data analysis

All the experiments were carried out in triplicate and data analysis was performed using IBM SPSS Statistics version 26 software. The data of each treatment during the storage period and the data of all the parameters (except sensory evaluation) at each storage day were analysed using one-way analysis of variance (ANOVA) to assess the impact of storage time and compare the results among three different treatments respectively. Data of sensory analysis during the storage and comparison among three treatments was carried out using Repeated Measure ANOVA. If a significant ($P < 0.05$) difference was found, Tukey comparison test was performed to further analyse the mean values. A two-way analysis of variance (ANOVA) was performed to determine whether there is any impact of the interaction between treatment and storage time on the meat quality attributes.

3.3 Results and discussion

3.3.1 Water-holding properties

The effect of storage time and coating treatments on purge loss of beef is presented in Figure 3.1. On average, there was a 3.2% increase in purge loss in uncoated steaks over 21 d, which was not significantly different from day 1. Gelatine and chitosan coated samples showed a 2.5% and 2.0% increase in purge loss over 21 d, respectively.

Colle et al. (2015) and Holman, Bailes, Kerr, and Hopkins (2019) reported increased purge losses in uncoated beef under vacuum packaging conditions over time. Storage time induced purge loss could be due to protein oxidation which causes low water-holding capacity (WHC) in muscle tissues (Traore et al., 2012). Protein oxidation is responsible for the loss of functional groups and formation of intra and inter protein disulphide cross-linking which impairs protein functionality to bind water molecules (Kim et al., 2018).

Gelatine and chitosan coated samples had significantly less purge loss compared to uncoated samples only on day 7. This indicates that edible coating treatments are not effective in controlling the purge loss and purge accumulation as the storage time increases. Gelatine and chitosan coatings have shown their effectiveness in controlling purge loss under aerobic storage conditions by acting as water barriers and moisture-sacrificing agents (Antoniewski et al., 2007; Cardoso et al., 2019; Cardoso et al., 2016). However, vacuum packaging causes significantly higher purge loss compared to aerobic packaging due to the physical compression caused by pressure applied during the vacuum packaging process (Sekar et al., 2006). Therefore, higher purge loss caused by vacuum packaging itself and increased storage time may not be controlled by coating materials as the purge loss may exceed the moisture-retaining threshold limit of the coating materials.

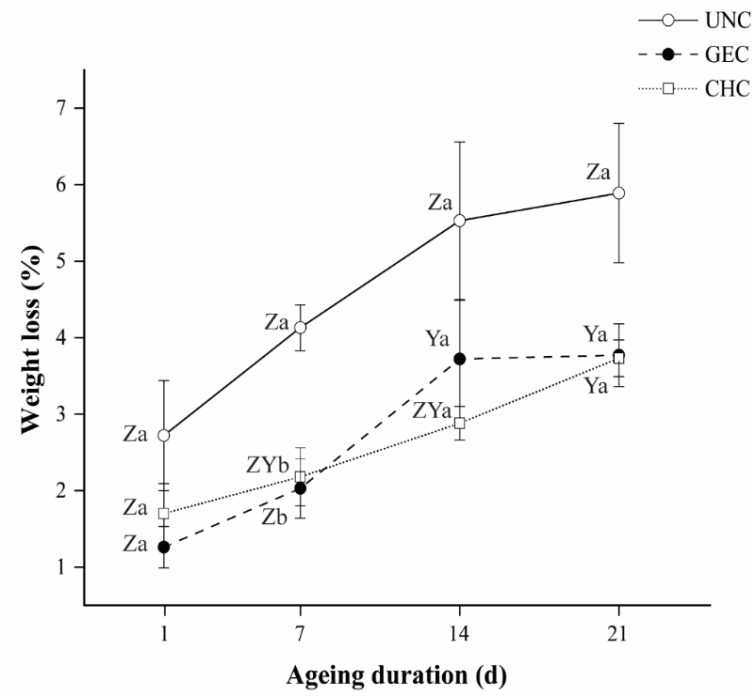

Figure 3.1 Changes in purge loss (%) of coated and uncoated samples at day 1, 7, 14 and 21. UNC: uncoated and vacuum-packaged; GEC: 10% gelatine coated and vacuum-packaged; CHC: 1% chitosan coated and vacuum-packaged. Different uppercase letters (Z-X) mean significant differences among different storage periods ($P < 0.05$). Different lowercase letters (a, b) mean significant differences in between treatments ($P < 0.05$).

No significant changes in drip loss were observed in all three treatments during the storage period (Table 3.1). Also, no significant differences in drip loss were found between different treatments in all storage days, except for day 21. Drip is the proteinaceous exudate that is lost from meat if the meat is hung in a closed container for a specific period at 4 °C (Honikel, 1998), whereas purge is the exudate that forms during storage and accumulates in the package. During vacuum-packaged storage, the majority of myowater is lost and this loss is recorded as purge loss. The remaining water is lost as drip when the meat is hung and stored for a specific period (Honikel, 1998). Our data shows that moisture loss due to drip is substantially smaller than moisture loss due to purge in vacuum packaging and this could be the reason why no significant differences were found in drip loss during storage and between treatments. No previous studies have looked at the purge loss and drip loss difference in vacuum-packaged uncoated and coated meat. Therefore, it may be more important to control the loss of purge instead of drip in vacuum packaging to minimize the financial damage associated with weight loss.

According to T_2 measurements (Table 3.1), two key water populations were detected in all meat samples, a major population between 40 and 52 ms (T_{21}), which corresponds to the intramyofibrillar water, and a minor population between 230 and 350 ms (T_{22}), which corresponds to the extramyofibrillar water. Peak areas of intramyofibrillar water and extramyofibrillar water are represented by A_{21} and A_{22}, respectively. The intramyofibrillar water population consists of immobilized water entrapped within the myofibrillar cell structure and the extramyofibrillar water population consists of more freely moving water within the myofibrillar network (Bertram et al., 2001). These results are similar to our previous findings for vacuum-packaged beef (Gedarawatte et al., 2020). No significant differences during storage were observed for all T_2 values (T_{21}, T_{22} and overall log mean transverse relaxation time T_{2lm}) in all samples. Gudjónsdóttir et al. (2015) also reported similar findings in beef which was vacuum-packaged and stored for 3 weeks. However, they observed a decreasing trend in the T_{21} and T_{2lm} values in beef which were unpackaged and stored for the same period. This may be due to the minimal muscle denaturation caused by storing under vacuum packaging conditions which contrasts with the significant protein denaturation caused by storing under aerobic conditions. This observation is in agreement with the tenderness results of the present study (Table 3.2) which shows that there were no significant differences in muscle firmness with the increase of storage time.

Table 3.1 Changes in drip loss (%) and LF-NMR relaxation parameters during vacuum-packaged storage of beef

Parameters	Treatment	Storage period (d)				SEM	P-value
		1	7	14	21		
Drip loss (%)	**UNC**	0.58 ± 0.02 Za	0.79 ± 0.07 Za	0.80 ± 0.05 Za	0.69 ± 0.04 Zab	0.04	0.073
	GEC	0.61 ± 0.08 Za	0.73 ± 0.04 Za	0.62 ± 0.04 Za	0.49 ± 0.03 Za	0.04	0.121
	CHC	0.84 ± 0.02 Za	0.97 ± 0.10 Za	1.03 ± 0.15 Za	0.83 ± 0.04 Zb	0.05	0.433
	P-value	0.055	0.189	0.110	0.017		
A_{21} (%)	**UNC**	97.90 ± 0.30 Za	94.15 ± 1.52 Za	95.88 ± 0.45 Za	95.94 ± 0.87 Za	0.56	0.108
	GEC	93.65 ± 0.61 Zb	94.44 ± 0.87 Za	94.66 ± 0.64 Za	95.96 ± 0.57 Za	0.37	0.163
	CHC	95.38 ± 0.68 Zb	97.29 ± 0.33 ZYa	96.71 ± 0.43 ZYa	97.81 ± 0.16 Ya	0.33	0.023
	P-value	0.005	0.134	0.078	0.082		
A_{22} (%)	**UNC**	2.10 ± 0.30 Za	5.86 ± 1.52 Za	4.12 ± 0.45 Za	4.06 ± 0.87 Za	0.56	0.108
	GEC	6.35 ± 0.61 Zb	5.56 ± 0.87 Za	5.34 ± 0.64 Za	4.04 ± 0.34 Za	0.37	0.163
	CHC	4.62 ± 0.68 Zb	2.71 ± 0.33 ZYa	3.29 ± 0.43 ZYa	2.19 ± 0.16 Ya	0.33	0.023
	P-value	0.005	0.134	0.078	0.082		
T_{21} (ms)	**UNC**	51.79 ± 0.00 Za	44.75 ± 0.00 Za	45.07 ± 3.79 Za	42.72 ± 2.03 Za	1.38	0.075
	GEC	44.75 ± 0.00 Zb	49.45 ± 2.35 Za	44.75 ± 0.00 Za	44.75 ± 0.00 Za	0.79	0.052
	CHC	44.75 ± 0.00 Zb	49.45 ± 2.35 Za	44.75 ± 0.00 Za	44.75 ± 0.00 Za	0.79	0.052
	P-value	0.000	0.216	0.993	0.422		
T_{22} (ms)	**UNC**	258.64 ± 00.00 Za	246.92 ± 11.73 Za	263.93 ± 35.43 Za	272.21 ± 13.57 Za	8.91	0.835
	GEC	285.79 ± 13.57 Za	315.07 ± 15.71 Za	317.20 ± 29.28 Za	285.79 ± 13.57 Za	9.38	0.511
	CHC	246.92 ± 11.73 Za	274.06 ± 25.30 Za	258.64 ± 00.00 Za	299.36 ± 00.00 Za	8.39	0.121
	P-value	0.089	0.101	0.298	0.296		
T_{2lm} (ms)	**UNC**	48.97 ± 0.91 Za	45.71 ± 2.14 Za	44.66 ± 3.00 Za	43.87 ± 1.46 Za	1.05	0.361
	GEC	47.26 ± 1.55 Za	49.74 ± 1.41 Za	47.64 ± 1.69 Za	44.12 ± 0.46 Za	0.84	0.103
	CHC	45.23 ± 1.54 Za	46.70 ± 2.85 Za	44.26 ± 1.35 Za	44.51 ± 0.56 Za	0.81	0.769
	P-value	0.230	0.455	0.512	0.891		

[a-b] Means within the same column for the same index with different lowercase letters differ significantly among the treatments ($P < 0.05$).

[Z-Y] Means within the same row with different uppercase letters differ significantly among different storage periods ($P < 0.05$).

UNC: uncoated and vacuum-packaged; GEC: 10% gelatine coated and vacuum-packaged; CHC: 1% chitosan coated and vacuum-packaged; A_{21} (%): population area ratio of T_{21}; A_{22} (%): population area ratio of T_{22}; T_{21} (ms): T_{21} relaxation time; T_{22} (ms): T_{22} relaxation time; T_{2lm} (ms): overall log mean transverse relaxation time; SEM: standard error of mean.

Table 3.2 Changes in pH, tenderness and lipid oxidation (TBARS) of beef during vacuum-packaged storage

Parameters	Treatment	Storage period (d)				SEM	P-value
		1	7	14	21		
pH	**UNC**	5.64 ± 0.03 Za	5.52 ± 0.03 Ya	5.44 ± 0.01 Xa	5.37 ± 0.01 Xa	0.03	0.000
	GEC	5.73 ± 0.07 Za	5.52 ± 0.01 Ya	5.48 ± 0.01 Yab	5.42 ± 0.04 Yab	0.04	0.004
	CHC	5.57 ± 0.02 Za	5.56 ± 0.03 Za	5.51 ± 0.03 Zb	5.51 ± 0.00 Zb	0.01	0.120
	P-value	0.131	0.410	0.022	0.019		
Tenderness (N)	**UNC**	39.47 ± 2.37 Za	36.30 ± 1.17 Za	36.97 ± 1.13 Za	34.90 ± 1.04 Za	0.79	0.228
	GEC	31.46 ± 1.55 Zb	35.61 ± 1.75 Za	33.86 ± 1.21 Za	33.06 ± 1.14 Za	0.74	0.260
	CHC	32.28 ± 0.74 Zb	35.29 ± 1.99 Za	33.20 ± 0.77 Za	30.73 ± 1.19 Za	0.69	0.117
	P-value	0.008	0.910	0.051	0.059		
Lipid oxidation (mg MDA/kg)	**UNC**	0.053 ± 0.00 Za	0.053 ± 0.01 Za	0.126 ± 0.00 Ya	0.281 ± 0.01 Xa	0.03	0.000
	GEC	0.082 ± 0.00 Zb	0.080 ± 0.01 Zb	0.133 ± 0.01 Ya	0.256 ± 0.02 Xa	0.02	0.000
	CHC	0.088 ± 0.00 Zb	0.079 ± 0.00 Zb	0.100 ± 0.00 Zb	0.179 ± 0.01 Yb	0.01	0.000
	P-value	0.001	0.095	0.001	0.005		

[a-b] Means within the same column for the same index with different lowercase letters differ significantly among the treatments ($P < 0.05$).

[Z-X] Means within the same row with different uppercase letters differ significantly among different storage periods ($P < 0.05$).

UNC: uncoated and vacuum-packaged; GEC: 10% gelatine coated and vacuum-packaged; CHC: 1% chitosan coated and vacuum-packaged; SEM: standard error of mean.

Furthermore, no significant differences in T_2 values were observed between treatments after one day of storage which indicates that coating treatments did not negatively impact muscle protein degradation. The amount of free water in the meat is represented by A_{22} % (Cheng et al., 2019) and, besides chitosan coated samples, no significant changes in this were found in UNC and GEC samples during storage. Similarly to the findings of Gudjónsdóttir et al. (2015), A_{22} % decreased significantly from 4.62 to 2.19 % in CHC samples within 3 weeks of storage. This trend negatively correlates to the trend of purge loss of CHC samples during the storage period. This suggests that the loss of free water is mainly responsible for the purge loss during the storage period. A similar pattern could not be observed in the A_{22} % of UNC and GEC samples, even though both these samples had a significantly increased purge loss during the storage period.

3.3.2 Physicochemical evaluation

The effect of storage time and coating treatments on meat pH is presented in Table 3.2. The uncoated and gelatine coated samples showed a significant ($P < 0.05$) reduction in pH from 5.64 to 5.37 and from 5.73 to 5.42, respectively, over the storage period. These findings are similar to the pH results for vacuum aged beef steaks reported by Gudjónsdóttir et al. (2015) and Gedarawatte et al. (2020). The decrease in meat pH could be attributed to the growth of LAB during anaerobic storage and is further discussed in section 3.3.3. Chitosan coated samples had consistent pH values throughout the storage period. This is similar to the findings of Clarke et al. (2017) who observed consistent pH values in beef sub-primal vacuum-packaged with antimicrobial coated polyamide films. This could be due to the significant reduction of LAB counts achieved in CHC samples compared to UNC and GEC samples (Figure 3.2C). Chitosan coating did not significantly impact the meat pH, indicating that chitosan is unlikely to create adverse effects on meat WHC or tenderness by altering the meat pH.

No significant differences in tenderness were observed in all samples subjected to three different treatments during the storage period (Table 3.2). Colle et al. (2015) also reported no differences in tenderness during the 63 d storage of vacuum-packaged *Gluteus medius* steaks. This was further confirmed by no significant changes in T_2 values and can be attributed to the minimal changes in soluble and insoluble collagen

during vacuum-packaged storage (Colle et al., 2015) at chilled temperatures (Sentandreu, Coulis, & Ouali, 2002). Coating treatments significantly improved the tenderness of meat samples on day 1, a finding which is in agreement with the results reported by Vital et al. (2016). This could be due to the significantly higher A_{22} % maintained by coated samples compared to the uncoated samples. This indicates that both coating treatments were effective in retaining free water and improving meat tenderness on day 1. However, the coating treatments have not significantly improved tenderness throughout the storage period which could be due to their inefficiency in retaining water loss during cooking or controlling the free water leakage over time. This is indicated by there being no significant differences in A_{22} % values between coated and uncoated meat samples after day 1.

TBARS values of all the samples increased after 14 d of storage (Table 3.2). Similar results have been reported by Mungure, Bekhit, Birch, and Stewart (2016) in a study conducted to evaluate vacuum ageing of hot boned *M. Semimembranosus* beef muscle. Ageing-induced lipid oxidation may result due to the decay of endogenous antioxidants with increased storage time (Mungure et al., 2016). Samples coated with gelatine and chitosan had significantly higher TBARS values compared to uncoated samples until the end of the 1st week of storage. The acidic nature of coating solutions (pH of gelatine and chitosan solutions was respectively 5.0 and 3.8) could have increased the susceptibility of meat to lipid oxidation (Ke, Huang, Decker, & Hultin, 2009). However, after 7 d of storage, this pH difference could not be observed and was probably due to purge exudate neutralising the coating solutions. Interestingly, chitosan coated samples showed significantly lower lipid oxidation compared to both uncoated and gelatine coated samples at day 14 and 21. Similarly, Xiong, Chen, Warner, and Fang (2020) observed significantly lower lipid oxidation in chitosan coated pork loin samples during cold storage and this could be attributed to the chitosan's ability to inhibit catalytic activity by chelating metal ions (Chen, Zheng, Wang, Lee, & Park, 2002). This suggests that chitosan may be more effective in controlling lipid oxidation in vacuum-packaged beef with the increase of storage time.

The coating treatments have significantly influenced the meat colour at both pre-bloom and post-bloom conditions (Table 3.3). All the samples showed stable lightness values at the end of the storage compared to day 1 results. This could be due to the combined

effect of vacuum packaging and super-chilled storage conditions (Chen et al., 2019). This is evident for both pre-bloom and post-bloom conditions, indicating that meat lightness was not impacted by the storage time. However, both gelatine and chitosan coated samples showed significantly lower lightness compared to uncoated samples after 14 d of storage and this was observed for both pre and post-bloom conditions. The L^* value measures the light reflectance from the surface; meat with water on the surface will have higher L^* values which could be the reason why the uncoated samples were lighter than the coated samples. The coating applications have significantly reduced light scattering at the end of the storage and made meat samples appear darker than the uncoated samples (Cardoso et al., 2019).

No significant changes in meat redness were observed in both uncoated and chitosan coated meat samples with an increase of storage time. This was observed under both pre and post-blooming conditions, which indicates that chitosan coating does not influence the meat redness with the increase of storage time. Conversely, gelatine coated samples had significantly lower redness after 14 d of storage at both pre-bloom and post-bloom conditions. In addition, gelatine coated samples were significantly less red compared to uncoated and chitosan coated samples on day 14 and 21. Antoniewski et al. (2007) evaluated the colour of gelatine itself and observed significantly reduced a^* values with increased storage time. Besides, gelatine coating may slow down the oxygenation process during post-bloom conditions, thereby reducing the formation of oxymyoglobin (Vital et al., 2016) by acting as a barrier between meat and the atmosphere. Therefore, gelatine coating may negatively influence the redness of meat as the storage time increases and this may result in dissatisfied consumers if it is applied for long-term vacuum package storage purposes. Chitosan coated samples showed significantly lower redness than other samples only on day 1 which may be associated with the high lipid oxidation values at the beginning of the storage period. High lipid oxidation diminishes the number of oxygen molecules available for the formation of oxymyoglobin and results in decreased redness. This condition was not observed after day 1 which may be related to decreased lipid oxidation (Table 3.2) as the storage time progressed and indicates that chitosan coating will be effective in preserving the redness of vacuum-packaged meat.

Table 3.3 Changes in colour parameters at pre-blooming and post-blooming conditions during vacuum-packaged storage of beef

Parameters	Treatment	Storage period (d)				SEM	P-value
		1	7	14	21		
*L** preb	**UNC**	47.83 ± 1.66 Za	50.85 ± 1.27 Za	50.48 ± 1.15 Za	50.82 ± 0.70 Za	0.65	0.321
	GEC	41.18 ± 1.20 ZYb	45.30 ± 0.96 XYb	39.11 ± 1.41 Zb	46.55 ± 1.10 Xb	1.04	0.007
	CHC	50.87 ± 0.74 ZYa	51.92 ± 0.67 Za	41.64 ± 1.80 Xb	46.45 ± 0.37 Yb	1.30	0.000
	P-value	0.004	0.007	0.004	0.012		
*a** preb	**UNC**	15.85 ± 1.23 Za	12.89 ± 0.55 Za	12.70 ± 1.57 Za	12.27 ± 0.69 Za	0.63	0.160
	GEC	15.56 ± 0.55 Za	14.05 ± 1.27 Za	6.44 ± 1.22 Yb	6.32 ± 0.11 Yb	1.34	0.000
	CHC	10.66 ± 0.09 Zb	10.81 ± 0.95 Za	12.30 ± 0.52 Za	11.68 ± 0.40 Za	0.32	0.233
	P-value	0.005	0.134	0.017	0.000		
*b** preb	**UNC**	17.20 ± 0.58 Za	15.77 ± 0.80 Za	15.48 ± 0.74 Za	14.69 ± 0.83 Za	0.42	0.195
	GEC	16.38 ± 0.81 ZYa	17.40 ± 0.54 Za	9.02 ± 0.74 Xb	13.91 ± 0.57 Ya	1.02	0.000
	CHC	15.37 ± 0.11 Za	16.10 ± 0.08 Za	11.64 ± 0.25 Yb	12.67 ± 0.40 Ya	0.57	0.000
	P-value	0.161	0.174	0.001	0.150		
*L** postb	**UNC**	46.05 ± 0.89 Za	49.73 ± 1.13 ZYab	49.32 ± 1.38 ZYa	51.79 ± 0.85 Ya	0.78	0.034
	GEC	43.34 ± 0.81 Za	45.24 ± 1.34 Za	38.89 ± 2.68 Zb	45.11 ± 0.22 Zb	1.02	0.064
	CHC	52.42 ± 0.40 Zb	51.66 ± 0.86 Zb	42.96 ± 2.37 Yab	46.14 ± 1.42 ZYb	1.34	0.005
	P-value	0.000	0.018	0.042	0.005		
*a** postb	**UNC**	17.85 ± 0.74 Za	18.49 ± 0.48 Za	13.78 ± 1.04 Ya	15.34 ± 0.30 ZYa	0.63	0.005
	GEC	17.15 ± 0.60 Za	15.41 ± 1.48 Zab	9.37 ± 0.94 Yb	10.03 ± 0.37 Yb	1.09	0.001
	CHC	11.39 ± 0.31 Zb	12.55 ± 1.52 Zb	15.35 ± 0.76 Za	14.93 ± 0.68 Za	0.63	0.065
	P-value	0.000	0.042	0.009	0.000		
*b** postb	**UNC**	17.60 ± 0.70 ZYa	19.73 ± 0.62 Ya	16.65 ± 0.28 Za	17.50 ± 0.12 Za	0.40	0.012
	GEC	17.74 ± 1.16 Za	18.79 ± 0.62 Za	11.97 ± 0.36 Yb	16.37 ± 0.14 Za	0.84	0.001
	CHC	15.95 ± 0.38 ZYa	17.10 ± 0.59 Za	13.91 ± 0.76 Yb	15.06 ± 0.41 ZYb	0.43	0.020
	P-value	0.297	0.057	0.002	0.002		

[a-b] Means within the same column for the same index with different lowercase letters differ significantly among the treatments ($P < 0.05$).

[Z-X] Means within the same row with different uppercase letters differ significantly among different storage periods ($P < 0.05$).

UNC: uncoated and vacuum-packaged; GEC: 10% gelatine coated and vacuum-packaged; CHC: 1% chitosan coated and vacuum-packaged; preb: colour evaluation at pre-blooming conditions (just after opening of the vacuum package); postb: colour evaluation at post-blooming conditions (after allowing to bloom at 4 ºC for 1 h); SEM: standard error of mean.

Uncoated meat maintained consistent yellowness throughout the storage period and this was apparent for both pre and post-bloom conditions. Both types of coated meat showed a significant reduction in yellowness after 7 d of storage under pre-blooming conditions. This was not evident once the meat samples had bloomed at the end of the storage period. No significant differences in yellowness were observed between uncoated and coated meat samples, except for day 14. As explained by Cardoso et al. (2019) and Xiong et al. (2020), the yellow colouration of coating solutions may have interfered with light reflectivity and caused differences in meat yellowness. However, this could not be observed at the end of storage period and suggests that the edible coating treatments will not negatively influence the yellowness of vacuum-packaged meat as the storage time increases.

3.3.3 Microbiological evaluation

Microbiological analysis of purge at day 1 could not be conducted due to the low amount of purge collected at the beginning of the storage. *Escherichia coli* were not detected in either meat or purge at any sampling point. These results are in agreement with the findings of Ercolini et al. (2011) and Gedarawatte et al. (2020) who did not detect *Enterobacteriaceae* in vacuum-packaged beef during the first 3 weeks of storage. Both meat and purge collected from all three treatments had a variable count of *B. thermosphacta* during the storage period (Figure 3.2A and B). Similar counts and variations of *B. thermosphacta* have been reported by Pennacchia, Ercolini, and Villani (2011) who evaluated the enumeration of spoilage bacteria populations in vacuum-packed beef for 20 d. Lactic acid bacteria showed a significant increase in counts in all meat samples during the storage period (Figure 3.2C). Clarke et al. (2017) also reported a significant increase in LAB counts in vacuum-packaged beef during chilled storage and this indicates that the vacuum packaging conditions were favourable for the growth of LAB (Reid et al., 2017). The decrease in pH (Table 3.2) reported in this study could have been due to the significant growth of LAB during storage has led to severe protein denaturation and higher purge losses.

No significant differences in *B. thermosphacta* or LAB counts were apparent between uncoated and gelatine coated meat and purge samples. Similar results have been reported by Azarifar, Ghanbarzadeh, and Abdulkhani (2020) who studied microbiological growth differences in beef which were unwrapped and wrapped with gelatine films. This indicates that gelatine coating does not enhance the bacterial growth under anaerobic conditions. Both meat and purge collected from chitosan coated samples had significantly lower counts of both organisms compared to uncoated and gelatine coated samples. This feature was observed for most of the days that the analysis was carried out. Duran and Kahve (2020) also reported significantly lower counts of LAB in chitosan coated vacuum-packaged meat than the meat only subjected to vacuum packaging. Chitosan acts as an antimicrobial agent against Gram-positive bacteria (Xiong et al., 2020) and inhibits bacterial growth by interacting with negative charges on the bacterial cell membrane, thereby permeabilizing the membrane which leads to leakage of intracellular substances (Elsabee & Abdou, 2013). Therefore, chitosan coatings may be effective in inhibiting the growth of bacteria in vacuum-packaged meat and could extend its shelf life.

3.3.4 Sensorial evaluation

Changes in visual sensory properties of meat during storage are shown in Table 3.4. The sensorial colour of uncoated samples was not significantly influenced by the storage time. The gelatine coated samples were scored the lowest in colour on day 10 and 21 compared to other samples which could be due to the significantly lower redness values they had after day 7. The chitosan coated samples had significantly lower scores for colour only on day 1 and the panellists found significant improvement in colour thereafter. These results are in agreement with the instrumental colour measurements which showed significantly lower post-bloom redness in chitosan coated samples only on day 1. According to colourimetric measurements improvements in colour was evident afterwards (Table 3.3).

There were no significant differences observed in firmness in uncoated and chitosan coated samples with the increase of storage time which is in agreement with the instrumental tenderness results. Despite the instrumental tenderness results, panellists found significant decreases in firmness in gelatine coated samples with increased storage time. It might be possible that panellists detected the gelatinous layer formed

by the coating around meat and reported a difference in firmness (Vital et al., 2016). No significant differences in odour were detected in all samples, as the storage time increased. This confirms that despite the ageing induced lipid oxidation and microbial growth, panellists could not detect any odour variations with the progression of the storage time since the TBARS values were well below the detectable levels (0.6 – 2.0) (Pabast et al., 2018). Chitosan coated samples had the most agreeable odour at day 10 and 21 compared to all the other samples confirming that barrier properties of chitosan were effective in preventing the transfer of odour substances once the vacuum packages were opened. This is in agreement with the sensory findings reported by Abdallah et al. (2017) on uncoated versus chitosan coated and dry-cured beef product.

Panellists found no significant difference in overall acceptability in both uncoated and chitosan coated samples with the increase of storage time. However, they found gelatine coated samples significantly less acceptable after the first week of storage and this was evident compared to all the other samples. This could be attributed to the significantly lower scores obtained by gelatine coated samples for both colour and firmness and indicate that the chitosan coating does not negatively impact the acceptability of the beef (Langroodi et al., 2018).

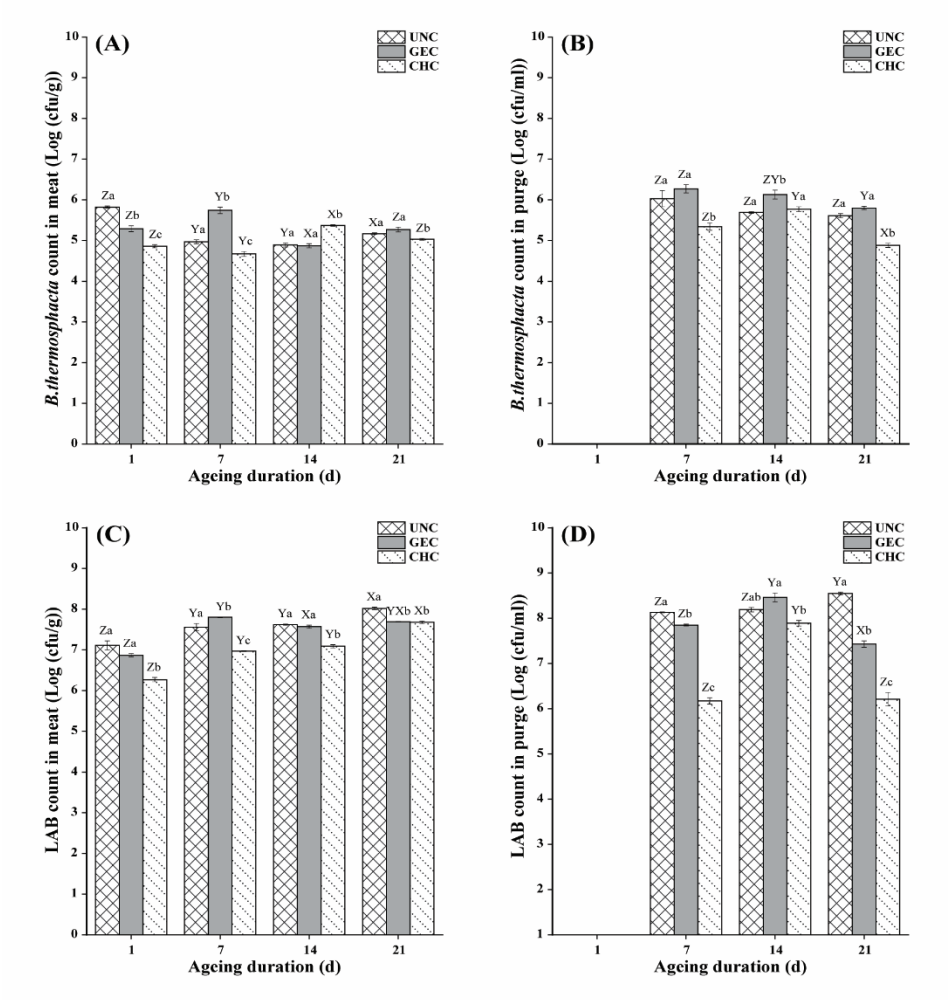

Figure 3.2 Changes in counts of *B. thermosphacta* of meat (a), *B. thermosphacta* of purge (b), lactic acid bacteria of meat (c) and lactic acid bacteria of purge (d) at day 1, 7, 14 and 21. UNC: uncoated and vacuum-packaged; GEC: 10% gelatine coated and vacuum-packaged; CHC: 1% chitosan coated and vacuum-packaged. Different uppercase letters (Z-X) mean significant differences among different storage periods ($P < 0.05$). Different lowercase letters (a, b) mean significant differences in between treatments ($P < 0.05$).

Table 3.4 Changes in sensory parameters during vacuum-packaged storage of beef

Parameters[A]	Treatment	Storage period (d)			P-value
		1	10	21	
Colour	**UNC**	14.35 ± 0.71 Za	13.12 ± 1.22 Zab	15.05 ± 0.74 Za	0.371
	GEC	16.68 ± 0.66 Za	10.97 ± 0.96 Ya	10.11 ± 1.33 Yb	0.001
	CHC	7.56 ± 1.24 Zb	15.84 ± 0.75 Yb	14.04 ± 1.11 Yab	0.000
	P-value	0.000	0.016	0.029	
Firmness	**UNC**	15.15 ± 0.75 Za	12.60 ± 1.37 Za	14.54 ± 0.96 Za	0.183
	GEC	15.47 ± 1.01 Za	10.87 ± 0.92 ZYa	10.27 ± 1.02 Yb	0.022
	CHC	11.74 ± 0.63 Zb	15.23 ± 1.07 Ya	13.98 ±0.90 ZYab	0.022
	P-value	0.002	0.065	0.018	
Odour	**UNC**	12.88 ± 0.93 Za	12.46 ± 0.97 Za	12.30 ± 1.32 Za	0.936
	GEC	13.30 ± 1.33 Za	9.49 ± 1.87 Za	9.60 ± 1.43 Za	0.122
	CHC	11.71 ± 1.05 Za	15.25 ± 0.89 Zb	12.99 ± 0.33 Za	0.052
	P-value	0.593	0.023	0.079	
Overall acceptability	**UNC**	14.34 ± 0.72 Za	12.09 ± 1.39 Zab	14.94 ± 1.03 Za	0.249
	GEC	15.64 ± 0.72 Za	8.60 ± 1.39 Ya	9.02 ± 1.03 Yb	0.004
	CHC	10.16 ± 0.72 Zb	15.17 ± 1.39 Zb	12.12 ± 1.03 Za	0.064
	P-value	0.002	0.022	0.050	

[a-b] Means within the same column for the same index with different lowercase letters differ significantly among the treatments ($P < 0.05$).

[Z-Y] Means within the same row with different uppercase letters differ significantly among different storage periods ($P < 0.05$).

UNC: uncoated and vacuum-packaged; GEC: 10% gelatine coated and vacuum-packaged; CHC: 1% chitosan coated and vacuum-packaged; SEM: standard error of mean. [A] Values represent the mean of nine independent replicates ± standard error.

3.3.5 Impact of interaction between storage time and treatments

A two-way ANOVA analysis was performed to find the impact of the interaction between storage time and treatments on the meat quality attributes (Table 3.5). The results indicate that there was no impact of joint interaction between storage time and treatments on purge loss, drip loss, T_{22}, T_{2lm} and tenderness. All the other meat quality parameters were impacted by the interaction between storage time and the treatments. This analysis was not carried out in the following chapters due to inconsistent results reflected in chapter 3.

Table 3.5 Impact of interaction between storage time and treatments on the meat quality attributes

Meat quality attribute	P-value
Purge loss %	0.840
Drip loss %	0.539
A_{21} (%)	0.011
A_{22} (%)	0.011
T_{21} (ms)	0.014
T_{22} (ms)	0.418
T_{2lm} (ms)	0.588
pH	0.008
Tenderness	0.184
Lipid oxidation	0.000
L^* preb	0.000
a^* preb	0.000
b^* preb	0.000
L^* postb	0.001
a^* postb	0.000
b^* postb	0.008
LAB count in meat	0.000
LAB count in purge	0.000
B.thermosphacta count in meat	0.000
B.thermosphacta count in purge	0.002

3.4 Conclusion

The results of this study showed that chitosan coating had a strong antimicrobial and antioxidant effect on meat as compared to uncoated and gelatine coated meat. Chitosan coating did not influence the other physicochemical attributes of meat and exhibited desirable sensory attributes over the storage period. Both coatings were only effective in controlling purge loss in short term storage. Gelatine coating significantly reduced meat redness and had lower sensorial acceptability compared to all other treatments. This study indicates that chitosan spray coating is more effective in preserving vacuum-packaged meat than using vacuum packaging alone. As this method relies on a simple spray coating application and the coating formulation does not affect the organoleptic properties of the product, this same methodology could be used to extend the shelf life of other red meat products. Edible coatings are not well optimised for industrial settings and spray techniques could help the industry to easily adapt coating applications to meat facilities.

Chapter 4. Effectiveness of bacterial cellulose in controlling purge accumulation and improving physicochemical, microbiological, and sensorial properties of vacuum-packaged beef

Information contained in this chapter has been published as follows:

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Abstract

The application of bacterial cellulose (BC) as a wrapping material for vacuum-packaged beef was studied and compared against unwrapped beef for up to 3 weeks. The impact of BC wrap on the weight loss, purge accumulation, and drip loss was assessed along with low-field nuclear magnetic resonance, physicochemical, microbiological, and sensorial evaluations. The BC wrap significantly ($P < 0.05$) reduced purge accumulation in vacuum packages which was confirmed by an increased swelling ratio and scanning electron microscopy images. Colourimetric measurements showed significantly ($P < 0.05$) increased redness and yellowness values in wrapped samples compared to unwrapped samples. BC wrap did not affect pH, tenderness, and odour of meat, but significantly ($P < 0.05$) increased lipid oxidation, and numbers of lactic acid bacteria and *Brochothrix thermosphacta* counts. This study shows that BC wrap has potential as a purge absorbent in vacuum-packaged meat.

4.1 Introduction

Purge can be defined as a red aqueous solution that exudes from the cut surface of a piece of meat during storage. One of the key challenges faced by the meat industry is reducing purge accumulation during the product shelf life. Excessive purge formation can cause economic losses due to the reduction of saleable product weight (Rooyen, Allen, Kelly-Rees, & O'Connor, 2018), rejection of product by the consumers (Kim, Warner, & Rosenvold, 2014) and loss of export markets. Loss of purge can severely impact meat juiciness and tenderness (Warner, 2017). Accumulation of purge inside

the meat packages causes an unattractive appearance to the product (Antoniewski, Barringer, Knipe, & Zerby, 2007; Hope-Jones, Strydom, Frylinck, & Webb, 2012). In addition, purge can jeopardize product shelf life and safety by providing a growth substrate for microorganisms (Lagerstedt, Ahnstrom, & Lundstrom, 2011).

The degree of purge loss significantly varies with the packaging technique deployed by different meat processors. Vacuum packaging is the most common packaging technique used for the transportation of meat to distant or export markets (Kerry, O'Grady, & Hogan, 2006). Several studies have shown that vacuum packaging results in greater purge loss in beef compared to modified atmospheric packaging (Hur, Jin, Park, Jung, & Lyu, 2013; Yang et al., 2016) and aerobic packaging (Sekar, Dushyanthan, Radhakrishnan, & Babu, 2006). High purge formation is mainly due to the physical compression of meat by the pressure applied during the vacuum packaging (Payne, Durham, Scott, & Devine, 1998).

Although several studies have investigated reducing purge loss via application of edible coatings or wraps, they have mainly evaluated the impact of these applications on beef at either retail display conditions (without vacuum packaging; Cardoso et al., 2016; Jridi et al., 2018) or dry-ageing conditions (Gudjonsdottir et al., 2015). To the best of our knowledge, only one study has evaluated the impact of coating (20% gelatine) on vacuum-packaged beef (Antoniewski et al., 2007). They found no significant change in the amount of purge in the vacuum-packaged beef during 14 d storage compared to control samples. However, no study has been conducted to evaluate the impact of edible wrapping treatment on controlling purge accumulation in vacuum-packaged beef. Therefore, it is appropriate to determine alternative methods to reduce the purge accumulation in vacuum-packaged meat.

Bacterial cellulose (BC) is a microbial polysaccharide with a nanofibrillar network produced by *Komagataeibacter xylinus.* Bacterial cellulose demonstrates beneficial mechanical properties such as high tensile strength, biocompatibility, biodegradability (Padrão et al., 2016) and water-holding capacity (WHC; Paximada, Tsouko, Kopsahelis, Koutinas, & Mandala, 2016). In contrast to plant cellulose, BC is free from lignin and hemicellulose (Helenius et al., 2006). Due to these unique properties, BC is widely used in a range of applications in the medical industry such as topical haemostatic agents, artificial microvessels, artificial skin, and scaffolds for tissue

engineering of cartilage (Helenius et al., 2006; Peng et al., 2011). In addition, BC is used in certain food applications such as “nata de coco”, mainly in Asian countries (Shi, Zhang, Phillips, & Yang, 2014). There are only a few published food application studies of BC which are mainly limited to sausage casings. Nguyen, Gidley, and Dykes (2008) used BC with nisin incorporated as a casing for vacuum-packaged frankfurters. Zhu et al. (2010) and Padrão et al. (2016) utilized BC with Ɛ-polylysine and lactoferrin incorporated, respectively, as casings for fresh sausages. In addition, Pirsa and Shamusi (2019) used BC modified by polypyrrole-ZnO nanocomposite as an intelligent and active packaging for chicken thigh meat. Jebel and Almasi (2016) and Malheiros et al. (2018) have developed ZnO nanoparticles loaded BC-films and bacteriocins immobilized BC-films as antimicrobial food active packaging, respectively. All these studies evaluated the use of BC as an intelligent or active food packaging. However, none have used BC as a packaging material in any type of vacuum-packaged meat. Almeida, Prestes, Woiciechowski, and Wosiacki (2011) have shown significant mass loss reduction in strawberries and apple slices coated with BC. Bacterial cellulose fibres have the ability to absorb fluids several times their own weight (Benedetto & Tarantino, 2014) and have been shown to be effective as a topical haemostatic agent. As such, researchers are exploring the use of styptic materials such as BC as potential packaging materials for meat (Ravensdale, Coorey, & Dykes, 2018). Though BC has been assessed as a potential active food packaging, its use as a purge absorption material in vacuum-packaged meat has not yet been investigated. This study will contribute to advances in packaging technology in the meat industry by providing an assessment of one of the most available and biodegradable substances as a potential meat packaging material. The aim of this research was to investigate the effectiveness of BC in controlling purge accumulation and improving physicochemical, microbiological and sensorial properties of vacuum-packaged beef.

4.2 Materials and methods

4.2.1 Experimental setup

4.2.1.1 Preparation of bacterial cellulose wrap

Bacterial cellulose sheets were prepared according to the method described by Tan, Rahman, and Dykes (2016) with some modifications. A primary inoculum of *Komagataeibacter xylinus* ATCC 53524 was propagated in Hestrin and Schramm (HS) broth medium. The media was adjusted to pH 5.0 with 5 M HCl (Merck, Darmstadt, Germany). The BC-sheets were manufactured in enclosed plastic vessels (27.5 cm X 20.0 cm X 4.0 cm) which were incubated statically at 30 °C for 120 h. The collected BC-sheets were washed with distilled water to remove excess media, chemically treated to remove bacterial by-products and any remaining media as per the Harris, Serafica, Damien, and Nonnenmann (2010) method with some modifications. The cellulose sheets were treated with 0.5 M NaOH (Scharlab S.L., Sentmenat, Spain) at 70 °C for 1 h in a shaking incubator (ES-20/60, Biosan Ltd., Riga, Latvia) followed by a continuous rinse with filtered water until a neutral pH was attained in drained water. Then the wet sheets were positioned between two sheets of polypropylene mesh (4.8 mm hole, RS Components Pty Ltd., Smithfield, Australia) and incubated at 37 °C for 18 h.

4.2.1.2 Steak preparation and wrapping treatment

Beef eye round (*Musculus semitendinosus*) primal cuts were obtained from a local butcher (Perth metropolitan area, Western Australia, Australia) within 24 h post-slaughter. The cuts were transported to our laboratory under refrigeration and were stored at - 1°C ± 1.0 °C for a maximum of 2 h until they were used. Visible fat and connective tissue were trimmed off under aseptic conditions and steaks averaging 100 g, 10 cm x 8 cm and 1.5 cm thick were cut. A total of 56 steaks were obtained and 28 steaks were randomly chosen and wrapped with BC-sheets and vacuum-packaged (BCW). The remaining 28 steaks acted as a control and were unwrapped and vacuum-packaged (UNW).

For the BCW meat, the dried BC-sheets were hydrated by surface contact with steak samples and then the wrapped steaks were placed in vacuum pouches (Nylon Co-ex, thickness 65 µm, oxygen transmission rate < 40 cc/m^2/24 h at 25 °C, moisture vapour transmission rate < 7 g/m^2/24 h at 38 °C, Vital Packaging, Perth, Australia). All the samples were vacuum-packaged (easyPACK-mk^2, Webomatic, Bochum, Germany) and stored under chilled storage conditions (- 1°C ± 1.0 ºC) for 3 weeks. Sub-zero chilled temperatures (- 1.5 ºC to 2 ºC) are considered as the ideal temperature for the transportation of vacuum-packaged meat to export markets (Rosenvold & Wiklund, 2011). Samples were withdrawn from all treatments on day 1, 7, 14 and 21 of storage as per Morsy, Khalaf, Sharoba, El-Tanahi, and Cutter (2014). Seven steaks were randomly sampled from each treatment at each sampling point and analysed as in section 4.2.2.4 to section 4.2.2.11 below.

4.2.2 Analytical methods

4.2.2.1 Morphological characterization of bacterial cellulose wrap

The dried BC morphology was viewed using a Neon 40EsB field emission scanning electron microscope (FESEM, Zeiss, Göttingen, Germany). For scanning electron microscopy (SEM) imaging, BC-sheet particles (0.5 cm X 1.0 cm) were placed on aluminium stubs with carbon paint. All the samples were observed under SEM without coating. In order to compare the surface morphology of BC at three different stages (dried BC before wrapping with meat; wet BC with purge absorbed at the end of 21 days of storage; dry BC with purge absorbed at the end of 21 d of storage) was examined by variable pressure field emission scanning electron microscope (VP-FESEM, Mira3, Tescan, Kohoutovice, Czech Republic) with field emission gun operated at 5kV.

4.2.2.2 Crystallinity analysis of bacterial cellulose wrapping

X-ray diffraction (XRD) pattern of BC at three different stages as mentioned under section 4.2.2.1 was analysed by powder diffractometer D8 Advance (Bruker AXS, Karlsruh, Germany). The 2θ diffraction angle ranged from 7.5 to 50º. The radiation source of copper K alpha generated an accelerating voltage of 40 kV and a filament emission of 40 mA.

The crystallinity index (CI) of BC at three different stages were calculated by the following equation (Kim et al., 2011).

$CI\ \% = ((I_{200} - I_{am}) / I_{200}) \times 100$

I_{200}: the maximum intensity of the lattice diffraction; I_{am}: the minimum intensity between (110) and (200) peak.

In addition, crystallinity % of the three stages of BC was calculated by the method explained by Vazquez, Foresti, Cerrutti, and Galvagno (2013).

4.2.2.3 Purge absorptivity of bacterial cellulose wrap

Purge absorptivity of BC wrap was determined according to the method described by Lin, Lien, Yeh, Yu, and Hsu (2013) with some modifications. The dry weight of BC-sheets (W_{dry}) was measured prior to wrapping the beef steaks. The wet weight of swollen BC-sheets (W_{wet}) was measured after unwrapping the beef steaks at the end of each storage period. Prior to measuring the wet weight, they were gently blotted with filter paper to remove the excess purge. The swelling ratio and moisture ratio was calculated using the following equations.

Swelling ratio (%) $= ((W_{wet} - W_{dry})/ W_{dry}) \times 100$

Moisture content ratio (%) $= ((W_{wet} - W_{dry})/ W_{wet}) \times 100$

4.2.2.4 Weight loss, purge accumulation and drip loss analysis

Weight loss during chilled storage was determined as per the method of Cardoso et al. (2016). The steaks were removed from the vacuum packages at each storage time period, gently blotted with filter paper to remove excess purge, weighed and results were expressed as a percentage of weight loss relative to the initial weight (day 0). The purge accumulation percentage was calculated according to the following formulas (Wiklund, Finstad, Johansson, Aguiar, & Bechtel, 2008).

Purge accumulation (%) for UNW $= ((W_T - (W_{MFinal} + W_{VInitial}))/ W_{MInitial}) \times 100$

Purge accumulation (%) for BCW $= ((W_T - (W_{MFinal} + W_{VInitial} + W_{wet}))/ W_{MInitial}) \times 100$

W_T: total weight of the vacuum-packaged meat measured at day 0; W_{MFinal}: final weight of the meat measured at day 1, 7, 14 and 21; $W_{VInitial}$: weight of the empty vacuum package measured at day 0; $W_{MInitial}$: initial weight of the meat measured at day 0; W_{wet}: weight of swollen BC-sheets measured at day 1, 7, 14 and 21.

Drip loss analysis was carried out according to the method of Honikel (1998).

4.2.2.5 Low-field nuclear magnetic resonance relaxation

The water distribution in the muscle was analysed by performing transverse relaxation time (T_2) analysis using a benchtop nuclear magnetic resonance analyser (GeoSpec, Oxford Instruments, Abingdon, UK). The nuclear magnetic resonance (NMR) analyser has a proton resonance frequency of 12 MHz and the T_2 analysis was carried out as described by Gudjonsdottir et al. (2015) with some modifications. At each sampling day, approximately 3 g of samples were cut from both wrapped and unwrapped steaks, placed in 10 mm sample tubes and analysed in the NMR. A Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence was used with a τ value of 100 µs. A number of 5500 echoes were collected in each scan with 8 repeated scans for each and recycling delay was maintained as 6 s. The obtained T_2 data was analysed to produce T_2 probability distributions using in-house regularisation techniques (Fridjonsson, Hasan, Fourie, & Johns, 2013; Hollingsworth & Johns, 2003) using REGEDS software produced by the fluid science and resources research group at the University of Western Australia.

4.2.2.6 Meat pH determination

Meat pH at each storage time was measured in triplicate by inserting a calibrated pH probe (standard buffers of pH 4.0 and 7.0, TPS Pty. Ltd., Brisbane, Australia) directly into the steak as described by Kim, Kemp, and Samuelsson (2016).

4.2.2.7 Meat colour measurements

The colour of the steaks was measured immediately after removal from the vacuum pack followed by unwrapping the BC-sheet (pre-blooming conditions) and after allowing the meat to bloom at 4˚C for 1 h (post-blooming conditions). A BYK colourimeter (BYK-Gardner GmbH, Geretsried, Germany) was used to collect L^*

(lightness), a^* (redness) and b^* (yellowness) values (D 65 light source and a 10° observer with an 11 mm aperture) at three random locations on each sample. Before collecting measurements, the instrument was calibrated using a white tile ($L^* = 96.06$, $a^* = -0.78$, $b^* = -0.34$) as indicated by the manufacturer.

4.2.2.8 Tenderness

Meat tenderness was measured using a Perten texture analyser (TVT 6700, Perten instruments, Hägersten, Sweden) coupled with a V-shaped Warner–Bratzler shear blade (p-WBT). Steak samples (approximately 6 × 6 × 3 cm) were removed from both treatments for every 7 d, cooked and cooled as per Li et al. (2012). Six cores (1 × 1 cm, parallel to muscle fibre orientation) were removed from each sample and sheared perpendicular to the fibre orientation with the blade maintained at a crosshead speed of 4 mm/s. The average peak force results for each treatment were expressed as load in Newton (N).

4.2.2.9 Lipid oxidation

The steaks were analysed for lipid oxidation by measuring 2-thiobarbituric acid reactive substances (TBARS) according to Cardoso et al. (2016) without any modifications. The samples were removed from each treatment on day 1 and every 7th consecutive day and immediately frozen at -80 $^{\circ}$C until the analysis was conducted (Measurements were completed within 3 d). Finally, the extracts were analysed for optical absorbance at 532 nm using a spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan). The TBARS values were reported in mg of malonaldehyde (MDA) per kg of the sample by averages of a standard curve using 1, 1, 3, 3-tetraethoxypropane (Sigma-Aldrich Pty. Ltd., Sydney, Australia) in 20% trichloroacetic acid (Sigma-Aldrich Pty. Ltd., Sydney, Australia).

4.2.2.10 Microbiological analysis

Lean meat (at day 1, 7, 14 and 21) and purge (at day 7, 14 and 21) were collected from each steak sample and analysed individually for the presence of lactic acid bacteria (LAB) and *Brochothrix thermosphacta.* The growth of *E. coli* was also evaluated in meat samples on day 1 and 21 and in purge samples on day 7 and 21.

From each sample, 10 g of meat trimmings were placed in 90 ml of buffered peptone water (BPW; Oxoid Ltd., Hants, UK) and homogenized for 2 mins using a homogenizer (PRO250, PRO Scientific Inc., Oxford, USA). For purge samples, a 1:10 dilution was prepared using BPW and homogenized as mentioned above. Decimal dilutions were prepared and spread plated onto the respective growth mediums, namely, de Man, Rogosa and Sharpe (MRS; Oxoid Ltd., Hampshire, England) agar for LAB, Streptomycin-Thallous Acetate-Actidione (STAA; Oxoid Ltd., Hampshire, England) agar for *B. thermosphacta* and Eosin-Methylene Blue (EMB; Oxoid Ltd., Hants, UK) agar for *E. coli*. MRS plates were anaerobically incubated at 30 °C ± 1°C for 120 ± 3 h, STAA and EMB plates were aerobically incubated at room temperature (22 °C ± 1°C) for 48 ± 3 h and at 37 °C ± 1°C for 24 ± 3 h, respectively. All microbiological experiments were performed in triplicate and counts were converted to $\log_{10}$CFU/g for meat samples and $\log_{10}$CFU/ml for purge samples.

4.2.2.11 Sensory evaluation

Raw beef samples stored for 1, 10 and 21 d were analysed by 9 semi-trained panellists (5 females and 4 males, between 25-55 years) per day for the sensory evaluation. The sensory evaluation followed methods recommended by Lawless and Heymann (2010) and AS 2542.1.3:2014 (Standards Australia, 2014). Ethics approval was obtained from the Curtin University Human Research Ethics Committee before conducting the sensory assessment (Approval number: HRE2019-0038). Panellists were screened based on their beef consumption every fortnight and whether they purchase raw beef and cook or prepare it for eating fortnightly. Prior to conducting the sensory evaluation, consent of all the panellist were obtained. The panellists were then trained on beef colour standards, odour, texture and firmness standards and descriptive tests in two separate training sessions.

The panellists were presented with raw beef samples from each treatment after allowing them to bloom at 4 °C for 1 h in order to mimic the retail display conditions. Samples were evaluated for colour, firmness, odour and overall acceptability using the general Labeled Magnitude Scale (Kalva, Sims, Puentes, Snyder, & Bartoshuk, 2014). They were instructed to mark the scale which was a structured line of 15 cm (Appendix D; separate scale for each parameter) with the sample number assigned for each treatment based on their preferences.

4.2.3 Data analysis

Statistical analysis was conducted using IBM SPSS Statistics version 26 software. Triplicates were used for all the measurements except drip loss in which duplicate analysis was carried out. Data of all the parameters (except sensory evaluation) of unwrapped and BC wrapped beef samples at each storage time was analysed using Independent-samples T-test to compare the results between the two different treatments. The data of each treatment during the storage period were analysed using one-way analysis of variance (ANOVA). Data of sensory evaluation during the ageing period and comparison between two treatments were carried out using Repeated Measure ANOVA and Paired sample T-test, respectively. If a significant ($P < 0.05$) difference was found, mean values were further analysed using Tukey comparison test.

4.3 Results and discussion

4.3.1 Characterization of bacterial cellulose wrapping

The morphology of dried BC viewed under FESEM and dried BC and BC after 21 d (both wet and dried) viewed under VP-FESEM are shown in Figure 4.1. The FESEM image (Figure 4.1A) and VP-FESEM image (Figure 4.1B) of dried BC exhibits a porous dense network structure created by BC nanofibrils. The fibrillar network comprises highly organized nanofibrils with empty space in-between them (Lin et al., 2013), which may increase the material's capacity to absorb liquids. Swollen fibrillar structure with the presence of rod-shape bacteria can be seen in BC-sheets aged for 21 d in both wet and dry conditions (Figure 4.1C and D), which means BC seems to be a good substrate for microbial attachment. The swollen structure may be due to the penetration of purge into the pores of BC network and absorbance of purge by BC nanofibrils. This was further evidenced by the swelling and moisture content ratio (Figure 4.2) values of BC wrapping.

A steady increase in both swelling ratio and moisture content ratio of BC-sheets during the ageing period can be seen (Figure 4.2). The swelling ratio increased by more than 50% by the end of 21 d ageing period, while the moisture ratio increased by ~14%. The continuous rise of these values demonstrated the increased amount of purge absorption of BC wrap over the ageing period. Bacterial cellulose is capable of

physically trapping water molecules by its delicate porous structure (Ul-Islam, Khan, & Park, 2012). Water molecules can easily form hydrogen bonds with hydroxyl groups of glucosyl units in the BC chain (Jebel & Almasi, 2016). Therefore, BC has the capacity to lock the purge exudate from meat and prevent collecting it in the packaging.

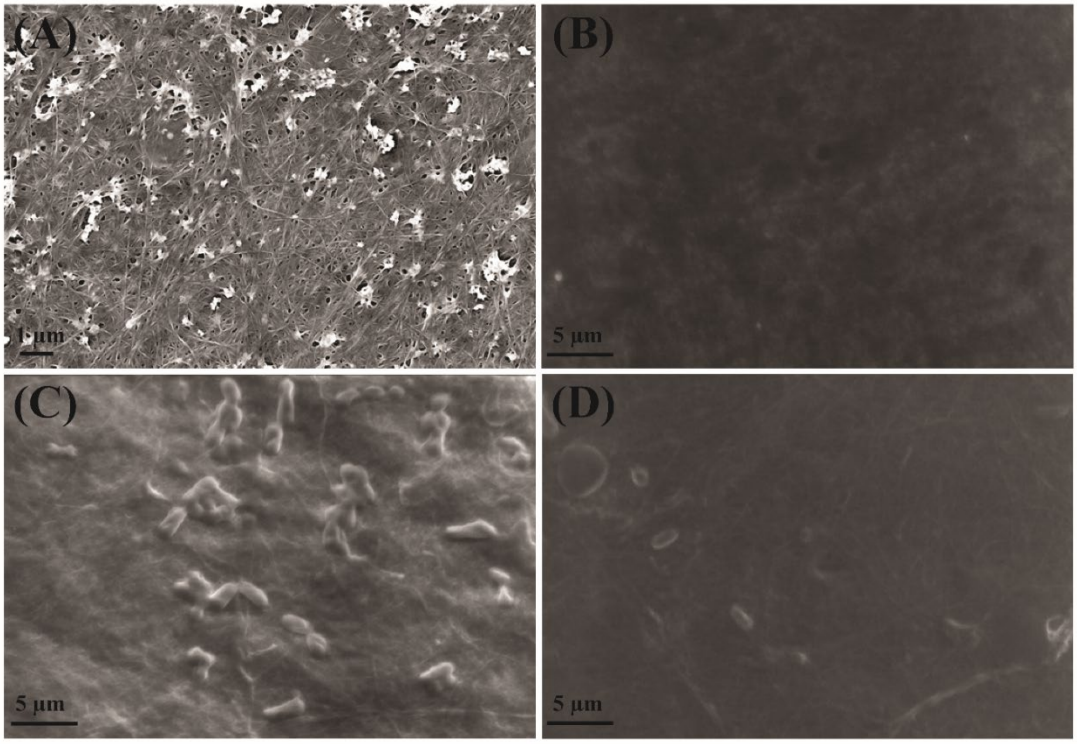

Figure 4.1 SEM images of BC-sheets at different ageing durations. FESEM image of dried BC (A), VP-FESEM image of dried BC (B), VP-FESEM image of BC aged for 21 d in wet condition (C) and dried condition (D).

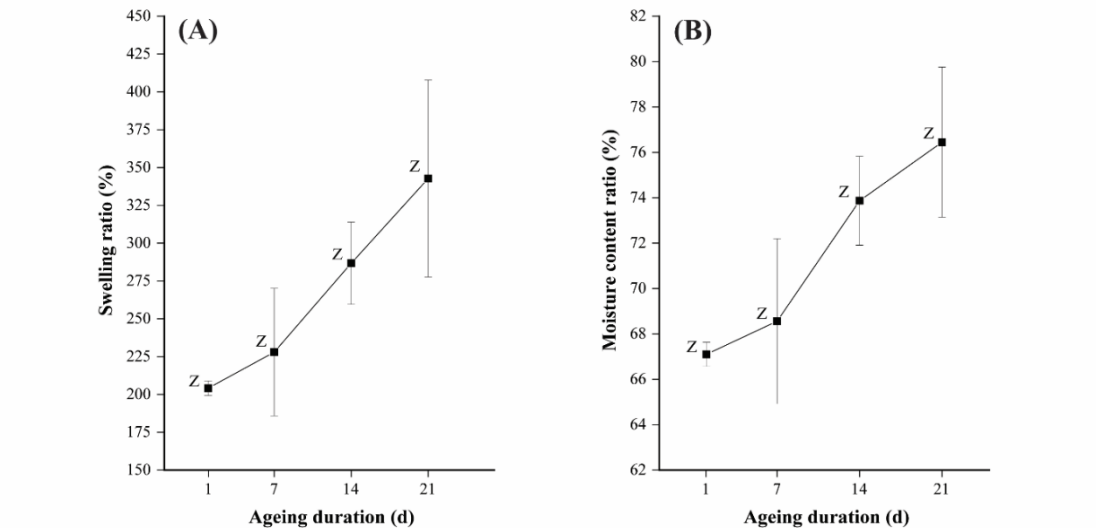

Figure 4.2 Changes in the swelling ratio (%) (A) and moisture content ratio (%) (B) of BC-sheets during ageing of meat. Different uppercase letters (Z-X) mean significant differences among different ageing durations ($P < 0.05$).

XRD patterns of initial dried BC and aged BC in both wet and dry conditions are presented in Figure 4.3. Three characteristics peaks positioned at 14.6º ($1\bar{1}0$), 16.9º (110) and 22.8º (200) suggests that only cellulose I was present in BC-sheets (Vazquez et al., 2013; Zhu et al., 2010). Cellulose I is a thermodynamically metastable crystalline cellulose (Moon, Martini, Nairn, Simonsen, & Youngblood, 2011). Crystallinity % and CI % values of BC at three stages are included in Table 4.1. These results were similar to the results shown by Vazquez et al. (2013). Increase in both peak intensities and CI % with ageing demonstrates the swelling of BC nanofibrils with the uptake of purge. Maintenance of high crystallinity % and CI % values even at the end of ageing confirm that the mechanical strength of BC-sheets remained stable throughout the beef ageing period. Therefore, BC has the ability to maintain its strength and integrity over the shelf life without any deterioration and continue to absorb purge.

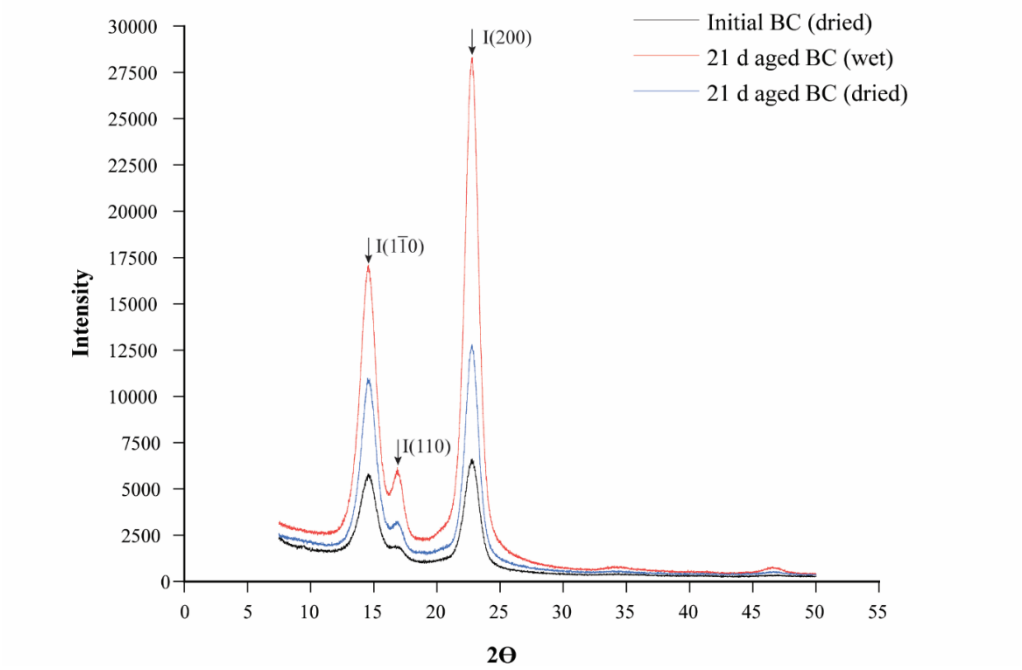

Figure 4.3 XRD patterns of BC at different stages.

Table 4.1 Crystallinity Index (CI) and crystallinity (%) of bacterial cellulose (BC) at different stages during ageing

Different stages of BC	CI (%)	Crystallinity (%)
Initial dried BC	84.18	75.98
21 d aged BC (wet state)	92.38	76.47
21 d aged BC (dry state)	87.92	77.18

4.3.2 Quality changes in beef during vacuum ageing

4.3.2.1 Water-holding properties

The effect of wrapping treatment and ageing duration on weight loss and purge accumulation is presented in Figure 4.4. It appeared that the weight loss values increased from 3% to 6%, but this did not reach a statistically significant level.in both UNW and BCW steaks during the ageing period. The increase in weight loss could be due to the myowater loss caused by protein denaturation (Apple & Yancey, 2013), leading to the loss of WHC. No significant differences between UNW and BCW treatments were observed for weight loss during the entire ageing period. This confirms that the BC wrap does not have the capability to plug the cut ends of meat capillaries. Significantly ($P < 0.05$) lower values of purge accumulation were

determined for BCW steaks compared to UNW steaks for each ageing period except for day 1. The reduced purge accumulation could explain the increased swelling and moisture content ratio results of BC-sheets during the ageing period. Bacterial cellulose wrap did not prevent purge loss from the meat itself, but it prevented the accumulation of purge inside the vacuum package which would make it more appealing to the customers.

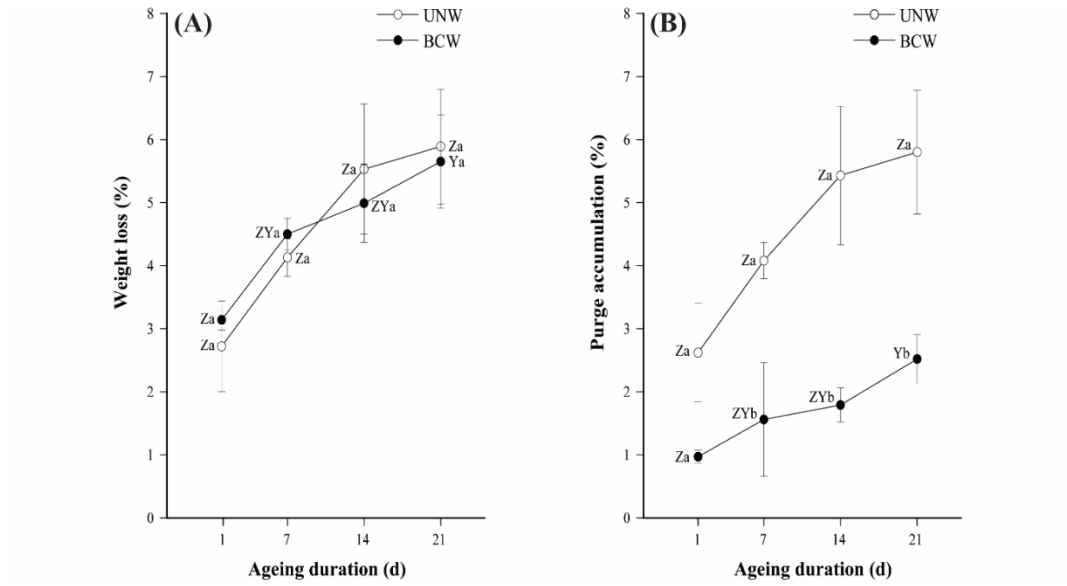

Figure 4.4 Changes in weight loss (%) (A) and purge accumulation (%) (B) of BC wrapped and unwrapped samples at day 1, 7, 14 and 21. UNW: unwrapped and vacuum-packaged; BCW: wrapped with BC-sheet and vacuum-packaged. Different uppercase letters (Z-X) mean significant differences among different ageing durations ($P < 0.05$). Different lowercase letters (a, b) mean significant differences between the two treatments ($P < 0.05$).

In UNW samples, drip loss values continued to increase for up to 14 d, but in BCW this was only up to 7 d (Table 4.2). On day 21, the drip loss value in both samples was reduced, which could be due to most of the myowater being already leached out into the purge. This is in agreement with the findings of Marino et al. (2014) who suggested that such changes may be due to the loosened muscle structure caused by myofibrillar and cytoskeletal protein degradation. BCW did not significantly influence the change in weight loss, drip loss or pH. As BCW did not have any impact on pH (Table 4.3), it should not have impacted WHC, confirming that the BC wrap is more appropriate as a purge absorbent.

According to T_2 analysis, two key water populations were seen in the samples, a dominant population (A_{21}) between 38 and 52 ms (T_{21}) representing intramyofibrillar water and a less prominent population (A_{22}) between 190 and 300 ms (T_{22}) representing extramyofibrillar water. These results are consistent with the findings of recent studies by Cheng et al. (2019) and Qian et al. (2019) who have also observed two key water populations in beef samples. No significant ($P > 0.05$) differences between either treatments or ageing were observed in terms of T_2 and population area ratio (A_{21}% / A_{22}%) values (Table 4.2). In this study, the change in A_{22}% which represents the extramyofibrillar myowater population was in accordance with the change in drip loss during the ageing period (Table 4.2), which is also in agreement with the study of Straadt, Rasmussen, Andersen, and Bertram (2007) on porcine muscle. Therefore, as the A_{22} water population follows the same trend as the drip loss over the 21 d ageing period and the A_{21} population is not significantly affected over the same time period, our data suggest that the extramyofibrillar water is likely the main initial myowater to be lost as drip in the meat samples. This behaviour could be observed in both UNW and BCW treatments. Also, a decrease in T_{21} and overall transverse relaxation time T_{2lm} during the ageing period could be observed in both treatments. A similar trend was reported by Gudjonsdottir et al. (2015) which indicates the partial denaturation of muscle proteins with ageing. Ageing induced partial denaturation reduced the ability of proteins to hold myowater and improved meat tenderness.

Table 4.2 Changes in drip loss (%) and LF-NMR relaxation parameters during ageing of beef

Parameters	Treatment	Ageing duration (d)				SEM	P-value
		1	7	14	21		
Drip loss (%)	**UNW**	0.58 ± 0.02 Za	0.79 ± 0.07 Za	0.80 ± 0.05 Za	0.69 ± 0.04 Za	0.04	0.073
	BCW	1.13 ± 0.13 ZYa	1.19 ± 0.14 Za	0.77 ± 0.04 ZYa	0.61 ± 0.05 Ya	0.10	0.036
	P-value	0.051	0.116	0.651	0.338		
A_{21} (%)	**UNW**	97.90 ± 0.30 Za	94.15 ± 1.52 Za	95.88 ± 0.45 Za	95.94 ± 0.87 Za	0.56	0.108
	BCW	96.66 ± 0.45 Za	96.43 ± 1.05 Za	96.92 ± 0.74 Za	97.04 ± 0.57 Za	0.32	0.935
	P-value	0.087	0.283	0.293	0.348		
A_{22} (%)	**UNW**	2.10 ± 0.30 Za	5.86 ± 1.52 Za	4.12 ± 0.45 Za	4.06 ± 0.87 Za	0.56	0.108
	BCW	3.34 ± 0.45 Za	3.57 ± 1.05 Za	3.08 ± 0.74 Za	2.96 ± 0.57 Za	0.32	0.935
	P-value	0.087	0.283	0.293	0.348		
T_{21} (ms)	**UNW**	51.79 ± 0.00 Za	44.75 ± 0.00 Za	45.07 ± 3.79 Za	42.72 ± 2.03 Za	1.38	0.075
	BCW	47.42 ± 4.38 Za	47.10 ± 2.35 Za	42.72 ± 2.03 Za	42.72 ± 2.03 Za	1.40	0.511
	P-value	0.374	0.374	0.614	1.000		
T_{22} (ms)	**UNW**	258.64 ± 0.00 Za	246.92 ± 11.73 Za	263.93 ± 35.43 Za	272.21 ± 13.57 Za	8.91	0.835
	BCW	223.46 ± 0.00 Za	272.21 ± 13.57 Za	246.92 ± 11.73 Za	235.19 ± 11.73 Za	7.11	0.059
	P-value		0.231	0.672	0.108		
T_{2lm} (ms)	**UNW**	48.97 ± 0.91 Za	45.71 ± 2.14 Za	44.66 ± 3.00 Za	43.87 ± 1.46 Za	1.05	0.361
	BCW	46.26 ± 2.92 Za	44.49 ± 0.12 Za	42.15 ± 3.14 Za	41.99 ± 1.87 Za	1.13	0.548
	P-value	0.424	0.598	0.594	0.475		

[a-b] Means within the same column for the same index with different lowercase letters differ significantly among the treatments ($P < 0.05$).

[Z-X] Means within the same row with different uppercase letters differ significantly among different ageing durations ($P < 0.05$).

UNW: unwrapped and vacuum-packaged; BCW: wrapped with BC-sheet and vacuum-packaged; A_{21} (%): population area ratio of T_{21}; A_{22} (%): population area ratio of T_{22}; T_{21} (ms): T_{21} relaxation time; T_{22} (ms): T_{22} relaxation time; T_{2lm} (ms): overall transverse relaxation time; SEM: standard error of mean.

4.3.2.2 Physicochemical properties

The effects of wrapping treatment and ageing duration on the steaks pH are presented in Table 4.3. No effect on the pH of meat wrapped with BC was observed. The pH of the BC-sheets was neutral, which is similar to a study by Wang et al. (2020). Therefore, it can be expected that the BCW did not impact the pH of the meat. However, ageing duration had a significant ($P < 0.05$) impact on pH in both treatments. A steady decrease in pH during the ageing period may have been caused by the growth of LAB in meat during the 21 d ageing period which is shown in Figure 4.5C and D and further discussed under section 4.3.2.3.

Wrapping treatment and ageing duration considerably affected the colour of meat (Table 4.4). An increase in lightness after 7 d and its persistence at the same level until the end of ageing could be seen in both treatments. This trend is evident for both pre-blooming and post-blooming scenarios and may occur due to the moisture loss and accumulation thereof at the surface, leading to higher light scattering during colour measurements (Hughes, Oiseth, Purslow, & Warner, 2014). No treatment effect on lightness could be observed except for day 1 which may be due to the difference of lightness of the individual meat samples.

Colour evaluations at pre-blooming revealed ageing did not ($P > 0.05$) have an impact on redness and yellowness values of meat from both treatments. A similar trend could be observed for the change in yellowness at post-blooming in both the treatments. A significant ($P < 0.05$) fall in redness after 21 d was observed compared to day 1 at post-bloom evaluations only for the UNW samples. This could be due to reduced mitochondrial respiration after 21 d of ageing (Mungure, Bekhit, Birch, & Stewart, 2016).

Table 4.3 Changes in pH, tenderness and lipid oxidation (TBARS) of beef during the ageing period

Parameters	Treatment	Ageing duration (d)				SEM	P-value
		1	7	14	21		
pH	**UNW**	5.64 ± 0.03 Za	5.52 ± 0.03 Ya	5.44 ± 0.01 Xa	5.37 ± 0.01 Xa	0.03	0.000
	BCW	5.76 ± 0.06 Za	5.51 ± 0.01 Ya	5.45 ± 0.00 Ya	5.40 ± 0.01 Ya	0.04	0.000
	P-value	0.151	0.777	0.116	0.067		
Tenderness	**UNW**	42.83 ± 1.42 Za	35.57 ± 1.15 Ya	36.23 ± 1.10 Ya	35.31 ± 0.89 Ya	0.84	0.000
(N)	**BCW**	42.08 ± 1.19 Za	34.63 ± 2.84 Ya	34.67 ± 1.18 Ya	34.55 ± 1.20 Ya	1.06	0.014
	P-value	0.696	0.764	0.359	0.626		
Lipid	**UNW**	0.053 ± 0.00 Za	0.053 ± 0.01 Za	0.126 ± 0.00 Ya	0.281 ± 0.01 Xa	0.03	0.000
oxidation	**BCW**	0.086 ± 0.00 Zb	0.082 ± 0.00 Zb	0.168 ± 0.00 Yb	0.314 ± 0.02 Xa	0.03	0.000
(mg MDA/kg)	P-value	0.001	0.028	0.001	0.295		

[a-b] Means within the same column for the same index with different lowercase letters differ significantly among the treatments ($P < 0.05$).

[Z-X] Means within the same row with different uppercase letters differ significantly among different ageing durations ($P < 0.05$).

UNW: unwrapped and vacuum-packaged; BCW: wrapped with BC-sheet and vacuum-packaged; SEM: standard error of mean.

Table 4.4 Changes in colour parameters at pre-blooming and post-blooming conditions during ageing of beef

Parameters	Treatment	Ageing duration (d)				SEM	P-value
		1	7	14	21		
*L** preb	**UNW**	33.63 ± 0.87 Za	41.34 ± 0.16 ZYa	42.81 ± 3.31 Ya	40.57 ± 1.16 ZYa	1.31	0.029
	BCW	38.97 ± 0.60 Zb	43.55 ± 1.02 Ya	42.40 ± 0.60 ZYa	42.64 ± 1.16 ZYa	0.65	0.028
	P-value	0.007	0.100	0.910	0.275		
*a** preb	**UNW**	11.61 ± 0.37 Za	8.97 ± 0.72 Za	9.78 ± 0.27 Za	9.35 ± 0.87 Za	0.40	0.063
	BCW	13.82 ± 0.75 Za	13.32 ± 0.57 Zb	15.37 ± 1.25 Zb	14.13 ± 0.45 Zb	0.41	0.382
	P-value	0.057	0.009	0.012	0.008		
*b** preb	**UNW**	8.25 ± 0.43 Za	10.32 ± 0.61 Za	10.77 ± 1.49 Za	9.59 ± 0.22 Za	0.46	0.236
	BCW	11.56 ± 0.47 Zb	13.78 ± 0.92 Zb	13.73 ± 1.06 Za	13.29 ± 0.36 Zb	0.42	0.210
	P-value	0.006	0.035	0.181	0.000		
*L** postb	**UNW**	36.49 ± 0.58 Za	43.32 ± 0.65 Ya	44.11 ± 1.68 Ya	43.01 ± 1.85 Ya	1.08	0.012
	BCW	39.70 ± 0.44 Zb	43.17 ± 0.64 Za	43.86 ± 1.81 Za	42.27 ± 0.34 Za	0.64	0.077
	P-value	0.012	0.880	0.924	0.714		
*a** postb	**UNW**	24.59 ± 1.50 Za	19.98 ± 0.64 ZYa	20.71 ± 1.57 ZYa	18.82 ± 0.40 Ya	0.82	0.035
	BCW	22.17 ± 0.42 Za	20.11 ± 0.73 Za	23.34 ± 1.38 Za	23.19 ± 0.95 Zb	0.56	0.132
	P-value	0.197	0.895	0.277	0.013		
*b** postb	**UNW**	16.48 ± 0.80 Za	17.81 ± 0.64 Za	18.09 ± 0.97 Za	17.80 ± 0.50 Za	0.37	0.468
	BCW	17.07 ± 0.16 Za	18.23 ± 0.88 Za	19.31 ± 1.16 Za	19.32 ± 0.61 Za	0.44	0.220
	P-value	0.509	0.722	0.465	0.125		

[a-b] Means within the same column for the same index with different lowercase letters differ significantly among the treatments ($P < 0.05$).

[Z-X] Means within the same row with different uppercase letters differ significantly among different ageing durations ($P < 0.05$).

UNW: unwrapped and vacuum-packaged; BCW: wrapped with BC-sheet and vacuum-packaged; preb: colour evaluation at pre-blooming conditions (just after opening of the vacuum package); postb: colour evaluation at post-blooming conditions (after allowing to bloom at 4 ºC for 1 h); SEM: standard error of mean.

The pre-bloom redness and yellowness significantly ($P < 0.05$) increased in wrapped samples compared to unwrapped samples. The colour of BCW samples became redder at pre-bloom that would make them more acceptable once the vacuum package is opened. The reason for these results is not fully understood. Bacterial cellulose membrane may act as an oxygen trapping material due to its high oxygen permeation capacity (Tomé et al., 2010). Oxidation of the iron molecule in myoglobin heme results in a red pigment. Therefore, the sustained release of oxygen molecules which were trapped in the BC network may maintain oxidation of the heme and prolong redness in the meat over a longer period of time. No treatment effect was determined in terms of redness and yellowness in post-blooming evaluations, except for the redness values of BCW steaks at day 21. Increased redness and yellowness at post-blooming may have resulted from the formation of oxymyoglobin with exposure to air.

TBARS values of UNW and BCW meat samples aged for 1 and 7 d were not statistically different ($P > 0.05$) while samples aged for 14 and 21 d showed a significant increase ($P < 0.05$; Table 4.3) compared to day 1 and 7 results. These results are in agreement with the findings of Mungure et al. (2016) who suggested the decay of endogenous antioxidants could be the reason for ageing enhanced lipid oxidation. Wrapped meat showed a significant ($P < 0.05$) increase in lipid oxidation compared to unwrapped meat until 14 d of ageing. With the uptake of purge, the porous structure of BC could have allowed guest molecules of purge to diffuse throughout its inner space easily and facilitate the release of entrapped oxygen molecules, increasing oxygen availability for chemical reactions. No significant ($P > 0.05$) difference of TBARS between UNW and BCW meat at 21 d of ageing was observed. Therefore, wrapping did not significantly impact the oxidative rancidity by the end of ageing and caused no impact on consumer acceptability in terms of odour as evidenced by the sensory evaluation (4.3.2.4).

Meat tenderness is expressed as the shear force required to cut through a piece of meat. Shear force was not influenced by wrapping treatment (Table 4.3). These results are similar to the findings of Gudjonsdottir et al. (2015) who reported no impact on beef tenderization due to different wrapping treatments and this may also be due to no significant influence on meat pH and WHC by the wrapping treatment. Ageing duration had a significant impact on tenderness. Mean shear force decreased

significantly ($P < 0.05$) from day 1 to day 7 and then remained stable until the end of ageing which, is in agreement with the findings of Lagerstedt, Lundstrom, and Lindahl (2011). Degradation of myofibrillar, cytoskeletal proteins (Lomiwes, Farouk, Wu, & Young, 2014) and intramuscular collagen (Starkey, Geesink, Collins, Oddy, & Hopkins, 2016) during post-mortem ageing causes meat structure to loosen and thus improve meat tenderness. In this study, tenderness was not significantly impacted by BC wrapping, although shear force values were consistently lower in the BCW samples compared to the UNW samples over 21 d ageing period.

4.3.2.3 Microbial evaluation

Microbial evaluation of purge on day 1 was not conducted since the amount of purge collected was negligible. The presence of *E. coli*. was not detected either in meat or purge collected from UNW and BCW at any time during the study which is in agreement with the results reported by Ercolini et al. (2011). Varied counts during the ageing were observed for *B. thermosphacta* in purge samples collected from both treatments (Figure 4.5B). The growth of *B. thermosphacta* in meat in both UNW & BCW samples (Figure 4.5A) significantly fluctuated during the study. However, changes in numbers were less than 1 log CFU/g and did not cause any visible meat spoilage. A significant ($P < 0.05$) increase in numbers of LAB during ageing was observed for both UNW and BCW meat (Figure 4.5C) which also did not cause any visible meat spoilage characteristics. A similar increase in numbers of LAB in vacuum aged beef during a 21 d storage period was reported by Gudjonsdottir et al. (2015) which shows that anaerobic packaging conditions were favourable for the growth of LAB (Pothakos, Devlieghere, Villani, Björkroth, & Ercolini, 2015). The increased numbers of LAB during ageing in the current study may account for the decreasing pH which could have caused protein denaturation and ultimately led to poor WHC of meat.

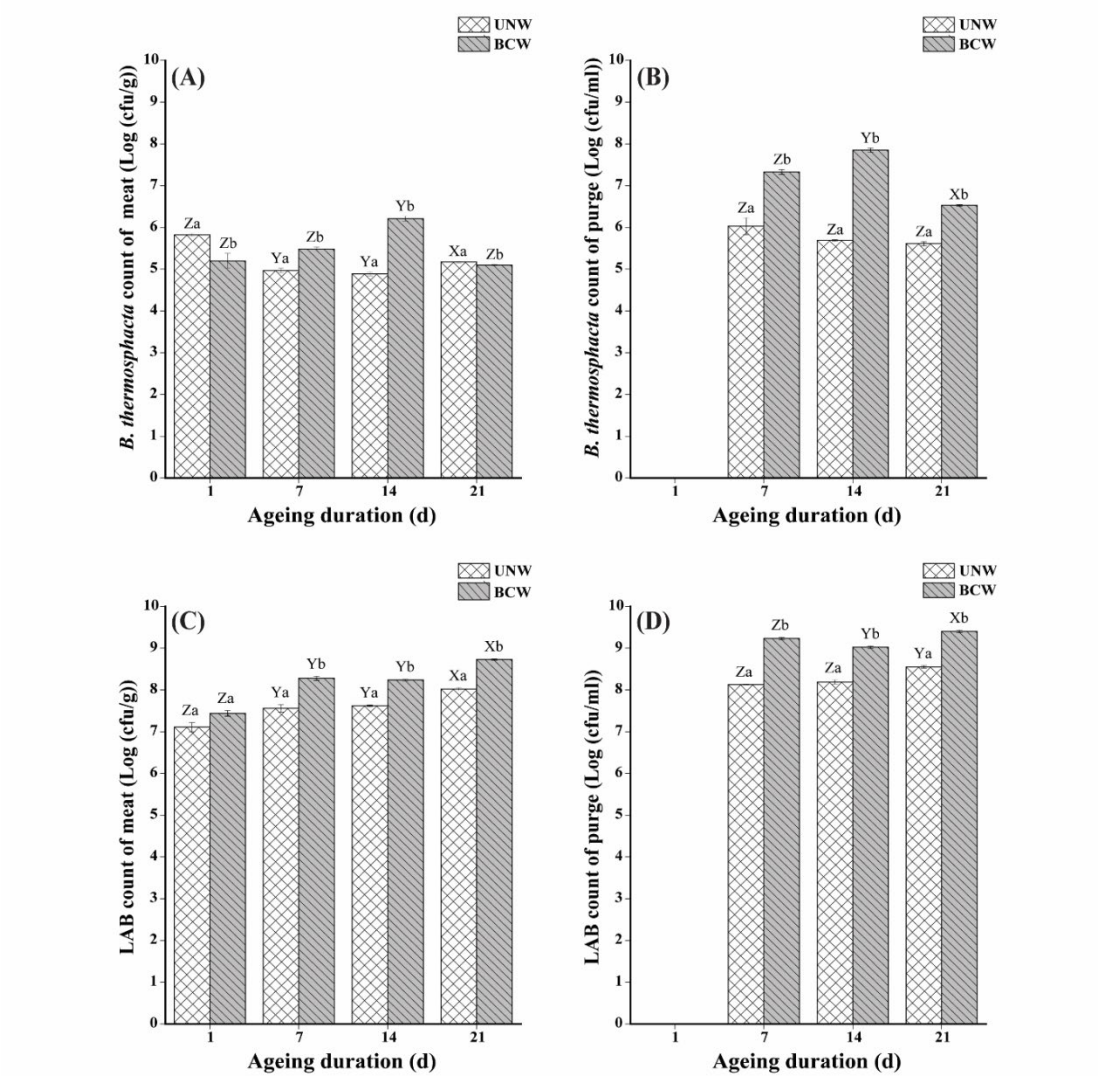

Figure 4.5 Changes in counts of *B. thermosphacta* of meat (A), *B. thermosphacta* of purge (B), lactic acid bacteria of meat (C) and lactic acid bacteria of purge (D) at day 1, 7, 14 and 21. UNW: unwrapped and vacuum-packaged; BCW: wrapped with BC-sheet and vacuum-packaged. Different uppercase letters (Z-X) mean significant differences among different ageing durations ($P < 0.05$). Different lowercase letters (a, b) mean significant differences between the two treatments ($P < 0.05$).

B. thermosphacta showed lower counts in meat and purge collected from both treatments compared to LAB at all time points. This may be due to the inhibition of *B. thermosphacta* growth by the production of hydrogen peroxide, organic acids or catalase by LAB (Castro, Palavecino, Herman, Garro, & Campos, 2011). Wrapping treatment resulted in about 1 log CFU/g or ml increase in LAB counts in both meat and purge and about 1.5 - 2 log CFU/ml increase in *B. thermosphacta* counts in purge. These results are in agreement with the results of total plate count reported by Nguyen et al. (2008). Jebel and Almasi (2016) and Dos Santos et al. (2018) also reported no inhibition of microorganism growth by pure BC-films. Bacterial cellulose in its innate form lacks antimicrobial properties (Wu et al., 2014) and multiple layers of nanofibrillar network of BC may have acted as a substrate for enhancing the attachment and growth of microorganisms to the meat surface during ageing (Figure 4.1C and D). The biocompatible fibres of BC create a surface with a high surface to voulme ratio due to its dense porous network. Therefore BC-sheets are an excellent medium for bacterial attachment. In addition, the BC network allows the diffusion of water molecules of purge, providing an aqueous environment suitable for the growth of microorganisms. Purge from UNW and BCW had higher counts of LAB and *B. thermosphacta* compared to meat at all time points. This indicates that purge can act as an excellent substrate for bacterial growth and may jeopardize product shelf life (Rooyen et al., 2018).

4.3.2.4 Sensory qualities

The panellists found no significant ($P > 0.05$) differences in firmness and odour between UNW and BCW samples at all three-time points at which the sensory evaluations were conducted (Table 4.5). The absence of detectable difference in odour indicates that the higher TBARS values of BCW samples were not significant enough to generate any off odours that could be detected. In addition, BC-sheets were odourless after the purification; thus no off-odours were introduced to the meat. No detectable differences in firmness were observed between the two treatments which are in agreement with the instrumental results of meat tenderness and suggest that BC may cause neither hardening nor softening of meat tissues. There were no significant ($P > 0.05$) differences detected in colour and overall acceptability on day 1. The post-blooming results of colourimetric analysis at day 1 further confirms the day 1 sensory

results. Similar results were reported for colour and overall acceptability by panellists on day 10. However, BCW samples were scored lower ($P < 0.05$) for colour compared to UNW steaks on day 21. The lower acceptability scores for BCW samples at day 21 could be due to the low scores gained for colour by the panellist, even though the colourimetric analysis did not show a significant difference on day 21.

4.4 Conclusion

The results obtained in this research revealed that BC wrap was effective in reducing purge accumulation in vacuum-packaged beef and in enhancing the redness and yellowness of meat at pre-blooming conditions. SEM imaging, swelling and moisture ratio measurements exhibited that BC was able to trap meat exudate in its porous network. XRD results revealed no deterioration of mechanical strength of the BC-sheet with the increase of meat shelf life. BC wrap showed no statistically significant impact on meat pH, tenderness, weight loss, drip loss at physicochemical evaluations and odour and firmness at sensory evaluations. However, BC enhanced microbial growth and lipid oxidation of wrapped meat compared to unwrapped meat. Incorporation of antimicrobial and antioxidant agents into BC could be an option to improve its effectiveness as a purge absorbent. This study shows the potential of using BC-sheets as an eco-friendly alternative for synthetic purge absorbent pads currently used in the meat industry.

Table 4.5 Changes in sensory parameters during ageing of beef

Parameters	Treatment	Ageing duration (d)			P-value
		1	10	21	
Colour	**UNW**	14.35 ± 0.71 Za	13.12 ± 1.22 Za	15.05 ± 0.74 Za	0.371
	BCW	13.28 ± 1.25 Za	12.69 ± 0.93 Za	11.98 ± 0.71 Zb	0.373
	P-value	0.490	0.549	0.024	
Firmness	**UNW**	15.15 ± 0.75 Za	12.60 ± 1.37 Za	14.54 ± 0.96 Za	0.183
	BCW	13.79 ± 1.01 Za	13.28 ± 0.92 Za	12.10 ± 1.02 Za	0.500
	P-value	0.144	0.562	0.140	
Odour	**UNW**	12.88 ± 0.93 Za	12.46 ± 0.97 Za	12.30 ± 1.32 Za	0.936
	BCW	11.84 ± 1.43 Za	10.34 ± 1.70 Za	10.61 ± 1.15 Za	0.726
	P-value	0.445	0.331	0.445	
Overall acceptability	**UNW**	14.34 ± 0.72 Za	12.09 ± 1.39 Za	14.94 ± 1.03 Za	0.249
	BCW	12.74 ± 1.63 Za	11.38 ± 1.43 Za	11.90 ± 1.27 Zb	0.816
	P-value	0.32	0.739	0.002	

[a-b] Means within the same column for the same index with different lowercase letters differ significantly among the treatments ($P < 0.05$).

[Z-X] Means within the same row with different uppercase letters differ significantly among different ageing durations ($P < 0.05$).

UNW: unwrapped and vacuum-packaged; BCW: wrapped with BC-sheet and vacuum-packaged; SEM: standard error of mean.

Chapter 5. Antimicrobial efficacy of nisin-loaded bacterial cellulose nanocrystals against selected meat spoilage lactic acid bacteria

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Abstract

This study aimed to explore the potential of bacterial cellulose nanocrystals (BCNs) loaded with nisin against selected meat spoilage lactic acid bacteria (LAB) *in vitro*. BCNs were produced by H_2SO_4 hydrolysis, and nisin-loaded BCNs were produced through the complexation method. All nanocrystals were assessed for their zeta-potential, encapsulation efficiency and antimicrobial activity. Different nisin concentrations were tested and the most effective nanocrystals were further characterised. BCNs had an average zeta-potential of - 43 mV and all nisin-loaded BCNs produced with 5 mg/ml BCNs suspension had zeta-potential values ≥ - 30 mV. The encapsulation efficiency of nisin varied from 80.5 to 93.3% and the crystallinity of BCNs was not influenced by nisin encapsulation. Microbial inactivation was achieved by BCN loaded with 2.0 and 2.5 mg/ml nisin. Therefore, nisin-loaded BCNs may be used as antimicrobial agents in active food packaging.

5.1 Introduction

Bacterial cellulose (BC) is a microbial polysaccharide with a three-dimensional nanofibrillar network produced predominantly by the bacteria in the genus *Gluconacetobacter* (Klemm, Heublein, Fink, & Bohn, 2005). Compared to plant cellulose, BC is chemically pure since it is free from hemicellulose, pectin and lignin (Klemm, Schumann, Udhardt, & Marsch, 2001; Vandamme, De Baets, Vanbaelen, Joris, & De Wulf, 1998). Bacterial cellulose has become one of the most versatile materials used in the biomedical, pharmaceutical and food industry due to its unique characteristics such as biocompatibility, biodegradability, non-toxicity, high water-holding capacity, high mechanical strength and crystallinity (Klemm et al., 2001). Due to these unique properties, bacterial cellulose nanocrystals (BCNs) are widely used in

biomedical applications such as scaffolds, wound dressings (Jonas & Farah, 1998; Klemm et al., 2005) and as fillers in nanocomposites (Klemm et al., 2011). The most common method employed for producing BCNs is sulphuric or hydrochloric acid hydrolysis of BC followed by centrifugation and ultrasonication. Acid hydrolysis produces BCNs by digesting the amorphous regions of BC by promoting cleavage of glycosidic bonds, thus breaking down the BC structure into individual nanocrystals (Martínez-Sanz, Lopez-Rubio, & Lagaron, 2011).

Bacterial cellulose in its innate form lacks antimicrobial properties (Gedarawatte et al., 2020; Wu et al., 2014). Therefore, BC has been suggested to be used in future food applications as an antimicrobial packaging by incorporating metal nanoparticles (Jebel & Almasi, 2016; Wang et al., 2020), lactoferrin (Padrão et al., 2016), postbiotics of lactic acid bacteria (LAB; Yordshahi, Moradi, Tajik, & Molaei, 2020) and probiotic bacteria (Moghanjougi, Bari, Khaledabad, Almasi, & Amiri, 2020). Currently, the usage of BCNs is limited to their use in stabilizing Pickering emulsion systems (Kalashnikova, Bizot, Cathala, & Capron, 2011; Yan et al., 2019; Yan et al., 2017), mainly in biomedical applications. The Australia New Zealand Food Standards Code contains no specific limitations or standards relevant to nanotechnologies (Fletcher & Bartholomaeus, 2011) and there is no commonly used regulation for the usage of nanocellulose in the EU (Mu et al., 2019). The use of bacterial and powdered cellulose in food products are accepted as “Generally Recognized As being Safe” (GRAS) by the US Food and Drug Administration (FDA, 2012). Several studies (Dong, Hirani, Colacino, Lee, & Roman, 2012; Ni et al., 2012) have shown that the nanocellulose exhibited low cytotoxicity. This may be due to the indigestible nature of nanocellulose which is expected to pass through the gastrointestinal tract and exit in the stool (Mu et al., 2019). However, to the best of our knowledge, the use of sulphuric acid hydrolysed BCNs as a food ingredient has not been investigated. Although BCNs may have physicochemical characteristics that could improve food quality when used as functional food ingredients or stabilizers, their lack of antimicrobial activity may limit their applications in the food industry.

Nisin is a bacteriocin composed of 34 amino acid residues which is produced by *Lactococcus lactis* subsp. *lactis* (Arthur, Cavera, & Chikindas, 2014). Nisin is widely used in the food industry and has been approved by the US Food and Drug

Administration, the European Food Safety Authority (Krivorotova, Cirkovas, et al., 2016) and the Food Standards Australia New Zealand (FSANZ, 2019). However, the antibacterial activity of nisin has been shown to be reduced in some food systems due to enzymatic degradation and its interaction with food components such as lipids (Gruskiene, Krivorotova, & Sereikaite, 2017). Various delivery systems have been developed to overcome this problem by nanoencapsulating nisin with other food-grade polysaccharides such as chitosan/alginate (Zimet et al., 2018; Zohri et al., 2013), chitosan/carrageenan (Chopra, Kaur, Bernela, & Thakur, 2014) and chitosan/monomethyl fumaric acid (Khan, Tango, Miskeen, & Oh, 2018). Krivorotova, Cirkovas, et al. (2016) and Krivorotova, Staneviciene, Luksa, Serviene, and Sereikaite (2016) have also been able to obtain nisin-loaded pectin and pectin-inulin nanoparticles. In addition, Nguyen, Gidley, and Dykes (2008) and Dos Santos et al. (2018) have successfully produced antimicrobial films by loading nisin into BC pellicles. However, no study to-date has reported the formation of antimicrobial agent-loaded BCNs.

Loading BCNs with nisin may make BCNs antimicrobial active and create potential preservative applications of BCNs in the food and packaging industry. This may also help food processors deliver nisin into food systems while ensuring its bioactivity. Given that meat products are highly perishable, bioactive packaging may be one of the most promising technologies which can be applied to reduce microbiological spoilage and extend the shelf life. High crystallinity and non-toxicity of BCNs make it suitable in the application of meat packaging as a carrier of antimicrobial agents and as a reinforcing agent in active packaging materials. Therefore, in this study, we aimed to produce nisin-loaded BCNs (NBCNs) by the simple and cost-effective complexation method and to evaluate the antimicrobial activity of NBCNs against two vacuum-packaged beef spoilage LAB.

5.2 Materials and methods

5.2.1 Production of BC and BCNs

Bacterial cellulose pellicles were produced according to Tan, Rahman, and Dykes (2016) with some modifications. An initial inoculum of *Komagataeibacter xylinus* ATCC 53524 was propagated in Hestrin and Schramm broth medium. The BC

pellicles were produced in 1 L Erlenmeyer flasks plugged with sterilized cotton. The flasks were incubated statically at 30 °C for 120 h. The collected pellicles were first washed with distilled water and then treated with 0.5 M NaOH (ThermoFisher, Australia) at 80 ºC for 1 h in a shaking water bath (OLS 200, Grant Instruments, England) to remove any remaining media and bacterial by-products. The pellicles were then continuously rinsed with filtered water until drained water achieved a neutral pH.

Bacterial cellulose pellicles were then acid hydrolysed as per Vasconcelos et al. (2017) with some modifications. The pellicles were cut into thin strips (1-2 mm) and homogenized using a stick blender (SM7200, Sunbeam, USA) with the addition of ultra-pure water to obtain a cellulosic pulp. The pulp was filtered through grade 1 Whatman filter paper and freeze-dried (Alpha 1-2 LD plus, Martin Christ, Germany) at - 30 °C at a pressure of 0.37 mbar for 24 h. The lyophilized BC was ground using a laboratory blender (LCG350SIL, Breville, China) and stored in a glass container until further use.

For the formation of BCNs, 0.6 g of ground BC was mixed with 60 ml of 60% (w/w) H_2SO_4 (ThermoFisher, Australia) at 45 °C for 1 h under mechanical stirring (500 rpm). The mixture was diluted ten-fold with ultra-pure water to stop the hydrolysis reaction. The resultant suspension was ultracentrifuged at 26,400 x g for 15 mins (JSE08J05, Beckman Coulter Inc., USA) as per Vasconcelos et al. (2017) to precipitate nanocrystals. The supernatant was removed and nanocrystal precipitate was separated. The nanocrystal precipitate was weighed and sterile water was added to make 1% (w/w; 10 mg/ml) BCN suspensions. Based on the dry weight basis, the yield of BCNs was 37.8%. The resulting BCN suspensions were ultrasonicated (S-4000-010, 20 kHz, 600 W, Misonix, USA) for 5 mins to separate the nanocrystals. The pH of the nanocrystal suspension was adjusted to neutral with the addition of 0.1 M NaOH.

5.2.2 Formation of nisin-loaded BCNs

A stock solution of 2.5 mg/ml nisin was prepared by dissolving 250 mg of nisin commercial powder (≥ 900 IU/mg, Glentham Life Sciences Ltd, Wiltshire, UK) in 100 ml of ultra-pure water as per Krivorotova, Cirkovas, et al. (2016) with some modifications. The solution was filtered through 0.45 µm filters and the pH was adjusted to neutral with the addition of 0.1 M NaOH. A dilution series (2.5, 2.0, 1.5,

1.0 and 0.5 mg/ml) of nisin commercial powder was prepared by diluting the stock solution with the addition of ultra-pure water. The dilution series of 2.5, 2.0, 1.5, 1.0 and 0.5 mg/ml nisin commercial powder was equivalent to the dilution series of 2250, 1800, 1350, 900 and 450 IU/ml nisin, respectively. The 10 mg/ml BCN suspension was also diluted with ultra-pure water to prepare 1 mg/ml and 5 mg/ml BCN suspensions for the formation of NBCNs. Two concentrations of BCNs (1 mg/ml (low-range) and 5 mg/ml (mid-range)) were used for the production of NBCNs to determine whether stability of NBCNs changes with varying BCNs concentration.

For the formation of different formulations of NBCNs, 1 ml of nisin solution was added dropwise to 10 ml of BCN suspension while stirring (500 rpm) at room temperature, as mentioned in Table 5.1. The stirring was continued for 30 mins to facilitate the ionic gelation reaction.

Table 5.1 Experimental design to obtain different formulations of NBCNs

BCN concentration (mg/ml)	Different concentrations of nisin (mg/ml)				
	0.5	**1.0**	**1.5**	**2.0**	**2.5**
a) 1.0 BCN	1_0.5 NBCN	1_1.0 NBCN	1_1.5 NBCN	1_2.0 NBCN	1_2.5 NBCN
b) 5.0 BCN	5_0.5 NBCN	5_1.0 NBCN	5_1.5 NBCN	5_2.0 NBCN	5_2.5 NBCN

BCNs: bacterial cellulose nanocrystals; NBCNs: nisin-loaded bacterial cellulose nanocrystals.

5.2.3 Zeta-potential and nanocrystal size

Zeta-potential of nanocrystals were determined using Zetasizer Nano (Malvern ZSP, Malvern Instruments Ltd, Worocestershire, UK) using a previously described protocol (Zimet et al., 2018). The hydrodynamic particle size of nanocrystals was determined using the same instrument as Singhsa, Narain, and Manuspiya (2018). Analyses were performed in an aqueous medium with a viscosity of 0.8872 cP, RI of 1.33 at 25 $^{\circ}$C and nanocrystal size was read in terms of z-average. All the samples were measured in triplicate. Based on the zeta-potential results, the most stable series of NBCNs formulated with either low-range (1 mg/ml) or mid-range (5 mg/ml) BCNs was chosen for further analysis of encapsulation efficiency, storage stability and antimicrobial activity (sections 5.2.4 to 5.2.6).

5.2.4 Nisin encapsulation efficiency analysis

Nisin encapsulation efficiency was determined using the bicinchoninic acid (BCA) colourimetric detection and quantitation method (Pierce™ BCA Protein Assay Kit, ThermoFisher). Nisin extraction, BCA analysis and encapsulation efficiency calculations were conducted as described by Zimet et al. (2018) without any modifications.

5.2.5 Analysis of storage stability

Each NBCN suspension was stored at 4 °C for 20 d and their zeta-potential was analysed on day 1, 4, 8, 12, 16 and 20 to determine the stability of nanocrystals with the increase of storage time. All the measurements were done in triplicate.

5.2.6 Antimicrobial activity analysis

The broth micro-dilution method was performed to analyse the antimicrobial effects of BCNs and NBCNs. Nisin-loaded BCNs were concentrated by centrifugation (5810R, Eppendorf AG, Germany) at 20,000 x g at 8 °C for 40 mins to remove free nisin (Zimet et al., 2018). The precipitated NBCNs were resuspended in a volume of sterile water which was equivalent to the removed supernatant. Nisin solutions without BCNs (free nisin) were prepared by dissolving 1 ml of each nisin concentration in 10 ml of sterile water to compare the antimicrobial efficacy of free nisin against NBCNs.

Meat spoilage LAB cultures of *Lactobacillus rhamnosus* LBM1 and *Leuconostoc mesenteroides* LBM2 were kindly donated by Huong Ho, Curtin University, and anaerobically grown in 10 ml of de Man, Rogosa and Sharpe (MRS; Oxoid Ltd., Hampshire, England) broth at 30 °C for 18 to 20 h. After the incubation period, the cultures were serially diluted to 10^6 CFU/ml in phosphate-buffered saline (PBS). For the determination of antimicrobial activity, 50 µl of each bacterial solution (10^6 CFU/ml) was added to 96 microtiter plate wells containing 100 µl of MRS broth. The wells were then supplemented with resuspended NBCN or free nisin solutions. In order to prepare positive controls of resuspended NBCN and free nisin, 50 µl of 5 mg/ml BCN solution was added instead of resuspended NBCN solution and 50 µl of sterile water was added instead of free nisin solution, respectively. All microtiter plates were incubated anaerobically at 30 °C for 48 h with shaking (150 rpm). The plates were

agitated linearly for 20 s (60 rpm) before the absorbance was measured at 0, 24 and 48 h using a multimode plate reader (HH3400, PerkinElmer Pte. Ltd, Singapore) at 590 nm. Every suspension was tested in triplicate. Antimicrobial activity was calculated as per Equation 1.

Absorbance relative positive control (%) = (A_{590} *of test sample / Average* A_{590} *of positive control*) * 100 (1)

After measuring absorbance readings at 48 h, 10 µl from the samples (free nisin and NBCNs) which exhibited the highest antimicrobial activity were spread plated on MRS agar plates in order to verify either inactivation of the microorganisms or inhibition of the microorganism growth. They were then incubated at 30 ºC and colony counts were taken after 96 h incubation period.

5.2.7 Physicochemical characterisation

The NBCN suspensions which showed the most effective antimicrobial activity against both strains were subjected to the physicochemical characterisations as mentioned in section 5.2.7.1 to 5.2.7.3 along with 5 mg/ml BCN suspension.

5.2.7.1 Electron microscopy imagining

The morphology of 5 mg/ml BCN suspension and selected NBCN suspension was viewed using a Neon 40EsB scanning electron microscope (SEM, Zeiss, Göttingen, Germany) operated with an acceleration voltage of 3 kV and aperture size of 20 µm. For SEM sample preparation, the suspensions were dropped onto aluminium stubs and allowed to dry for approximately 24 h. All the samples were observed under SEM without coating.

The same suspensions which were viewed using SEM were also viewed by the FEI Talos transmission electron microscope (TEM, ThermoFisher Scientific, Czech Republic) at an objective aperture 70. Before TEM imagining, samples were diluted ten times with ultra-pure water, placed on a carbon film-painted copper grid (300 mesh, Quantifoil®, Ted Pella Inc, CA, USA) and air-dried for nearly 48 h. The length (*L*) and width (*D*) of BCNs and NBCNs were determined from at least 50 measurements by Fiji ImageJ software.

5.2.7.2 FTIR

Fourier-transform infrared (FTIR) measurements were performed using a Nicolet™ iS50 FTIR Spectrometer (Thermo Scientific, WI, USA). For FTIR sample preparation, all nanocrystal samples were thoroughly washed with distilled water using centrifugation to remove any physically adsorbed nisin and then the samples were freeze-dried. Nisin powder was directly used without any treatment. Spectra of all samples were recorded from 400 – 4000 cm^{-1} at a resolution of 4 cm^{-1} with 64 scans.

5.2.7.3 XRD

X-ray diffraction (XRD) patterns of 5 mg/ml BCN suspension and selected NBCN suspensions were performed by powder diffraction, using a D8 Advance diffractometer (Bruker AXS, Karlsruh, Germany). The diffraction angle ranged from 10 to 50º 2θ. The radiation source of copper K alpha generated an accelerating voltage of 40 kV and a filament emission of 40 mA. All the samples were freeze-dried before XRD measurements. The baseline subtraction was carried out as per Coelho (2018) using TOPAS Academic version 7 software. The crystallinity index (CI) was calculated by Equation 2 (Singhsa et al., 2018).

$$CI\ \% = ((I_{200} - I_{am}) / I_{200}) \times 100 \qquad (2)$$

Where I_{200} is the overall intensity of the peak at 2θ = 22.8° and I_{am} (the minimum intensity between (200) and (110) planes) is the intensity of the baseline at 2θ = 18.4°.

5.2.8 Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics version 26 software. Two independent experiments with three replicates were conducted and the mean values ± SD were reported. The data on storage stability analysis and all the formulations at each storage day were analysed using one-way analysis of variance (ANOVA). The data on zeta-potential, nanocrystal size, encapsulation efficiency and antimicrobial activity of NBCNs were also analysed using ANOVA. The data on 1 and 5 mg/ml BCN samples obtained under analysis 5.2.3 and data on NBCN and free nisin obtained under analysis 5.2.6 were analysed using Paired sample T-test to compare the

results between the two treatments. If a significant ($P < 0.05$) difference was found, mean values were further analysed using the Tukey comparison test.

5.3 Results and discussion

5.3.1 Zeta-potential and nanocrystal size

The zeta-potential evaluates the presence of surface charges on the nanoparticles and can be used to assess the stability of a colloid solution. After hydrolysis, BCNs (10 mg/ml) had an average zeta-potential of - 33.5 ± 2.7 mV at pH 3.0 which increased up to - 43.0 ± 1.6 mV when the pH was adjusted to neutral, similar to the results reported by Yan et al. (2017). The progressive increase of deprotonated carboxyl groups may increase the zeta-potential by enhancing electrostatic repulsions to prevent the aggregation of BCNs (Chenglin et al., 2012). The BCNs gained relatively higher zeta-potential due to the anionic sulphate half-ester groups derived from the esterification of the hydroxyl groups present on the BC surface (Vasconcelos et al., 2017), as shown in Figure 5.1. Nisin was positively charged with an average zeta-potential of 16.3 ± 1.3 mV at neutral pH.

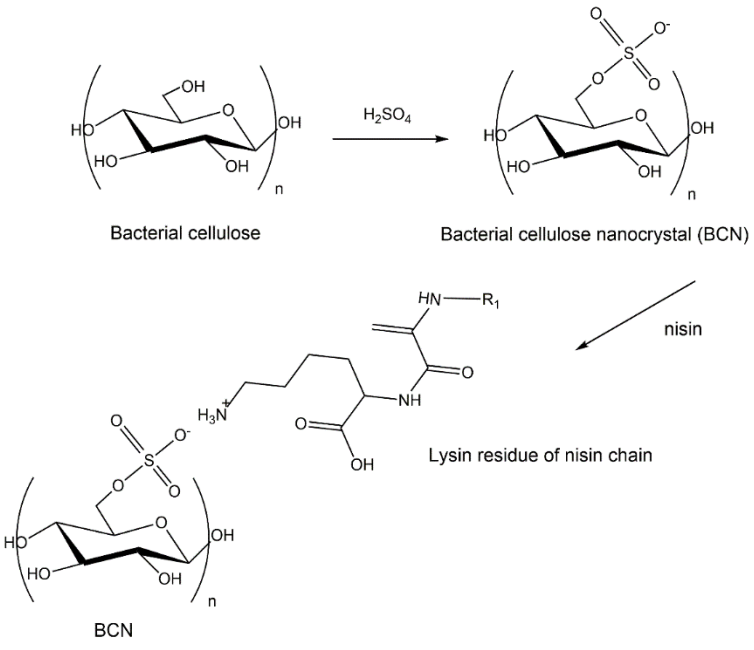

Figure 5.1 Formation of BCNs by acid hydrolysis and electrostatic interaction between BCNs and lysine residue of nisin molecule. BCNs: bacterial cellulose nanocrystals.

The zeta-potential measurements of NBCNs exhibited the interaction between negatively charged BCNs and positively charged nisin molecules. The negative zeta-potentials of NBCNs significantly decreased with the increase in nisin concentration for both BCN concentrations (Figure 5.2). Similarly, Krivorotova, Cirkovas, et al. (2016) and Krivorotova, Staneviciene, et al. (2016) have reported decreasing trends of zeta-potential with increasing nisin concentrations in the formation of nisin-loaded pectin and pectin-inulin nanoparticles, respectively. It is hypothesized that the ionic interactions between positively charged lysine residues of nisin and negatively charged $-OSO_3^-$ groups of BCNs (Figure 5.1) could have caused the reduction of negative surface charges and led to the formation of NBCNs. All NBCNs formulated with 5 mg/ml BCNs suspension had zeta-potential values which were greater than or equal to - 30 mV, whereas the zeta-potentials of NBCNs formed with 1 mg/ml BCNs fell below the accepted threshold of stability (zeta-potential ≥ 30 mV; Yan et al., 2016) with the increase in nisin concentration. This could be due to the low availability of anionic BCNs to bind with the increasing number of cationic nisin molecules, thus leading to agglomeration of the BCNs. Therefore, NBCNs formulated with 5 mg/ml BCNs suspension exhibited more stability in aqueous suspension, possibly due to the stronger repulsion forces between nanocrystals (Yan et al., 2016), and were chosen to carry out further experiments. This suggested that NBNCs formulated with 5 mg/ml BCNs had a greater number of free negative surface charges compared to the NBNCs formulated with 1 mg/ml BCNs, after interacting with cationic nisin molecules. As per Singhsa et al. (2018), the number of remaining negative surface charges was greater than the occupied positive ones, resulting in a higher net negative charge on the surfaces of NBCNs formulated with 5 mg/ml BCNs.

BCNs ranged in hydrodynamic particle size from 162 to 190 nm (Table 5.2) with a polydispersity index of 0.29 which was in line with previous studies (Yan et al., 2019). The small average size and narrow size distribution indicated the high uniformity of BCNs suspensions which could have resulted from the cleavage of amorphous components and the desquamate of the glycosidic bonds (Zhai, Lin, Li, & Yang, 2020). The nanocrystals formed with 5 mg/ml BCNs showed a tendency to decrease in hydrodynamic particle size with increased nisin concentrations. However, in the NBCNs formed with 1 mg/ml BCNs, first a drop and then an increase in hydrodynamic

particle size at the highest nisin concentration was observed. The change in hydrodynamic particle size with different nisin concentrations is possibly due to the ionic interaction between peptide and polymeric molecules (Krivorotova, Cirkovas, et al., 2016; Krivorotova, Staneviciene, et al., 2016). The drop in hydrodynamic particle size could have been caused by masking negative surface charges of BCNs by positive charges of nisin molecules which may have led BCNs to condense closer into small nanocrystals. However, when the nisin concentration reaches a critical limit (2.5 mg/ml nisin for the 1 mg/ml BCNs sample), the repulsive forces could have been neutralised and agglomeration of BCNs could have been initiated which led to the formation of bigger particles. Similar results on hydrodynamic particle sizes of BCNs modified with cationic amines have been reported by Singhsa et al. (2018). However, zeta-potential analysis has limitations in determining the actual particle size since acid hydrolysis results in particles with reduced sphericity. The size of BCNs and selected NBCNs were therefore further calculated using the images of SEM and TEM as discussed in section 5.3.5.

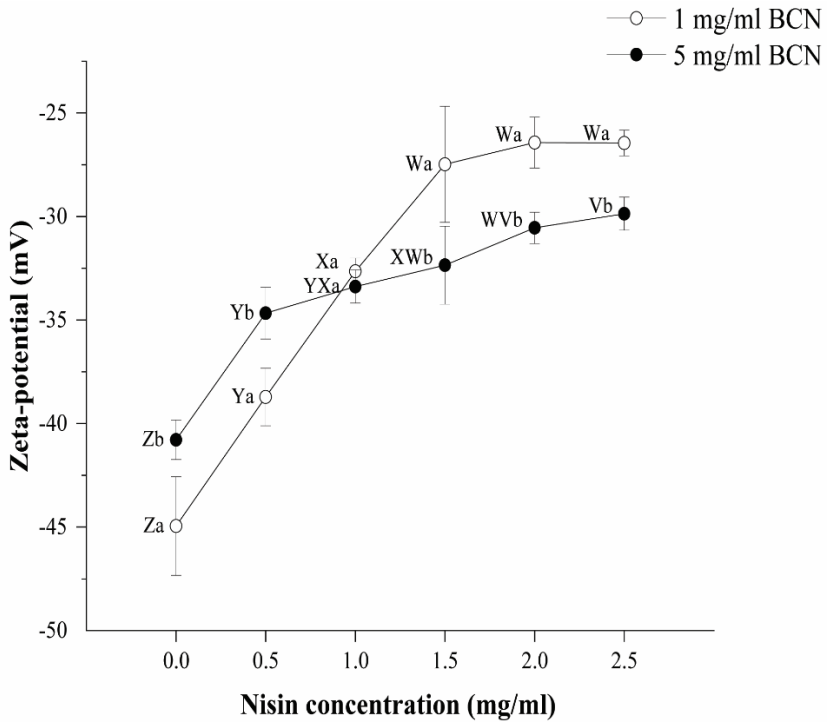

Figure 5.2 Zeta-potential of BCNs and NBCNs as a function of nisin concentration. Different uppercase letters (Z-V) represent significant differences among different nisin concentrations ($P < 0.05$). Different lowercase letters (a-b) mean significant differences between two different BCN concentrations ($P < 0.05$). BCNs: bacterial cellulose nanocrystals; NBCNs: nisin-loaded bacterial cellulose nanocrystals.

Table 5.2 Average hydrodynamic particle size (nm) of BCNs and NBCNs as a function of nisin concentration

BCN (mg/ml)	Nisin concentration (mg/ml)						SDM	P-value
	0.0	0.5	1.0	1.5	2.0	2.5		
1.0	179.87 ± 7.11 Za	171.10 ± 10.94 ZYa	162.52 ± 2.17 Ya	163.75 ± 6.79 Ya	161.42 ± 2.56 Ya	180.77 ± 2.04 Za	9.94	0.000
5.0	168.63 ± 5.65 Zb	164.43 ± 2.67 ZYa	158.05 ± 5.33 YXa	157.00 ± 5.02 Xa	157.07 ± 1.48 Xb	157.92 ± 3.07 YXb	5.93	0.000
P-value	0.000	0.280	0.158	0.184	0.006	0.000		

[a-b] Means within the same column for the same index with different lowercase letters differ significantly between two BCN concentrations ($P < 0.05$).

[Z-X] Means within the same row with different uppercase letters differ significantly among different nisin concentrations ($P < 0.05$).

BCNs: bacterial cellulose nanocrystals; SDM: standard deviation of mean.

5.3.2 Nisin encapsulation efficiency

The nisin encapsulation efficiencies of NBCNs formulated with 5 mg/ml BCN suspension are displayed in Figure 5.3. The nisin encapsulation efficiency of nanocrystals ranged from 80.5 ± 0.9 to 93.3 ± 0.2 %. The high encapsulation efficiencies indicate that hydrophilic properties and the large surface area of BCNs have facilitated the successful loading (Li et al., 2019) of the bacteriocin. In this study, the encapsulation of nisin into the BCNs was achieved by adsorbing the cationic peptide on the anionic surface of nanocrystals. According to Figure 5.3, the encapsulation efficiency has significantly increased with the increase of nisin concentration. Soto et al. (2016) and Zimet et al. (2018) have also reported increased encapsulation efficiencies as the concentration of nisin increased in the formation of nisin-loaded electrospun nanofibers and nisin-loaded alginate-chitosan nanoparticles, respectively. The saturation of nisin into nanocrystals could be seen above nisin concentrations of 1.5 mg/ml, probably due to the limited availability of BCNs to bind with the increasing number of nisin molecules. The encapsulation efficiency of nisin into BCNs was higher as compared to the findings of Hosseini et al. (2014), Cui, Wu, Li, and Lin (2017) and Zimet et al. (2018) who reported maximum encapsulation efficiencies of 59.8%, 49.3% and 35.6%, respectively. Hosseini et al. (2014), Cui et al. (2017) and Zimet et al. (2018) have used alginate/alginate-resistant starch, poly-γ-glutamic acid/chitosan and alginate/chitosan polymer combinations as the carriers of nisin in their studies, respectively. The BCNs have a large fibre surface due to their

nanometer dimension which enables them to form strong interactions with surrounding molecules, such as water and other polymer compounds, with functional groups active in ionic-bond and hydrogen-bond formation (Klemm et al., 2011). As suggested by Singhsa et al. (2018), physical adsorption of amines onto the BCN chain via ionic interaction between cationic nisin molecules and anionic sulphate groups on the BCN's surface could be the reason for BCNs showing high nisin encapsulation efficiency compared to other polymers. This suggests that the problem of the low throughput of nisin as a carrier in the food industry (Soto et al., 2016) could be overcome by encapsulating nisin with BCNs due to its high encapsulation efficiency.

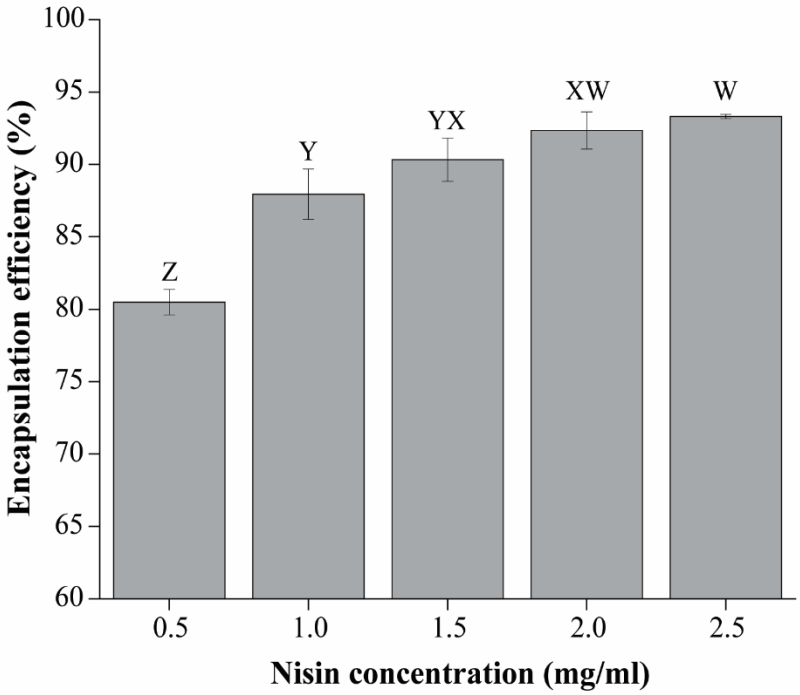

Figure 5.3 Encapsulation efficiency of BCNs as a function of nisin concentration. Different uppercase letters (Z-W) mean significant differences among different nisin concentrations ($P < 0.05$). BCNs: bacterial cellulose nanocrystals.

5.3.3 Stability of NBCNs

Figure 5.4 presents the changes in zeta-potentials of BCNs and NBCNs during the refrigerated storage for 20 d. Properties of nanoparticles may vary as a function of time and understanding the time dependency behaviour of nanoparticles is important to determine their shelf life stability (Baer, 2011), especially when considering their application in the food manufacturing industry. Pure BCNs appeared quite stable over

longer storage times. Their zeta-potential charges remained greater than - 40 mV during the 20 d storage period. However, in the case of NBCNs, the negative zeta-potential charges dropped significantly with the increase of storage time. This indicates that the stability of nisin-loaded nanocrystals may decrease over time in refrigerated storage. Nanocrystals formed with 2.0 and 2.5 mg/ml nisin concentrations displayed greater instability after 16 d of storage. The instability of NBCNs at longer storage times could be due to either agglomeration or dissolution processes (Izak-Nau et al., 2015). According to Izak-Nau et al. (2015), the dissolution process may lead to the release of encapsulated agents into the solution, and thus the zeta-potential value may be a combination of the zeta-potential of nanocrystals and that of the free agent. Based on these results, it is recommended to use NBCNs within the first two weeks from the day of production when they are used in industrial applications.

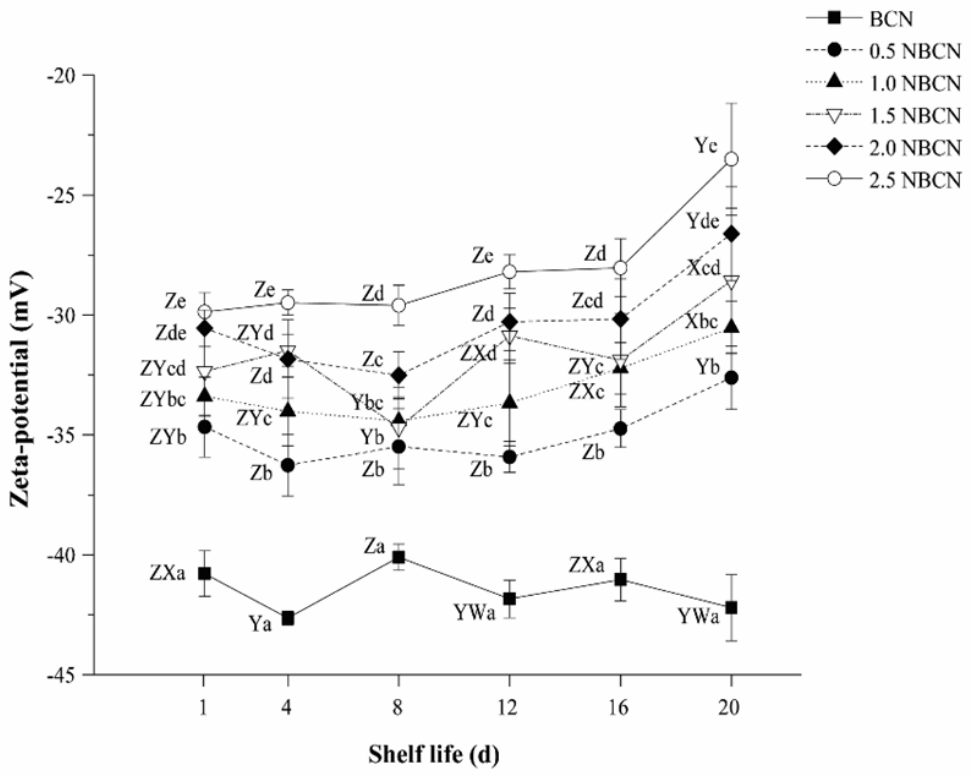

Figure 5.4 Change in zeta-potential values of BCNs and NBCNs during the shelf life. Different uppercase letters (Z-W) represent significant differences among different storage times ($P < 0.05$). Different lowercase letters (a-e) mean significant differences among different nisin concentrations ($P < 0.05$). BCNs: bacterial cellulose nanocrystals; NBCNs: nisin-loaded bacterial cellulose nanocrystals.

5.3.4 Antimicrobial efficiency

The antimicrobial activity of encapsulated nisin and free nisin was assessed against two Gram-positive vacuum-packaged beef spoilage bacteria, *L. rhamnosus* LBM1 and *L. mesenteroides* LBM2 (Figure 5.5). *L. rhamnosus* LBM1 and *L. mesenteroides* LBM2 are meat spoilage isolates that are responsible for the blowing of meat packages (Broda, Boerema, & Bell, 2003; Pothakos, Devlieghere, Villani, Björkroth, & Ercolini, 2015).

After 24 and 48 h incubation periods, resuspended NBCN solutions with low nisin concentrations (0.5 - 1.5 mg/ml) did not exhibit an inhibitory effect on *L. rhamnosus* LBM1 growth. However, free nisin solutions exhibited significantly higher antimicrobial activity against *L. rhamnosus* LBM1 after treatment with 1.0 and 1.5 mg/ml concentrations respectively at 24 and 48 h incubation periods. Only at higher nisin loadings (2.0 and 2.5 mg/ml) was a strong inhibitory effect of NBCNs against the growth of *L. rhamnosus* LBM1 observed. This could be due to the high surface to volume ratio provided by BCNs for bacterial attachment and growth. It is known that BC lacks antimicrobial properties (Wu et al., 2014) and is capable of entrapping microbial cells (Gedarawatte et al., 2020; Moghanjougi et al., 2020) due to its high surface to volume ratio (Jebel & Almasi, 2016) and these results suggest that the same applies to the BCNs. Also, the antimicrobial effect of nisin occurs through direct contact of nisin with the bacteria. On the NBCNs, only the nisin molecules that are exposed on the surface of the NBCNs can reach the bacteria, and therefore only these are effective in antimicrobials.

Both free nisin and NBCNs formed with low nisin concentrations (0.5 – 1.5 mg/ml) did not exhibit antimicrobial activity against the growth of *L. mesenteroides* LBM2. This was observed after incubation periods of 24 and 48 h. A significant inhibitory effect of free and loaded nisin against *L. mesenteroides* LBM2 was obtained after treatment at 2.0 and 2.5 mg/ml of nisin concentrations. No LAB growth was observed on MRS plates from the samples (2.0 and 2.5 mg/ml of free nisin and NBCNs) streaked plated after 48 h incubation period which further confirmed free nisin and NBCNs loaded with nisin concentrations $\geq$ 2 mg/ml were effective in inactivating both microorganisms. Overall, the individual application of BCNs in the food industry is not very promising since they may enhance the growth of microorganisms

(Gedarawatte et al., 2020). But the results of this study indicate that this problem could be mitigated and microbial inactivation could be achieved by loading BCNs with food-grade nisin.

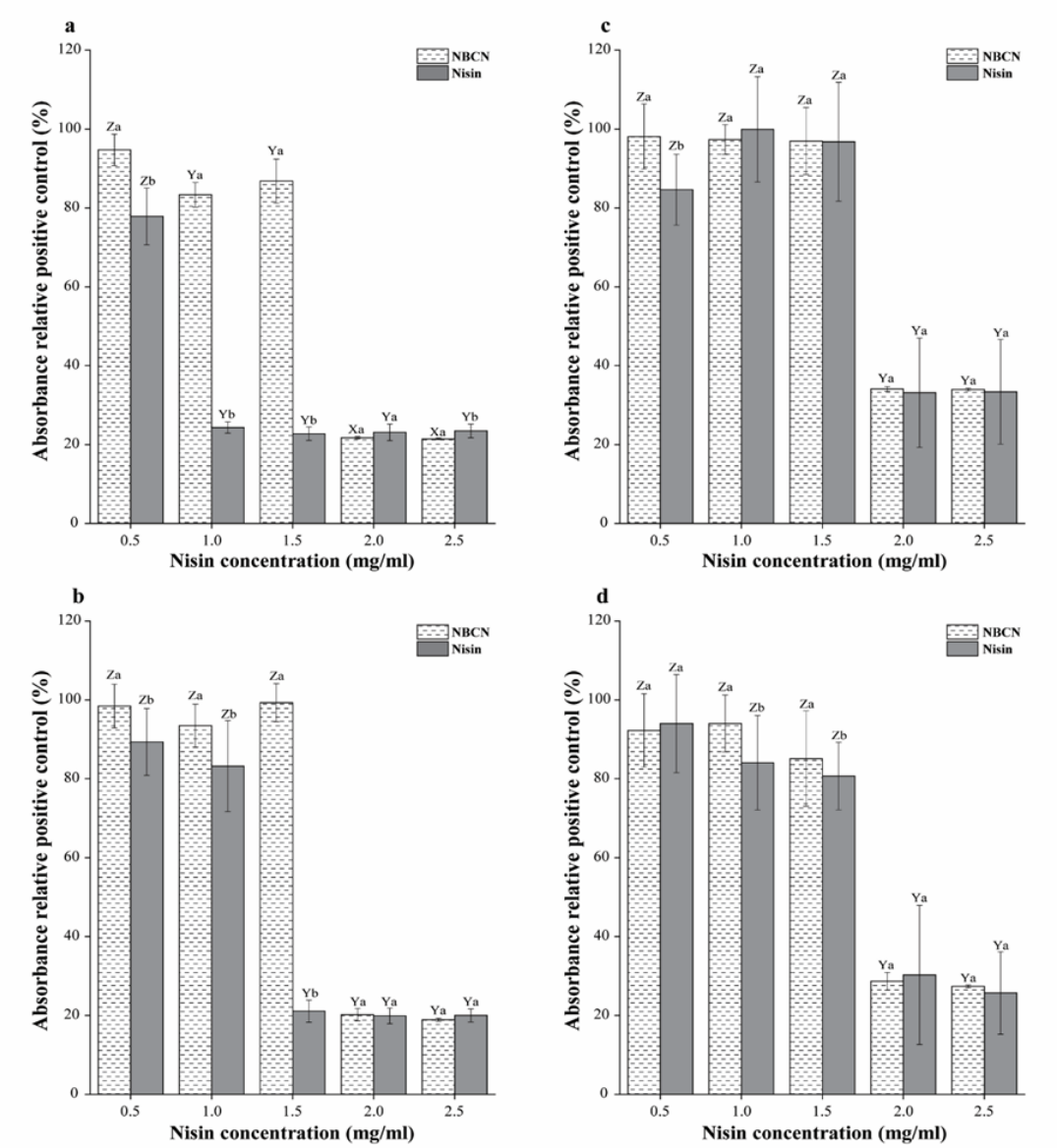

Figure 5.5 Absorbance relative to positive control (%) of *Lactobacillus rhamnosus* LBM1 after incubation at 30 ˚C for (a) 24 h, (b) 48 h and of *Leuconostoc mesenteroides* LBM2 after incubation at 30 ºC for (c) 24 h and (d) 48 h. Different uppercase letters (Z-W) mean significant differences among different nisin concentrations ($P < 0.05$). Different lowercase letters (a, b) mean significant differences between encapsulated nisin (NBCNs) and free nisin ($P < 0.05$). NBCNs: nisin-loaded bacterial cellulose nanocrystals.

5.3.5 Morphology

The SEM (Figure 5.6a) and TEM (Figure 5.6c) images of BCNs showed rod or needle-shaped nanocrystals, similar to the morphology described by Martínez-Sanz et al. (2011) and Yan et al. (2017). The BCNs solution (5 mg/ml) without any dilution was visualized using SEM and tightly packed networks of nanofibers could be observed as shown in Figure 5.6a. This is similar to the typical SEM image of the surface of a bacterial cellulose membrane as reported by Gedarawatte et al. (2020) which confirms that BCNs solutions are capable of creating a porous network of nanofibers when they are applied on a surface. Acid hydrolysed BCNs had a width ranging from 15 to 56 nm and a length ranging from 259 to 1142 nm. These measurements represented an average width of 32.2 ± 9.4 nm and an average length of 628.9 ± 207.4 nm, corresponding to an aspect ratio (L/D) of 19.5. These results were quite similar to the findings of Vasconcelos et al. (2017). Figure 5.6b and d illustrate SEM and TEM images of NBCNs formed with 2.0 mg/ml nisin solution, respectively. The geometrical structures observed in Figure 5.6b and 5d are considered as NBCNs. The dumbbell-shaped morphology of NBCNs in these images could be due to agglomeration of BCNs following neutralisation of negative surface charges of BCNs by positive charges of nisin molecules. These NBCNs had a width ranging from 16 to 128 nm (average width 55.5 ± 23.1 nm) and a length ranging from 161 to 1507 nm (average length 589.5 ± 288.4 nm) corresponding to an aspect ratio (L/D) of 10.6. The reduced aspect ratio could be due to the significantly increased width of nanocrystals after encapsulating with nisin solution.

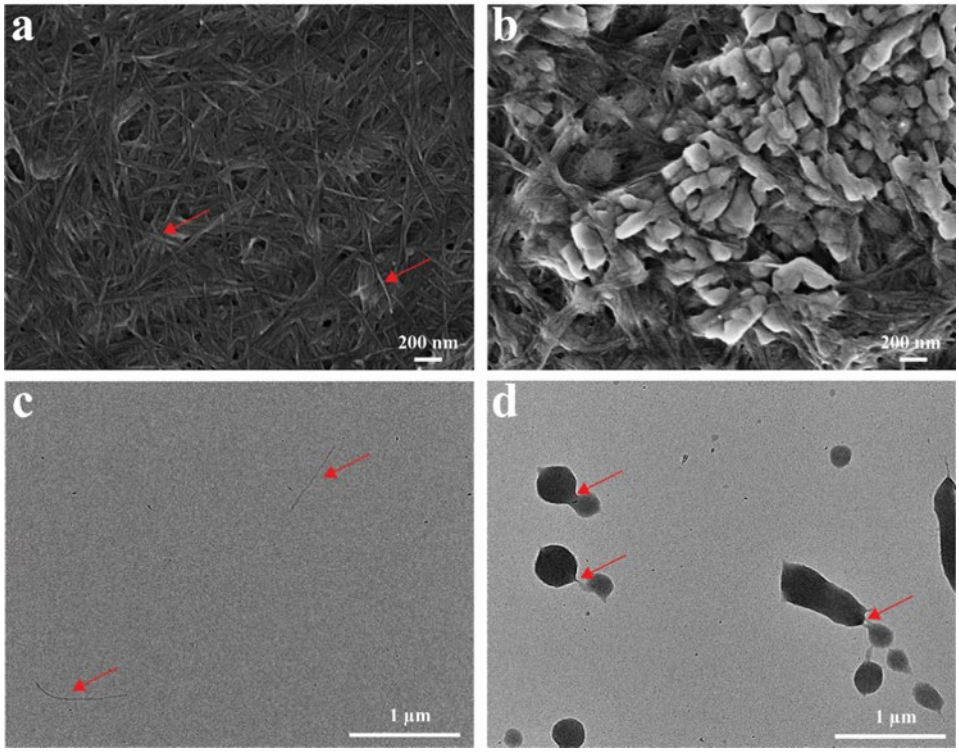

Figure 5.6 SEM images of (a) BCNs and (b) NBCNs; TEM images of (c) BCNs and (d) NBCNs. Red arrows point to BCNs. BCNs: bacterial cellulose nanocrystals; NBCNs: nisin-loaded bacterial cellulose nanocrystals.

5.3.6 FTIR

FTIR analysis is a useful method for examining the specific functional groups and structural changes of BCNs, NBCNs and nisin (Figure 5.7). In the BCNs and NBCNs (prepared with 2.0 and 2.5 mg/ml nisin) spectra, the peaks at 3340 and 1645 cm^{-1} were attributed to the stretching and bending vibrations of −OH bonds, respectively (Huang, Zhan, Li, Tian, & Chang, 2019; Taokaew, Seetabhawang, Siripong, & Phisalaphong, 2013). The peaks at 2895 and 1428 cm^{-1} correspond to the asymmetric stretching and deformation of $-CH_2-$ bonds, respectively (Huang et al., 2016). In addition, the peak at 1055 cm^{-1} was associated with the C−O−C stretching of cellulose pyranose ring (Yan et al., 2017). In a similar way to that reported by Sukhavattanakul and Manuspiya (2020), Vasconcelos et al. (2017) and Martínez-Sanz et al. (2011), the peak at 807 cm^{-1} which corresponds to the symmetric vibration of C−O−S (the bond derived due to the hydrolysis reaction) bond of BCNs could not be observed, possibly due to the small number of attached sulphate groups (Singhsa et al., 2018). The spectra of nisin gave three characteristic peaks at 1640, 1529 and 1447 cm^{-1} which were attributed to the

amide I group, $-NH_2$ bending and amide III group, respectively (Khan et al., 2018). It seems apparent that the peak of the amide group I overlaps with the peak of $-OH$ bonds of NBCNs at 1645 cm^{-1}. The spectra of NBCNs produced with 2.0 and 2.5 mg/ml of nisin demonstrated new characteristic peaks at 1556 and 1540 cm^{-1}, respectively, which are associated with the presence of N–H deformation in the amides combined with $-NH_3^+$ deformation of the amide II group of nisin (Saini, Sillard, Belgacem, & Bras, 2016; Wu et al., 2020). This clearly confirms physical adsorption of nisin molecules onto the BCNs chain via ionic interaction between cationic nisin molecules and anionic surface sulphate groups of BCNs. In addition, similarly to Saini et al. (2016), new peaks at 2916 and 2850 cm^{-1} were observed in NBCNs spectra which further confirms the loading of nisin.

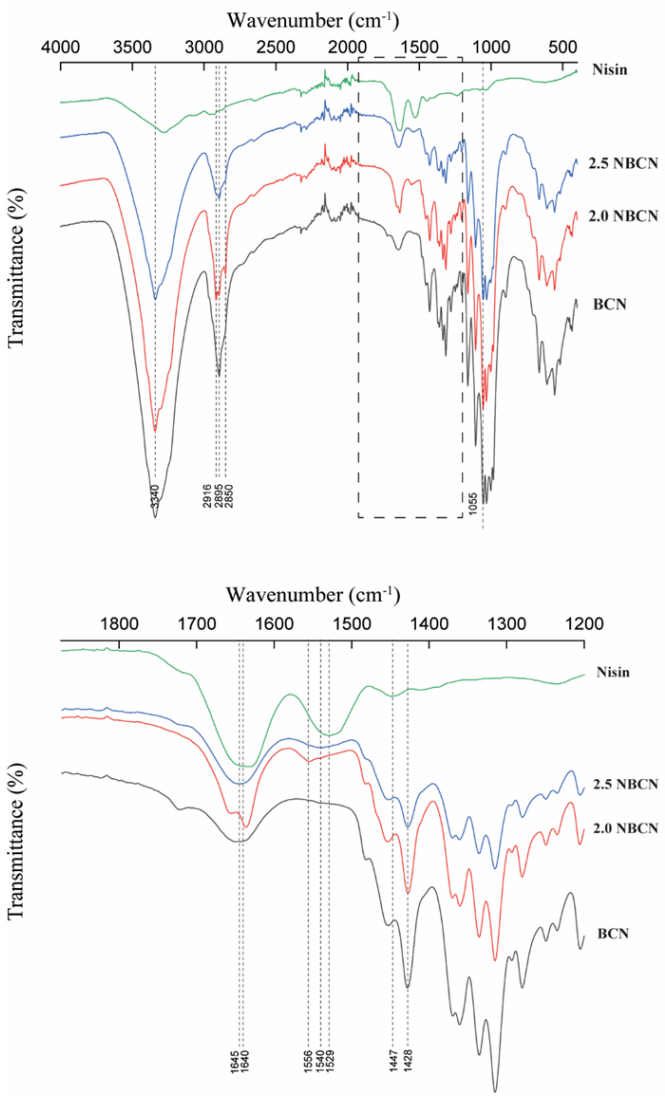

Figure 5.7 FTIR spectra of nisin, BCNs and NBCNs formed with 2.0 and 2.5 mg/ml nisin. BCNs: bacterial cellulose nanocrystals; NBCNs: nisin-loaded bacterial cellulose nanocrystals.

5.3.7 Crystallinity

The XRD patterns of BCNs and NBCNs (Figure 5.8) exhibited three characteristics peaks at 14.6º, 16.9º and 22.8º corresponding to the crystallographic planes of $(1\bar{1}0)$, (110) and (200), respectively. These results are similar to the findings of Vasconcelos et al. (2017) and Yan et al. (2017) and indicate that only cellulose Iα (triclinic) is present in the samples which confirm that the nanocrystals consist of pure cellulose free from lignin and hemicellulose. The CI calculated for the BCNs (83.12%) was close to that reported in a previous study for BCNs obtained through a similar hydrolysis process (Singhsa et al., 2018). NBCNs predominantly exhibited the characteristic peaks of BCNs, similar to previous findings (Li et al., 2019). The encapsulation process did not substantially change the CI values of NBCNs produced with 2.0 (89.01%) and 2.5 (89.05%) mg/ml of nisin which indicates that nisin loading did not impact the crystallinity of BCNs. In contrast, Hosseini et al. (2014) have reported reduced crystallinity upon the addition of nisin into their microparticles. Low nisin to BCNs ratio (1:10) maintained in this study could be the reason for relatively constant CI values obtained by NBCNs, whereas Hosseini et al. (2014) had used a relatively high nisin to alginate ratio (4:1, 2:1 and 1:1) in their microparticle formation. The complexation method suggested in this study allows the formation of antimicrobial active BCNs without reducing their crystallinity. This confirms that the mechanical strength of BCNs remained stable even after they were subjected to the encapsulation process. Therefore, the strength and integrity of BCNs will not be affected by nisin loading and this potentially allows its versatile use as a reinforcement agent in active food packaging as well as a direct food additive.

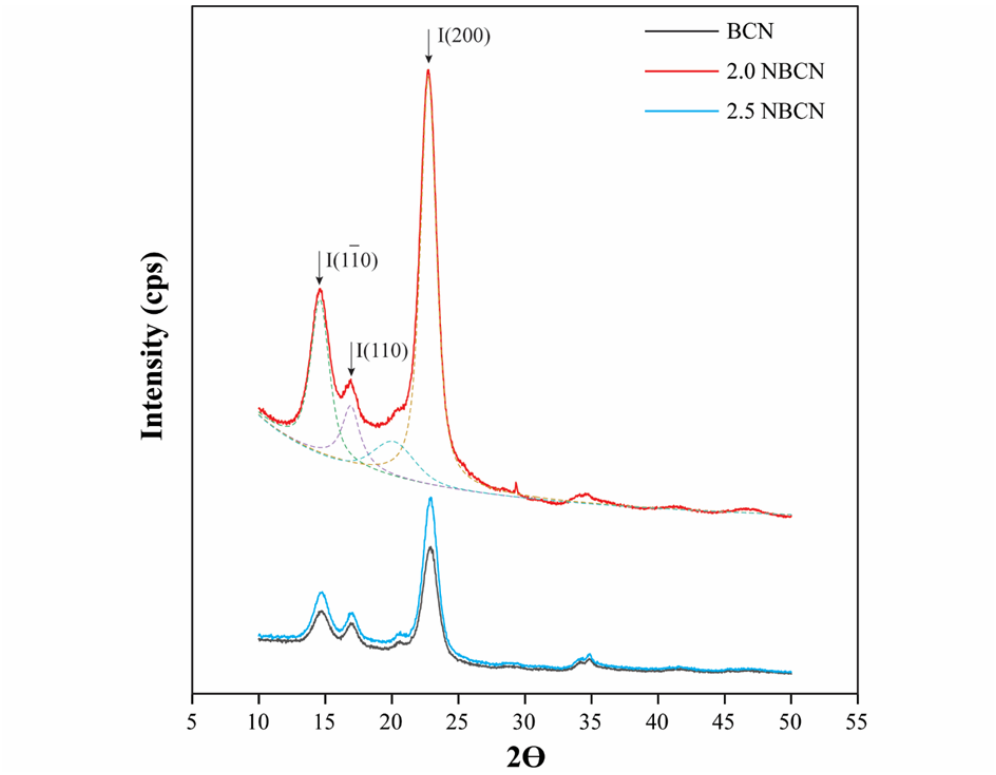

Figure 5.8 XRD patterns of BCNs and NBCNs formed with 2.0 and 2.5 mg/ml nisin. Arrows indicate the crystallographic planes of (1$\bar{1}$0), (110) and (200). BCNs: bacterial cellulose nanocrystals; NBCNs: nisin-loaded bacterial cellulose nanocrystals. XRD pattern of 2.0 NBCN has been offset by 10,000 cps for visibility.

5.4 Conclusion

This study was conducted to produce NBCNs by the simple and low-cost complexation method, which is applicable in an industrial scale and assess the nanocrystals structural and antimicrobial properties. Two concentrations (1 and 5 mg/ml) of BCNs were used and the most stable NBCNs were formed with the BCNs concentration of 5 mg/ml. BCNs were able to achieve a higher nisin encapsulation efficiency (80 – 93%) with increased nisin concentrations. Loading with nisin concentrations of 2.0 and 2.5 mg/ml greatly improved the nanocrystals antimicrobial activity which could make their use in the food industry desirable. XRD results confirmed no impact on mechanical strength due to loading with nisin. The results from this study show the potential of NBCNs as an antimicrobial biomaterial that could be used as a reinforcing agent in active food packaging. Further study on the toxicity and food spoilage characteristics of these biopolymers could aid in the development of bioactive packaging for long term storage of food.

Chapter 6. Evaluation of the water-holding and anti-spoilage effect of a bacterial cellulose nanocrystal coating for the storage of vacuum-packaged beef

Information contained in this chapter has been submitted for publication as follows:

Gedarawatte, S. T., Ravensdale, J. T., Johns, M. L., Ming, L., Al-Salami, H., Dykes, G. A., & Coorey, R. (2021). Evaluation of the water-holding and anti-spoilage effect of a bacterial cellulose nanocrystal coating for the storage of vacuum-packaged beef. *Food Chemistry* (under review).

Abstract

The preservative effect of applying bacterial cellulose nanocrystals (BCNs) and nisin-loaded BCNs (NBCNs) as spray coatings for vacuum-packaged beef was investigated and compared to uncoated beef for up to 4 weeks. The impact of nanocoatings on water-holding properties were investigated using purge loss, drip loss and LF-NMR analysis. Physicochemical, microbiological and sensory properties were also performed to analyse the anti-spoilage effect of the coatings. The electrochemical interaction between nanocrystals and the meat structure was evaluated by Kelvin probe force microscopy and zeta-potential analysis. BCNs alone significantly increased the microbial growth while treating beef with NBCNs reduced bacterial growth. No significant impact of nanocrystals on physicochemical and sensorial properties was observed. Nanocrystals were not effective in controlling purge loss. This study suggests that weak electrochemical interaction between nanocrystals and purge may relate to poor water-holding properties and this could aid in the design of new preservative coatings using nanocrystals.

6.1 Introduction

Vacuum packaging is routinely used to extend the shelf life of beef and transport it to distant markets by reducing microbial and other degradative processes related to spoilage. Super-chilled storage (-1.5 °C) further extends the shelf life of vacuum-packaged beef compared to conventional chilled (2 °C) and frozen (-18 °C) storage by reducing microbial growth and structural damage, respectively (Chen et al., 2020). However, vacuum packaging is reported to result in the highest purge loss compared to other packaging systems due to the physical compression applied during the packaging process (Sekar, Dushyanthan, Radhakrishnan, & Babu, 2006). Purge loss is

indicated by fluid accumulation inside the package and reflects weight loss during storage leading to unattractive product appearance, poor eating quality and substantial economic losses to red meat processors.

Nano systems are gradually being applied in the food industry and nanocoatings are being thoroughly researched as one of the options for extending the shelf life of food. Some researchers have investigated the impact of traditional edible coatings on controlling purge loss and shelf life extension of vacuum-packaged beef (Antoniewski, Barringer, Knipe, & Zerby, 2007; Duran & Kahve, 2020). To the best of our knowledge, no previous study has been published evaluating the possibility of nanocoating usage to extend the shelf life of fresh red meat. Many types of biopolymers, such as gelatine, chitosan, alginate and cellulose are currently used for the formation of nanocoatings for fresh food. The influence of cellulose nanofiber coating for shelf life extension of saffron (Jafari, Bahrami, Dehnad, & Shahidi, 2018), strawberries (Fakhouri et al., 2014), cherries (Jung, Deng, Simonsen, Bastías, & Zhao, 2016) and fresh-cut spinach (Pacaphol, Seraypheap, & Aht-Ong, 2019) have been investigated. Only one study has evaluated the use of bacterial cellulose nanocrystals (BCNs) for direct coating application on fresh-cut apples (Zhai, Lin, Li, & Yang, 2020) which showed potential for making edible films and coatings.

BCNs are produced by acid hydrolysis of microbial polysaccharide predominantly formed by bacteria in the genus *Komagataeibacter* (Klemm, Heublein, Fink, & Bohn, 2005). Compared to cellulose nanofibers originating from plant materials, BCNs are chemically pure since they are free from pectin, lignin and hemicellulose (Vandamme, De Baets, Vanbaelen, Joris, & De Wulf, 1998). BCNs have some remarkable characteristics such as non-toxicity, biocompatibility, high water-holding capacity and high mechanical strength (Klemm et al., 2005) making them ideal in food nanocoating applications. In a previous study, Gedarawatte et al. (2020) demonstrated that the application of bacterial cellulose as a wrapping material was effective in absorbing purge accumulated during the storage of vacuum-packaged beef. One of the key limitations of using BCNs in nanocoating applications is their lack of antimicrobial activity (Gedarawatte, Ravensdale, Al-Salami, Dykes, & Coorey, 2021). Gedarawatte et al. (2021) reported this could be overcome by loading BCNs with nisin antimicrobial

peptide which made them effective against selected meat spoilage lactic acid bacteria (LAB).

Investigating the use of BCNs and nisin-loaded BCNs (NBCNs) as an edible nanocoating to control purge loss and shelf life extension of fresh beef may contribute to the advancements in food packaging. Previous studies that assessed the direct application of nanocoating on food did not investigate the interaction between nanoparticles and the food matrix and their mechanism of controlling quality degradation. This study evaluated the impact of BCNs and NBCNs in controlling purge loss and improving physicochemical, microbiological and sensorial characteristics of vacuum-packaged beef. It also determined the electrochemical interaction between nanocrystals and the structure of the beef muscle.

6.2 Materials and methods

6.2.1 Experimental setup

6.2.1.1 Preparation of BCNs and NBCNs spray coating solutions

Bacterial cellulose pellicles were prepared and acid hydrolysed to produce 0.5% (w/w; 5 mg/ml) BCN suspension as per Gedarawatte et al. (2021) without any modifications. A 20 ml of 2.5 mg/ml nisin (2250 IU/ml, Glentham Life Sciences Ltd, Wiltshire, UK) was prepared and added dropwise to 200 ml of 5 mg/ml BCN suspension to form NBCNs as per Gedarawatte et al. (2021) without any modifications.

BCNs and NBCNs have been characterised by the methods of zeta-potential, encapsulation efficiency, storage stability, antimicrobial activity, electron microscopy imagining, FTIR and XRD and all the results can be found in our previous study, Gedarawatte et al. (2021).

6.2.1.2 Steak preparation and spray coating treatments

Beef eye round (*M. semitendinosus*) primal cuts derived from 3 cattle were sourced from a local butcher (Perth metropolitan area, Western Australia, Australia) within 24 h post-slaughter. Visible fat and connective tissues were trimmed off under aseptic conditions and a total of 75 steaks averaging 100 g and dimensions of 10 cm x 8 cm

and 1.5 cm were cut. Three groups of 25 steaks each, with steaks randomly assigned to each group were spray coated as follows;

Group A - Uncoated and vacuum-packaged (UNC)

Group B - Coated with BCNs and vacuum-packaged (BCN)

Group C - Coated with NBCNs and vacuum-packaged (NBCN)

The BCN and NBCN steaks were spray coated by a spray gun (W590 Flexio, Wagner GmbH, Markdorf, Germany) at 0.18 litre/min flow rate with the horizontal flat jet nozzle (1.8 mm nozzle opening) position under aseptic conditions. The nozzle was positioned 45 cm directly vertical to the beef sections. Steaks were flipped manually to ensure complete coating on both sides and each solution was sprayed for about 2 mins to ensure complete coverage. Once completed, all steaks were placed under a laminar air flow (HH 48, Holten LaminAir, Thermo Fisher Scientific, Bath, UK) at an air velocity of 0.40 – 0.45 m/s for 30 mins to remove any excess nanocrystal solution. The coated steaks were weighed and then vacuum-packaged (easyPACK-mk2, Webomatic, Bochum, Germany) in vacuum pouches (Vital Packaging, Perth, Australia; 65 µm thickness; < 40 cc/m^2/24 h at 25 ºC oxygen transmission rate; < 7 g/m2/24 h at 38 ºC moisture vapour transmission rate). The uncoated control samples were directly subjected to vacuum packaging as described above. All the samples were stored at sub-zero chilled temperatures (- 1 $^{\circ}$C ± 1.0 $^{\circ}$C) for 4 weeks and five steaks were randomly withdrawn from each treatment on each sampling day (day 1, 7, 14, 21 and 28) and analysed as per section 6.2.2.

6.2.2 Beef shelf life evaluation

6.2.2.1 Purge and drip loss analysis

Purge loss of steaks was determined as per Cardoso et al. (2016). The steaks were removed from the vacuum pouches, gently blotted with a filter paper to remove excess purge and weighed. The purge loss was expressed as percentage moisture loss relative to the steak's initial weight (day 0). Drip loss was determined as per Honikel (1998) without any modifications. The steaks were removed from the vacuum pouches, weighed and placed in a closed container on a supporting mesh and stored at 4 ºC ±

1.0 ºC for 18 h. The steaks were patted dry with absorbent paper, reweighed after 18 h and drip loss was expressed as moisture loss relative to the weight measured before placing them on the supporting mesh.

6.2.2.2 Low-field nuclear magnetic resonance relaxation

Transverse relaxation time (T_2) analysis was carried out by an NMR Rock Core analyser (Magritek Limited, Wellington, New Zealand) which has a proton resonance frequency of 2 MHz. The T_2 measurements were performed using the Carr-Purcell-Meiboom-Gill NMR method as per Gedarawatte et al. (2020) with some modifications. Approximately 3 g of samples were cut with the coating as the ratio of coating to the meat sample was negligible, placed in 8.5 mm glass bottles and analysed in the NMR. A number of 2000 echoes were collected in each scan with 32 repeated scans for each with a recycling delay of 7.5 s and an echo time of 200 µs. The collected T_2 data was analysed to produce T_2 probability distributions using the Lawson and Hanson regularisation technique based on non-negative least squares fit analysis using RoCA 4.26 software.

6.2.2.3 Meat pH determination

Meat pH was determined as per Kim, Kemp, and Samuelsson (2016) by directly inserting a calibrated meat pH tester (Hanna instruments, Hanna, Romania, standard buffers of pH 4.0 and 7.0) into the meat.

6.2.2.4 Meat colour analysis

Meat colour was measured before removing the vacuum pouch (pre-blooming state) and after allowing the meat to bloom at 4 °C ± 1.0 °C for 1 h (post-blooming state). A BYK colourimeter (BYK-Gardner GmbH, Geretsried, Germany, D 65 light source and a 10° observer with an 11 mm aperture) was used to measure L^* (lightness), a^* (redness) and b^* (yellowness) values at three random locations on each steak. Colourimeter was calibrated as per Gedarawatte et al. (2020) without any modifications.

6.2.2.5 Tenderness

Steak samples with 6 × 6 × 3 cm dimensions were cut from each treatment, cooked and cooled as per Li et al. (2012) without any modifications. Six core samples were obtained from each cooked steak and sheared perpendicular to the orientation of the muscle fibres with a V-shaped Warner–Bratzler shear blade (p-WBT, crosshead speed of 4 mm/s) attached to a Perten texture analyser (TVT 6700, Perten instruments, Hägersten, Sweden). The average shear force was expressed as load in Newtons (N).

6.2.2.6 Lipid oxidation

The lipid oxidation of steaks was determined as per Cardoso et al. (2016) by measuring 2-thiobarbituric acid reactive substances (TBARS) without any modifications. The samples were removed from each treatment, immediately frozen (-80 ºC) and TBARS measurements were carried out within 3 d. The standard curve preparation, optical absorbance measurements and reporting of TBARS were carried out as per Gedarawatte et al. (2020) without any modifications.

6.2.2.7 Microbiological analysis

Lean meat (at day 1, 7, 14, 21 and 28) and purge (at day 7, 14, 21 and 28) samples were collected from each treatment aseptically and numbers of LAB were determined. Briefly, 10 g of meat trimmings were placed in 90 ml buffered peptone water (BPW; Oxoid Ltd., Hants, UK) and homogenized for 2 mins using a homogenizer (PRO250, PRO Scientific Inc., Oxford, USA). For purge samples, a 1:10 dilution was prepared using BPW and then the samples were homogenized as described above. Decimal dilution series of both meat and purge were prepared, spread plated onto de Man, Rogosa, and Sharpe (MRS; Oxoid Ltd., Hampshire, England) agar and the plates were incubated anaerobically at 30 $^{\circ}$C ± 1°C for 120 ± 3 h. Microbial counts were reported as $\log_{10}$CFU/g for meat samples and $\log_{10}$CFU/ml for purge samples.

6.2.2.8 Sensory evaluation

A semi-trained panel conducted the sensory evaluation of raw beef on day 1, 14 and 28. The panel consisted of 12 members within the age range of 25 to 65 years. Before carrying out the sensory assessment, ethics approval was obtained from the Curtin

University Human Research Ethics Committee (Approval number: HRE2019-0038) and the panellist's consent was obtained at the beginning of the recruitment. Screening of panellists was based on whether they purchase and consume beef every 2 weeks. The panellists were then trained on beef colour standards, odour, texture and firmness standards and descriptive tests in two separate training sessions. All the panellists were trained and the assessment was carried out as per AS 2542.1.3:2014 (Standards Australia, 2014). Beef samples were allowed to bloom at 4 ºC for 1 h before presenting them to the panellists in order to mimic the retail display conditions.They were asked to evaluate the samples for their colour, odour, firmness and overall acceptability using general Labeled Magnitude Scale of a structured 15 cm line (Appendix D) as per Kalva, Sims, Puentes, Snyder, and Bartoshuk (2014).

6.2.3 Electrochemical characterization of beef tissue and purge

6.2.3.1 Beef tissue surface potential measurements

Beef tissue section preparation for Kelvin probe force microscopy (KPFM; Dimension Icon, Veeco instruments, USA) was carried out as per Graham et al. (2010) with some modifications. Beef blocks (1 x 1 x 1 cm) with coating were embedded in optimal cutting temperature (OCT) compound (Sakura Finetck USA, CA, USA) within a plastic mould and lowered into liquid nitrogen until frozen. The frozen blocks were removed from the plastic mould, wrapped it in aluminium foil and submerged in liquid nitrogen for 3 mins. Aluminium foil was removed, frozen meat blocks were cryo-sectioned (Leica CM1520, Leica Microsystems Pty Ltd, VIC, Australia) to a thickness of 20 µm and collected onto glass slides. Then the sections were allowed to defrost, washed with distilled water for 1 min to remove excess OCT and dried at room temperature until completely dry. A few sections were dipped in 0.5% BCN suspension and air-dried to determine nanocrystal's effect on the surface potential of meat. A few drops of 0.5% BCN suspension and 10% (mg/ml) L-lysine solution (Sigma-Aldrich Pty. Ltd., Sydney, Australia) were dropped onto glass slides and air-dried. All samples (uncoated meat, coated meat, BCN and L-lysine) were made conductive by connecting a wire to the sample deposited on the glass slide with carbon tape. Cantilever (MESP-V2, Bruker AFM Probes Americas, CA, USA) oscillation frequency (57 kHz) and drive amplitude (2127 mV) were determined by the

Nanoscope software. Height, amplitude, phase, and potential images at the scan size of 15 μm were captured at a scan rate of 0.996 Hz with 3.2 and 5.7 integral and proportional gains. A sample bias of 100 mV was applied under the interleave mode.

6.2.3.2 Zeta-potential measurements of purge

Zeta-potential of purge was measured by Zetasizer Nano (Malvern ZSP, Malvern Instruments Ltd, Worocestershire, UK). Four samples of purge were measured and each measurement was carried out in triplicate with a RI of 1.358 at 25 °C.

6.2.4 Data analysis

All the experiments related to beef shelf life evaluation were conducted in triplicate and IBM SPSS Statistics version 26 software was used for data analysis. The data of all experiments (except sensory evaluation) at each sampling day and the data of each treatment during the storage period were analysed using one-way analysis of variance (ANOVA) to compare the results among different treatments and to evaluate the impact of storage period, respectively. Repeated Measure ANOVA test was performed to assess the impact of storage period on sensory data and to compare the treatment effect on sensorial characteristics. Tukey comparison test was performed to analyse the mean values further if a significant ($P < 0.05$) difference was found.

6.3 Results and discussion

6.3.1 Changes in beef water-holding properties during vacuum-packaged storage

The effect of nanocoating and storage time on purge loss of beef is presented in Table 6.1. Both uncoated and nanocrystal coated samples (BCNs and NBCNs) showed a significant increase in weight loss with the increase of storage time. The increase in purge loss observed during this study was similar to previous studies on vacuum-packaged fresh beef (Holman, Bailes, Kerr, & Hopkins, 2019; Rooyen, Allen, Gallagher, & O'Connor, 2018). This could be attributed to the impairment of muscle protein functionality to hold myowater due to protein oxidation (Kim et al., 2018). No significant difference in purge loss was observed between uncoated and nanocrystal

coated samples at all sampling days, indicating that nanocrystals were not effective in reducing purge loss. Zhai et al. (2020), Fakhouri et al. (2014) and Pacaphol et al. (2019) achieved significant weight loss reduction in fresh-cut apples, strawberries and spinach leaves by coating the samples with BCNs, cellulose nanocrystals and nanofibrillated cellulose, respectively. Cellulose nanocrystals control water loss by reducing water evaporation (Zhai et al., 2020) by creating hydrogen bonds with the water molecules in the sample surface (Pacaphol et al., 2019). In vacuum-packaged meat storage, moisture loss due to evaporation is impossible to occur and weight loss is associated with poor water-holding capacity (WHC) due to protein denaturation. Nanocrystals' ineffectiveness in controlling purge loss could be due to the size gap between nanocrystals and the drip channels. The gaps between the muscle fibres, muscle bundles, and in the perimysial network are known as drip channels that facilitate purge flow and have been reported to be approximately 20-50 μm in size (Bertram & Ersen, 2004). BCNs and NBCNs had an average width and length of 32.2 ± 9.4 nm, 628.9 ± 207.4 nm and 55.1 ± 23.1 nm, 589.5 ± 288.4 nm, respectively (Gedarawatte et al., 2021). The openings of drip channels have widths almost 100x that of the nanocrystals which could explain why they were unable to prevent the flow of purge from inside to the meat surface.

Another reason could be the poor electrochemical interaction between the meat structure and nanocrystals. Both BCNs and NBCNs suspensions had zeta-potential values which were greater than or equal to – 30 mV (Gedarawatte et al., 2021). It can also be seen in the KPFM images (Figure 6.1A; right); BCNs appeared to have a distinctive negative surface potential with respect to the positive sample bias applied. The surface potential images of both L-lysine (Figure 6.1B; right) and uncoated beef sections (Figure 6.1C; right) displayed significant relative positive surface potential compared to the positive sample bias applied. L-lysine is one of the most abundant amino acids present in beef proteins and may contribute to the positive charge observed on the surface of the tissue section. The KPFM image of beef section coated with BCNs (Figure 6.1D; right) showed a general decrease in the surface potential compared to uncoated beef section. This could be due to the electrochemical interaction between the negative charges of the nanocrystals and the positive charges of the muscle fibres. These interactions could have helped the attachment of

nanocrystals to the muscle fibre wall, thereby controlling the purge loss by creating some hindrance to the purge flow.

Table 6.1 Changes in water-holding parameters during vacuum-packaged storage of beef

Parameters	Treatment	Vacuum storage (d)					SEM	P-value
		1	7	14	21	28		
Purge loss (%)	**UNC**	3.16 ± 0.32 Za	5.90 ± 1.17 ZYa	6.21 ± 0.46 ZYa	6.45 ± 0.14 ZYa	7.74 ± 1.02 Ya	0.49	0.016
	BCN	3.05 ± 0.57 Za	5.83 ± 0.79 ZYa	6.03 ± 0.92 ZYa	6.70 ± 0.88 ZYa	8.77 ± 1.19 Ya	0.60	0.015
	NBCN	3.23 ± 0.66 Za	4.66 ± 0.43 ZYa	5.64 ± 0.49 ZYXa	5.90 ± 0.76 YXa	8.01 ± 0.45 Xa	0.47	0.002
	P-value	0.972	0.548	0.833	0.707	0.736		
Drip loss (%)	**UNC**	1.12 ± 0.15 Za	0.87 ± 0.08 Za	0.92 ± 0.06 Za	1.13 ± 0.12 Za	0.96 ± 0.04 Za	0.05	0.341
	BCN	1.05 ± 0.03 Za	1.13 ± 0.02 Za	1.04 ± 0.04 Za	1.16 ± 0.23 Za	1.16 ± 0.04 Za	0.04	0.862
	NBCN	0.95 ± 0.15 Za	0.93 ± 0.08 Za	0.97 ± 0.14 Za	1.09 ± 0.04 Za	1.18 ± 0.18 Za	0.05	0.599
	P-value	0.638	0.124	0.664	0.940	0.400		
A_{21} (%)	**UNC**	95.89 ± 1.27 Za	97.28 ± 0.27 Za	96.19 ± 1.03 Za	96.64 ± 0.35 Za	96.41 ± 0.55 Za	0.33	0.779
	BCN	96.37 ± 1.24 Za	96.74 ± 1.10 Za	96.55 ± 0.51 Za	96.72 ± 0.43 Za	98.00 ± 0.52 Za	0.35	0.668
	NBCN	96.49 ± 1.47 Za	97.81 ± 0.86 Za	96.24 ± 0.32 Za	95.68 ± 0.71 Za	97.18 ± 0.36 Za	0.38	0.474
	P-value	0.946	0.670	0.923	0.353	0.146		
A_{22} (%)	**UNC**	4.11 ± 1.27 Za	2.72 ± 0.27 Za	3.81 ± 1.03 Za	3.36 ± 0.35 Za	3.59 ± 0.55 Za	0.33	0.779
	BCN	3.63 ± 1.24 Za	3.26 ± 1.10 Za	3.45 ± 0.51 Za	3.28 ± 0.43 Za	2.00 ± 0.52 Za	0.35	0.668
	NBCN	3.51 ± 1.47 Za	2.19 ± 0.86 Za	3.76 ± 0.32 Za	4.32 ± 0.71 Za	2.82 ± 0.36 Za	0.38	0.474
	P-value	0.946	0.670	0.923	0.353	0.146		
T_{21} (ms)	**UNC**	43.38 ± 0.64 Za	46.04 ± 0.00 Ya	44.69 ± 0.67 ZYa	40.24 ± 0.00 Xa	40.86 ± 0.62 Xab	0.62	0.000
	BCN	46.04 ± 0.00 Zb	43.38 ± 0.64 Yb	43.38 ± 0.64 Ya	40.24 ± 0.00 Xa	39.65 ± 0.59 Xa	0.65	0.000
	NBCN	43.38 ± 0.64 ZYa	42.09 ± 0.00 Zb	44.69 ± 0.67 Ya	44.02 ± 0.00 ZYb	42.09 ± 0.00 Zb	0.32	0.004
	P-value	0.017	0.001	0.337	0.018	0.035		
T_{22} (ms)	**UNC**	204.84 ± 50.05 Za	203.66 ± 14.34 Za	210.13 ± 41.04 Za	174.78 ± 6.85 Za	156.14 ± 14.10 Za	12.80	0.678
	BCN	216.52 ± 50.95 Za	257.77 ± 80.22 Za	209.47 ± 11.52 Za	198.11 ± 17.07 Za	210.10 ± 29.14 Za	18.02	0.899
	NBCN	252.22 ± 66.80 Za	224.48 ± 23.90 Za	201.32 ± 18.07 Za	191.51 ± 26.34 Za	191.58 ± 11.11 Za	14.68	0.703
	P-value	0.831	0.745	0.967	0.675	0.226		
T_{2lm} (ms)	**UNC**	45.91 ± 0.53 Za	48.00 ± 0.58 Ya	47.80 ± 0.24 Ya	42.79 ± 0.11 Xa	42.40 ± 0.27 Xa	0.65	0.000
	BCN	48.69 ± 0.39 Zb	45.94 ± 0.71 Ya	46.35 ± 0.10 Yb	42.17 ± 0.39 Xa	40.92 ± 0.32 Xb	0.78	0.000
	NBCN	46.72 ± 0.578 Zab	42.90 ± 0.48 Yb	47.27 ± 0.25 Za	47.42 ± 1.78 Zb	43.87 ± 0.27 Yc	0.52	0.000
	P-value	0.020	0.003	0.007	0.000	0.001		

[a-c] Means within the same column for the same index with different lowercase letters differ significantly among the treatments ($P < 0.05$).
[Z-X] Means within the same row with different uppercase letters differ significantly among different vacuum storage periods ($P < 0.05$).
UNC: uncoated and vacuum-packaged; BCN: 5 mg/ml BCNs coated and vacuum-packaged; NBCN: 5 mg/ml NBCNs coated and vacuum-packaged; A_{21} (%): population area ratio of T_{21}; A_{22} (%): population area ratio of T_{22}; T_{21} (ms): T_{21} relaxation time; T_{22} (ms): T_{22} relaxation time; T_{2lm} (ms): overall transverse relaxation time; SEM: standard error of mean.

However, this was not quite evident from the weight loss results which could be attributed to the low zeta-potential values of the purge samples (Figure 6.1E). Two purge samples tested in this study had average positive zeta-potentials and the other two had average negative zeta-potentials which indicate that purge consists of molecules which are both positively and negatively charged and average zeta-potential is dependent on their respective concentrations. BCNs may not form strong interactions with molecules in purge due to their very low zeta-potential. In addition, BCNs may not be able to form strong interactions with negatively charged molecules present in purge. The weak electrochemical interaction between nanocrystals and purge may have led to poor control in purge loss.

The size of the drip channel openings and myowater hydrostatic pressure increase with storage time. The drip channels become wider as the storage time increases (Offer & Cousins, 1992) which may prevent BCNs from blocking the channel openings on the meat surface. As it is evident in the weight loss results, the amount of purge exudate from meat increased as the storage life extended which may lead to an increase in hydrostatic pressure within the meat sample. The nanocrystals may not be able to control the increased hydrostatic pressure, making them ineffective in controlling the exudation of purge.

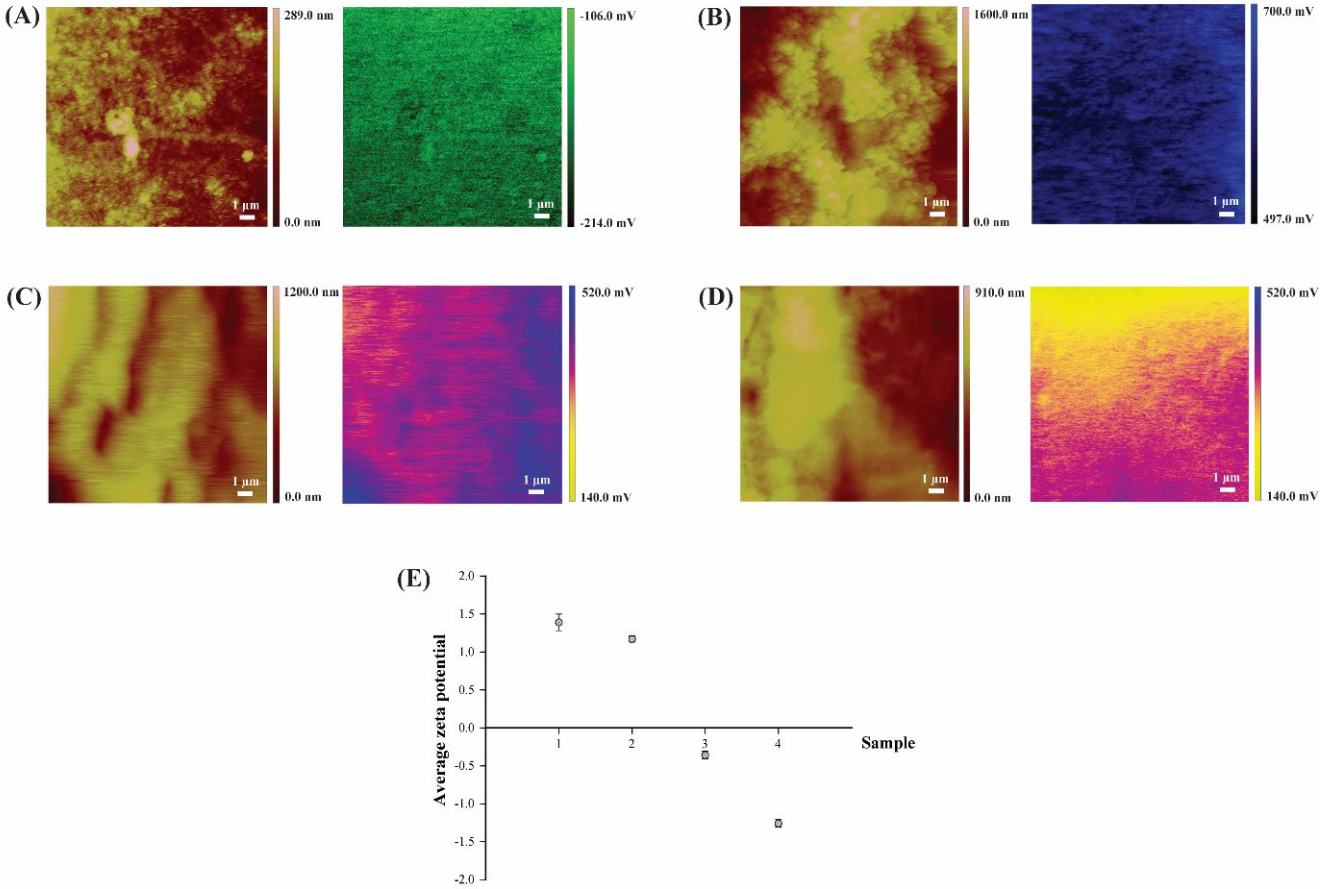

Figure 6.1 Topography (left) and surface potential (right) maps of 5 mg/ml BCNs solution (A); topography (left) and surface potential (right) maps of L-Lysine solution (B); topography (left) and surface potential (right) maps of uncoated meat section (C); topography (left) and surface potential (right) maps of meat section coated with 5 mg/ml BCNs solution (D); average zeta-potential values of purge samples (E). BCNs: bacterial cellulose nanocrystals.

Drip loss refers to the weight loss incurred when the meat is hung in a closed container at refrigeration temperature for a specific period (Honikel, 1998). In this study, no significant changes were observed in drip loss in all the treatments as the storage time progressed (Table 6.1). Also, there were no significant differences in drip loss between the treatments at all storage days. These observations indicate that weight loss associated with purge loss is much greater compared to the weight loss associated with drip loss. Hence, the majority of water is already lost during the storage as purge and only the remaining water is lost as drip, leading to no significant changes in drip loss.

The LF-NMR T_2 analysis (Table 6.1) identified two key water populations in all meat samples; a major population (A_{21} = 93.7 – 98.9 % of the water) with 38.47 to 46.04 ms T_{21} relaxation time, corresponding to the intramyofibrillar water and a minor population (A_{22} = 1.1 – 6.3 % of the water) with 129.35 to 415.82 ms T_{22} relaxation time, corresponding to the extramyofibrillar water. These results are in agreement with the previous studies on beef which presented two major water populations with similar relaxation times (Bertram & Ersen, 2004; Qian et al., 2019). No significant changes in A_{21}, A_{22} and T_{22} relaxation time were observed among the treatments and during storage. Similar results have been reported in previous studies (Gedarawatte et al., 2020; Gudjónsdóttir et al., 2015) in vacuum-packaged meat. No changes in T_{22} relaxation time correlate to no changes observed in drip loss values (Zhu et al., 2017). A significant reduction in T_{21} and overall relaxation time T_{2lm} was observed in all the treatments with the increase of storage time which indicates a decrease in intramyofibrillar water mobility. These results are in accordance with the findings of Straadt, Rasmussen, Andersen, and Bertram (2007) and could be due to the degradation of cytoskeletal structure (Zhu et al., 2017). Some significant changes in T_{21} and overall relaxation time T_{2lm} were seen between uncoated and nanocrystals coated samples which could be due to the ionic interaction between nanocrystals and myowater.

6.3.2 Physicochemical evaluation of beef during vacuum-packaged storage

The effect of coating treatment and storage time on meat pH is presented in Table 6.2. Meat pH did not differ between the treatments, but a significant decrease in pH was observed in all three treatments with increased storage time. The pH of both BCNs and NBCNs spray solutions were adjusted to neutral with the addition of 0.1 M NaOH

(Gedarawatte et al., 2021). No impact of coatings on meat pH could be due to the neutral pH of the spray solutions. This confirms that the nanocrystal coating will not adversely affect meat WHC or tenderness by altering meat pH. The significant decrease in meat pH observed with the progress of storage is in agreement with the data obtained by Gedarawatte et al. (2020) and Gudjónsdóttir et al. (2015). This is probably attributed to the increase of LAB counts detected in all treatments as the storage time prolonged which is further discussed in Section 6.3.3.

No effect of storage time on meat tenderness was observed in all treatments (Table 6.2). Similar results have been reported by Crivelli, Tirloni, Bernardi, Rossi, and Stella (2019) who found no tenderizing effect of ageing on vacuum-packaged heifers meat. It has been observed previously that collagen remains insoluble at refrigeration temperatures which may explain why ageing at chilled temperatures does not increase beef tenderness (Silva, Patarata, & Martins, 1999). In addition, *Semitendinosus* muscle is known to remain tough and not improve in terms of tenderness with ageing when compared to other muscles such as *Longissimus*. Meat tenderness was also not influenced by the coating treatment. The steaks coated with BCNs, NBCNs and uncoated samples retained similar tenderness during the storage period. Pilon et al. (2015) reported similar observations on the firmness of apple slices coated with chitosan nanoparticles.

Based on TBARS results (Table 6.2), no storage effect was found for lipid oxidation, except for the increase in TBARS observed in uncoated samples after 1 week and then it was consistent until the end of storage. No storage effect on lipid oxidation was seen in coated samples. Likewise, Holman et al. (2019) reported no differences in TBARS values of vacuum-packaged beef samples across 12 weeks of ageing at 1.5 $^{\circ}$C. Sub-zero chilled temperatures may have inhibited the negative impact of reactive species and thereby prevented excessive oxidation (King et al., 2009). Lipid oxidation was not affected by the BCNs or NBCNs coating treatments, except on day 01. This is in agreement with the findings of Marchetti, Muzzio, Cerrutti, Andrés, and Califano (2017) who reported no significant changes in TBARS values of beef sausages formulated with the application of BCNs which was stored over a 30 d period. These findings confirm that BC's application as nanocrystals may not influence lipid

oxidation and it may help prevent the increased lipid oxidation observed when BC was applied as a wrapping material (Gedarawatte et al., 2020).

Some of the uncoated meat as well as BCNs and NBCNs coated meat maintained stable lightness, redness and yellowness values over the storage period at both pre and post-bloom conditions (Table 6.3). Chen et al. (2019) and Antoniewski et al. (2007) also reported minimal colour changes during vacuum-packaged beef storage. The combined effect of vacuum packaging and super-chilled storage can maintain stable lightness (Bellés, Alonso, Roncalés, & Beltrán, 2017), redness and yellowness values (Chen et al., 2019). Spray coating treatments with BCNs and NBCNs did not significantly influence the meat redness at either pre-bloom or post-bloom condition. This could be attributed to no significant changes observed in lipid oxidation among the three treatments. Nanocrystal coatings showed a significant increase in lightness at pre-bloom condition only on day 7 and at post-bloom condition only on day 28 compared to uncoated samples. Similar to this observation, a significant increase in yellowness was observed in coated samples at pre-bloom condition after 1 week and 4 weeks and at post-bloom condition after 4 weeks of storage compared to uncoated samples. Huq, Riedl, Bouchard, Salmieri, and Lacroix (2014) also observed a significant increase in the lightness values of ham samples coated with alginate-cellulose microbeads compared to uncoated samples. These results are not fully understood and may be due to the opaque nature of nanocrystals and their interactions with the purge layer on the meat surface.

Table 6.2 Changes in pH, tenderness and lipid oxidation (TBARS) of beef during vacuum-packaged storage

Parameters	Treatment	Vacuum storage (d)					SEM	P-value
		1	7	14	21	28		
pH	**UNC**	5.61 ± 0.03 Za	5.49 ± 0.05 ZYa	5.39 ± 0.01 YXa	5.33 ± 0.01 Xa	5.37 ± 0.01 YXa	0.03	0.000
	BCN	5.56 ± 0.03 Za	5.49 ± 0.03 ZYa	5.42 ± 0.03 YXa	5.33 ± 0.01 Xa	5.36 ± 0.01 Xa	0.02	0.000
	NBCN	5.52 ± 0.03 Za	5.52 ± 0.02 Za	5.39 ± 0.01 Ya	5.34 ± 0.01 Ya	5.38 ± 0.01 Ya	0.02	0.000
	P-value	0.249	0.750	0.444	0.765	0.317		
Tenderness (N)	**UNC**	42.66 ± 1.65 Za	42.79 ± 1.62 Za	42.54 ± 1.15 Za	43.00 ± 0.77 Za	41.79 ± 1.27 Za	0.56	0.974
	BCN	40.56 ± 2.53 Za	43.10 ± 1.33 Za	39.99 ± 2.26 Za	41.55 ± 2.29 Za	40.62 ± 1.17 Za	0.85	0.825
	NBCN	43.38 ± 2.23 Za	42.35 ± 1.48 Za	41.00 ± 1.41 Za	42.05 ± 1.02 Za	40.15 ± 1.74 Za	0.71	0.675
	P-value	0.643	0.936	0.567	0.792	0.707		
Lipid oxidation (mg MDA/kg)	**UNC**	0.060 ± 0.00 Za	0.066 ± 0.00 ZYa	0.073 ± 0.00 Ya	0.073 ± 0.01 Ya	0.069 ± 0.00 Ya	0.00	0.003
	BCN	0.086 ± 0.01 Zb	0.066 ± 0.01 Za	0.065 ± 0.00 Za	0.071 ± 0.00 Za	0.075 ± 0.00 Za	0.00	0.129
	NBCN	0.086 ± 0.00 Zb	0.066 ± 0.00 Za	0.071 ± 0.00 Za	0.062 ± 0.01 Za	0.071 ± 0.00 Za	0.00	0.075
	P-value	0.021	0.558	0.281	0.549	0.418		

[a-b] Means within the same column for the same index with different lowercase letters differ significantly among the treatments ($P < 0.05$).

[Z-X] Means within the same row with different uppercase letters differ significantly among different vacuum storage periods ($P < 0.05$).

UNC: uncoated and vacuum-packaged; BCN: 5 mg/ml BCNs coated and vacuum-packaged; NBCN: 5 mg/ml NBCNs coated and vacuum-packaged; SEM: standard error of mean.

Table 6.3 Changes in colour parameters at pre-blooming and post-blooming conditions during vacuum-packaged storage of beef

Parameters	Treatment	Vacuum storage (d)					SEM	P-value
		1	7	14	21	28		
*L** preb	**UNC**	47.44 ± 1.07 Za	42.01 ± 0.75 Ya	45.46 ± 0.44 ZYa	45.98 ± 1.24 ZYa	44.16 ± 1.37 ZYa	0.63	0.036
	BCN	49.14 ± 1.02 Za	47.45 ± 0.42 Zb	47.50 ± 2.35 Za	45.84 ± 0.47 Za	45.61 ± 1.00 Za	0.59	0.336
	NBCN	47.45 ± 0.62 ZYa	48.86 ± 0.52 Yb	49.92 ± 0.80 Ya	47.60 ± 0.40 ZYa	46.07 ± 0.43 Za	0.41	0.007
	P-value	0.389	0.000	0.175	0.295	0.429		
*a** preb	**UNC**	8.00 ± 0.18 Za	7.57 ± 0.39 Za	7.00 ± 0.30 Za	6.52 ± 0.67 Za	6.44 ± 0.57 Za	0.24	0.146
	BCN	6.12 ± 0.40 Za	7.31 ± 0.39 Za	6.66 ± 0.59 Za	6.27 ± 0.18 Za	7.01 ± 0.75 Za	0.22	0.456
	NBCN	5.91 ± 0.90 Za	7.03 ± 0.24 ZYa	6.40 ±0.32 ZYa	6.53 ± 0.21 ZYa	8.66 ± 0.58 Ya	0.32	0.030
	P-value	0.080	0.580	0.628	0.888	0.109		
*b** preb	**UNC**	13.05 ± 0.89 Za	9.61 ± 0.34 YXa	12.16 ± 0.91 ZXa	12.05 ± 0.76 ZXa	7.80 ± 0.30 Ya	0.58	0.002
	BCN	12.49 ± 1.07 Za	12.70 ± 0.24 Zb	13.02 ± 0.73 Za	12.44 ± 0.73 Za	11.24 ± 0.18 Zab	0.30	0.454
	NBCN	11.73 ± 0.52 Za	12.65 ± 0.85 Zb	14.26 ± 0.69 Za	13.39 ± 0.19 Za	11.92 ± 1.42 Zb	0.40	0.256
	P-value	0.580	0.011	0.240	0.356	0.028		
*L** postb	**UNC**	50.07 ± 2.30 Za	41.25 ± 0.78 YXa	48.13 ± 1.14 Za	46.28 ± 0.71 ZXa	40.40 ± 0.37 Ya	1.12	0.001
	BCN	50.27 ± 0.54 Za	45.94 ± 2.19 Zab	48.26 ± 1.01 Za	47.15 ± 1.49 Za	45.38 ± 0.57 Zb	0.68	0.142
	NBCN	48.55 ± 1.27 Za	50.06 ± 0.94 Zb	50.87 ± 0.20 Za	48.68 ± 1.36 Za	46.67 ± 1.83 Zb	0.61	0.231
	P-value	0.704	0.015	0.123	0.432	0.017		
*a** postb	**UNC**	14.78 ± 2.37 Za	14.31 ± 1.10 Za	11.39 ± 0.87 Za	10.44 ± 0.80 Za	13.13 ± 0.88 Za	0.67	0.182
	BCN	13.57 ± 1.40 Za	12.36 ± 0.49 Za	11.64 ± 1.16 Za	10.04 ± 0.51 Za	11.84 ± 0.46 Za	0.46	0.166
	NBCN	14.20 ± 0.28 Za	11.10 ± 0.54 Ya	10.45 ± 0.42 Ya	10.78 ± 0.51 Ya	13.48 ± 0.34 Za	0.44	0.000
	P-value	0.868	0.064	0.620	0.713	0.207		
*b** postb	**UNC**	16.62 ± 1.37 Za	12.71 ± 1.00 Ya	15.26 ± 0.51 ZYa	14.35 ± 0.36 ZYa	13.32 ± 0.26 ZYa	0.48	0.045
	BCN	17.51 ± 0.33 Za	15.13 ± 0.23 Ya	15.44 ± 0.87 ZYa	13.67 ± 0.45 Ya	14.59 ± 0.17 Yab	0.39	0.003
	NBCN	16.32 ± 0.56 Za	14.87 ± 0.13 Za	15.77 ± 0.72 Za	15.89 ± 0.14 Zb	15.64 ± 0.74 Zb	0.24	0.460
	P-value	0.629	0.054	0.880	0.010	0.035		

[a-b] Means within the same column for the same index with different lowercase letters differ significantly among the treatments ($P < 0.05$).

[Z-X] Means within the same row with different uppercase letters differ significantly among different vacuum storage periods ($P < 0.05$).

UNC: uncoated and vacuum-packaged; BCN: 5 mg/ml BCNs coated and vacuum-packaged; NBCN: 5 mg/ml NBCNs coated and vacuum-packaged; preb: colour evaluation at pre-blooming conditions (just after opening of the vacuum package); postb: colour evaluation at post-blooming conditions (after allowing to bloom at 4 ºC for 1 h); SEM: standard error of mean.

6.3.3 Changes in meat microbiology during vacuum-packaged storage

In all treatment groups, LAB counts increased by ~ 1.0 to 4.5 log cfu in meat and by ~ 0.2 to 1.0 log cfu in purge over the storage period (Figure 6.2). This was evident in both meat and purge and agree with the results reported by Duran and Kahve (2020) and Gudjónsdóttir et al. (2015) on vacuum-packaged beef. Vacuum packaging may favour the LAB's growth since they are facultatively anaerobic and may become the dominant microflora by inhibiting the growth of aerobic spoilage bacteria in vacuum-packaged meat (Duran & Kahve, 2020). Increased LAB counts may have attributed to the rise in pH observed over the storage period which may have also led to the increase in purge loss. Microbiological assessment of purge on day 1 was not carried out due to the low volume of purge collected.

BCNs coating resulted in about 1.5 log cfu and 0.6 log cfu increase in LAB counts in meat and purge, respectively, compared to both uncoated and NBCNs coated samples. BCNs lack antimicrobial properties and they enhance the growth of beef spoilage LAB by providing a substrate for bacterial attachment due to their high surface to volume ratio (Gedarawatte et al., 2021). This diminishes the possibility of direct application of BCNs in vacuum-packaged meat products. However, direct application of BCNs may be ideal for products such as cheese, yoghurt and sour beers that involve microbiological fermentation. The results show no significant differences in LAB counts between uncoated and NBCNs coated samples. This could be observed in both meat and purge samples which suggests that loading BCNs with nisin could make them suitable for application in vacuum-packaged meat cuts. Rollini et al. (2020) and Mapelli et al. (2019) reported that no antimicrobial properties were observed with BCNs and cellulose nanofibers. They were also able to achieve significant growth reduction of *Listeria innocua* in soft cheese and smoked salmon samples by loading the nanocrystals with the antimicrobial agent Sakacin-A. Nisin is a food-grade antimicrobial agent and studies conducted by Huq et al. (2014) and Zimet et al. (2018)

found significant growth reduction of *Listeria monocytogenes* on ham and lean beef by loading alginate-cellulose nanocrystals and alginate-chitosan nanoparticles with nisin.

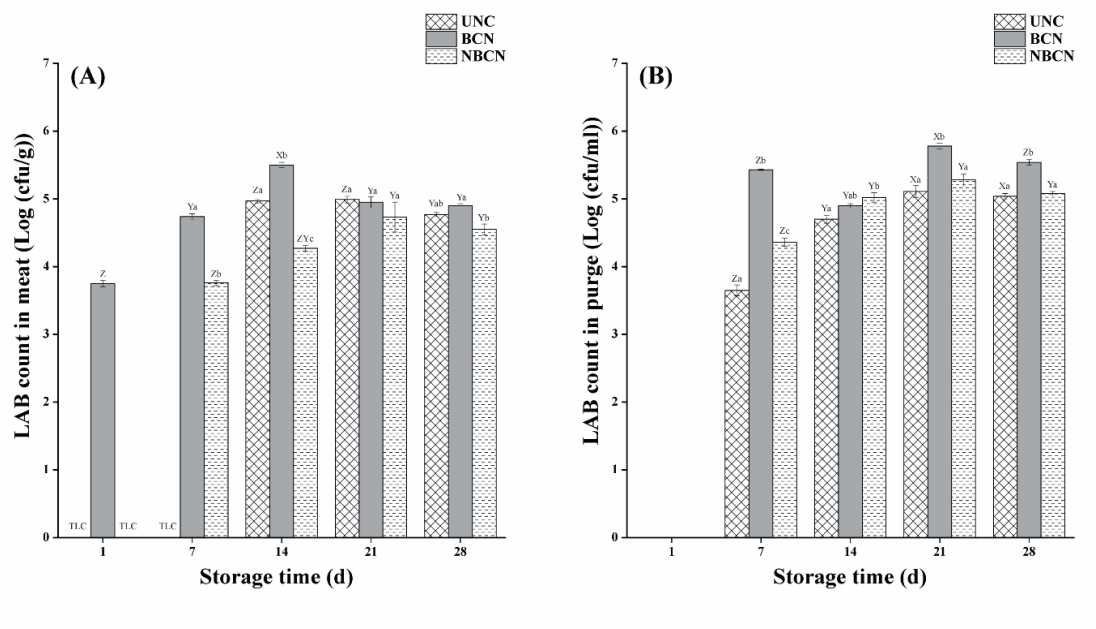

Figure 6.2 Changes in counts of lactic acid bacteria of meat (A) and lactic acid bacteria of purge (B) at day 1, 7, 14, 21 and 28. UNC: uncoated and vacuum-packaged; BCN: 5 mg/ml BCNs coated and vacuum-packaged; NBCN: 5 mg/ml NBCNs coated and vacuum-packaged; TLC: too low to count. Different uppercase letters (Z-X) mean significant differences among different storage periods ($P < 0.05$). Different lowercase letters (a-c) mean significant differences between treatments ($P < 0.05$).

6.3.4 Sensorial evaluation of beef during vacuum-packaged storage

Sensory results (Table 6.4) showed that panellists did not make any colour distinction between uncoated and nanocrystal coated samples during the storage period. No significant changes observed in redness at post-bloom conditions (Table 6.3) further support the sensory results. Similarly, Jafari et al. (2018) reported that panellists found no detectable differences in colour between uncoated and nanocellulose coated saffron samples in both their dry and solution states. These findings indicate that either BCNs or NBCNs coating may not alter the colour of meat as the storage time increases.

Table 6.4 Changes in sensory parameters during vacuum-packaged storage of beef

Parameters	Treatment	Vacuum storage (d)			P-value
		1	14	28	
Colour	**UNC**	13.05 ± 1.26 Za	10.22 ± 0.99 Za	14.00 ± 0.87 Za	0.066
	BCN	13.90 ± 1.24 Za	10.32 ± 1.30 Za	13.96 ± 0.79 Za	0.060
	NBCN	12.49 ± 0.95 Za	11.35 ± 1.11 Za	11.91 ± 1.45 Za	0.820
	P-value	0.745	0.771	0.294	
Firmness	**UNC**	12.25 ± 1.28 Za	10.59 ± 0.62 Za	12.90 ± 0.90 Za	0.322
	BCN	13.76 ± 0.99 Za	11.60 ± 1.28 Za	12.80 ± 0.96 Za	0.476
	NBCN	11.81 ± 0.74 Za	12.28 ± 0.86 Za	11.87 ± 1.09 Za	0.925
	P-value	0.420	0.513	0.670	
Odour	**UNC**	10.92 ± 1.20 Za	10.37 ± 0.79 Za	12.51 ± 0.93 Za	0.336
	BCN	12.96 ± 0.89 Za	11.31 ± 1.14 Za	10.88 ± 1.01 Za	0.384
	NBCN	11.59 ± 0.72 Za	12.06 ± 0.77 Za	10.85 ± 1.09 Za	0.681
	P-value	0.328	0.298	0.330	
Overall acceptability	**UNC**	11.82 ± 1.34 Za	10.01 ± 0.87 Za	13.21 ± 0.95 Za	0.178
	BCN	12.85 ± 1.16 Za	11.85 ± 1.06 Za	12.30 ± 1.02 Za	0.826
	NBCN	11.70 ± 0.99 Za	11.82 ± 1.14 Za	11.03 ± 1.21 Za	0.870
	P-value	0.769	0.331	0.310	

[a-b] Means within the same column for the same index with different lowercase letters differ significantly among the treatments ($P < 0.05$).

[Z-Y] Means within the same row with different uppercase letters differ significantly among different vacuum storage periods ($P < 0.05$).

UNC: uncoated and vacuum-packaged; BCN: 5 mg/ml BCNs coated and vacuum-packaged; NBCN: 5 mg/ml NBCNs coated and vacuum-packaged; SEM: standard error of mean.

There were no detectable changes observed in firmness between uncoated and coated meat samples during the storage period, which agrees with the instrumental tenderness measurements (Table 6.2). Panellists found no significant differences among the treatments in the sample's odour at all-time points where the sensory evaluation was carried out. This confirms that though there were significant differences in TABRS values between uncoated and coated meat samples on day 1, the panellists could not detect any odour changes. No significant changes in lipid oxidation between uncoated and coated samples were observed on day 14 and 28 (Table 6.2) and panellists could not distinguish between samples based on the odour tests. The panellists found similar acceptability in all the samples across the three treatments. This indicates that coating meat samples with both BCNs and NBCNs would not affect the natural vacuum-ageing of beef.

6.4 Conclusion

This study indicated that the spray coating of BCNs or NBCNs did not improve either physicochemical or sensorial properties of beef compared to uncoated beef Nanocrystal coatings were not effective in controlling purge exudation from meat. This could be due to the poor electrochemical interaction and the size gap between drip channels and the nanocrystals and increase in the size of the drip channels and hydrostatic pressure with the progress of the storage time. Therefore, to make this treatment into a promising solution, several alterations may need to be evaluated. This includes formulation of a mixture of microparticles with both positive and negative zeta-potentials which will be enough to create a considerable hindrance in drip channels by a blockage due to strong interaction between microparticles and the wall of the drip channels. The findings of this study will mainly contribute in determining the key parameters that need to be considered when optimizing the nanoparticles for future nanocoating applications. Direct application of BCNs on meat enhanced the microbial growth which could be mitigated by loading BCNs with nisin antimicrobial peptide. KPFM and zeta-potential measurements also showed to be excellent tools for understanding the electrochemical interaction between nanoparticles and food matrixes in future food nanotechnological applications.

Chapter 7. General conclusion and future work

This research aimed to employ novel methods to extend the shelf life of vacuum-packaged beef by reducing the moisture loss associated with purge and drip exudation. The application of edible coatings or films for meat is a fast-evolving field of study in food science and technology. However, most studies conducted to date have assessed the impact of edible coatings, applied using the dipping technique, on the quality of aerobically stored meat at 4 °C. Vacuum packaging causes the highest purge loss over the storage period compared to all other packaging techniques (Sekar, Dushyanthan, Radhakrishnan, & Babu, 2006). This could be due to the physical compression of meat caused by pressure applied during the packaging operation (Payne, Durham, Scott, & Devine, 1998) and the negative pressure applied to the meat in the vacuum that squeezes the liquid out of the tissue. This thesis addresses the limitations of previous studies and provides insights into the factors that need to be considered when developing edible coatings or films to reduce moisture accumulation inside vacuum-packaged meat. Using a combination of meat and material sciences, this study has generated a comprehensive understanding of the interaction of edible coatings with the structure of the meat.

This study has shown that moisture in vacuum-packaged beef is mainly lost due to purge formation rather than lost as drip. Therefore, the key focus of this thesis was to determine how novel methods can be employed to reduce purge accumulation in vacuum bags. The novel methods tested in this study can be categorized into two main sections; edible spray coating or wrapping treatments and the application of the nanocoating to the meat. Key conclusions, limitations and recommendations for future studies (Figure 7.1) are discussed below.

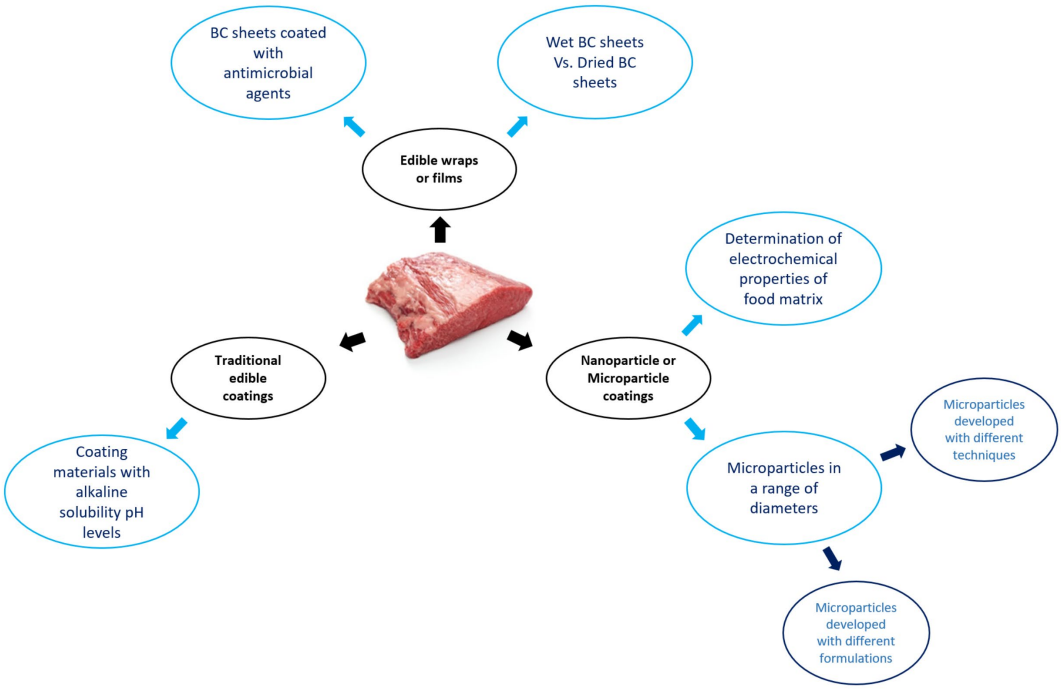

Figure 7.1 Suggestions for future studies

7.1 Edible spray coating or wrapping treatments for meat

Previous research has shown that gelatine and chitosan coatings were effective in reducing moisture loss in aerobically stored beef. The findings of chapter 3 revealed that the same was not observed when gelatine and chitosan coatings were applied to meat prior to vacuum-packaging operation. When the meat is vacuum-packaged, the coated surface of the meat is in direct contact with the packaging material, leaving purge in constant contact with meat until the vacuum bags are opened. This is not the same when the meat is packaged in aerobic packages. When the meat is packaged on a tray wrapped with shrink-wrap, the purge has more space to flow out within the tray, and only the bottom surface of the meat is in direct contact with the purge. The pH of both meat and purge significantly drops with the increase of storage time under anaerobic conditions, which is likely due to lactic acid bacteria's growth. This pH is very close to the pH of the solubility state of both chitosan and gelatine coating materials as they easily become soluble in acidic solutions (Musso, Salgado, & Mauri, 2016; Sogias, Khutoryanskiy, & Williams, 2010). Therefore, gelatine and chitosan may solubilise when exposed to purge which could compromise the integrity of the coating. This could be the key limitation in applying gelatine and chitosan coatings to control purge loss in vacuum-packaged beef. A future study may need to determine if

a reduction in purge loss can be achieved by applying coating materials that are less likely to solubilize in acidic pH levels such as cod myofibrillar protein (Lacroix & Vu, 2014).

Bacterial cellulose (BC) has not been used as a meat wrapping material in any packaging study to date. Chapter 4 showed that BC was effective as a purge absorbent and reduced purge accumulation inside the vacuum bags. Red meat processors could benefit by replacing synthetic absorption pads with this eco-friendly alternative as consumer demand for environmentally friendly packaging solutions grow (Ketelsen, Janssen, & Hamm, 2020). In this chapter, the physical and chemical changes in BC with the increase of shelf life were also evaluated by scanning electron microscopy, XRD and purge absorptivity measurements. This chapter showed how using techniques common in microbiology, food chemistry, and material science can give a better understanding of how effective different coating materials can be at preserving perishable meat.

One key limitation of this meat packaging study is that only dried BC-sheets were evaluated as a wrapping material. Sanchavanakit et al. (2006) mentioned that wet BC-sheets have a significantly higher water retention capacity than dried BC-sheets. Therefore, it is suggested that future studies compare the purge absorption capacity between BC in wet and dried states which may aid in developing meat wraps with higher purge absorption capacities. Results of chapter 4 indicated that BC enhanced microbial growth by acting as a substrate for microbial attachment. This could be mitigated by incorporating BC with antimicrobial agents such as chitosan, as reported by Lin, Lien, Yeh, Yu, and Hsu (2013). They achieved significantly higher antimicrobial efficiency in BC-sheets coated with chitosan compared to uncoated BC-sheets. Most importantly, they reported no significant changes in water absorption capacity due to the incorporation of chitosan. Therefore, it would be interesting to study the impact of BC-chitosan membranes on purge loss and microbial growth on meat surfaces when applied as wraps. Bacterial cellulose was identified as the most suitable material to produce nanocrystals for nanocoating application based on the results of chapters 3 and 4.

7.2 Nanocoating and meat science

In chapter 5, BCNs were produced by acid hydrolysis and antimicrobial active nanocrystals were formed by loading BCNs with nisin as BCNs alone enhanced the growth of meat spoilage bacteria *Lactobacillus rhamnosus* and *Leuconostoc mesenteroides*. This was the first study to report on BCNs loaded with an antimicrobial agent. BCNs loaded with 2.0 and 2.5 mg/ml nisin were identified as the optimal concentration for antimicrobial activity and stability. Physicochemical tests were carried out to characterize the most stable NBCNs and XRD results confirmed there was no impact on the mechanical strength of BCNs due to loading with nisin. Chapter 5 revealed that NBCNs could be used as an antimicrobial biomaterial in active food packaging applications. However, further studies should be carried out to confirm their toxicity and efficacy against other types of food spoilage and pathogenic microorganisms. This will facilitate their versatility as an active food packaging material. The formation of NBCNs was successful since the BCNs are negatively charged and nisin is positively charged. In the future, it would be interesting to determine whether BCNs can form stable nanocrystals with other positively charged antimicrobial agents.

The effectiveness of BCNs and NBCNs as nanocoatings in controlling purge loss of vacuum-packaged beef was studied in chapter 6. Both BCNs and NBCNs were not effective in reducing purge loss by creating a hindrance in the drip channels. Farouk, Mustafa, Wu, and Krsinic (2012) explained that an increase in WHC with ageing is possible due to the "sponge effect" in which the flow of purge through drip channels could be hindered by debris formed due to the breakdown of cytoskeleton muscle proteins. A similar effect was expected by creating a hindrance through interaction between nanocrystals and the wall of the drip channels. In chapter 6, KPFM data showed a drop in positive surface potential in meat sections coated with BCNs compared to uncoated meat sections. Zeta-potential results revealed that weak electrochemical interaction between purge and nanocrystals could be the reason for not achieving the expected outcomes. This chapter outlines the significance of understanding the food matrix's electrochemical properties before designing the nanocoating system, which will be important in producing an effective nanocoating mechanism for reducing the quality degradation of muscle food.

The effectiveness of the nanocoating method could have been limited due to the physical compression applied by vacuum packaging operation. Therefore, it is suggested that a future study determines the impact of nanocoating on moisture loss of aerobically or modified atmospherically packaged meat. Another possible limitation would be the nanocrystals being too small to create a "plug" in the drip channels due to the considerable size gap between drip channels and the nanocrystals. Future studies on coating systems designed with microparticles should be carried out to determine whether microparticles will reduce purge loss by creating a hindrance to the flow of purge. It is recommended to develop microparticles in a range of different diameters that could block the drip channels with different sizes of cut openings. Microparticles could be developed using various formulations and techniques and their preservative effect on meat could be assessed in future studies. In-depth investigations into the human safety of nanoparticles and microparticles are limited (Borel & Sabliov, 2014; Luo, 2020). It is vital to determine the safety of these particles in the human food chain. Filling this knowledge gap may help satisfy the concerns of both consumers and food processors before applying them on a commercial scale.

7.3 The best practices for red meat processors and researchers

Bacterial cellulose wrap was the most effective application in controlling purge accumulation among all the treatments investigated in this thesis. It can be used as a purge absorption film to improve product appearance by reducing purge accumulation inside the vacuum bags. This alternative can ensure there will not be any migration of synthetic microplastics from the purge absorption pad to the meat as it is completely edible. The introduction of BC as purge absorption films will benefit red meat processors as they can improve customer satisfaction by promoting eco-friendly alternatives over synthetic absorption pads. One of the key limitations of industrial-scale use of edible coatings is the negative perception of consumers towards the consumption of foreign materials together with the food component (Wan, Lee, & Lee, 2007). The application of BC in the form of a film will help mitigate negative consumer perception towards edible coatings since they can easily remove the film before they prepare the meat for consumption.

This thesis showed that the use of advanced microscopy and other analytical techniques such as KPFM, LF-NMR, XRD and zeta-potential measurements could

help in understanding the mechanisms responsible for determining the effectiveness of either coating or wrapping treatments on maintaining the quality of meat during storage. The application of KPFM is rare in food shelf life studies to explore quality degradation mechanisms. Combining these advanced analytical techniques with food shelf life evaluation methods such as accelerated shelf life study, time-temperature study and sensory evaluation is highly encouraged in future food science investigations which will aid in providing comprehensive scientific explanations.

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APPENDICES

Appendix A Sensory evaluation-Participant information statement

Curtin University

Beef Sensory Evaluation

PARTICIPANT INFORMATION STATEMENT

HREC Project Number:	HRE2019-0038
Project Title:	Development of an Edible Coating to Limit the Effect of Moisture Loss on the Shelf Life of Beef
Chief Investigator:	Dr Ranil Coorey
Student researcher:	Shamika Thushara Gorokgaha Gedarawatte
Version Number:	2.0
Version Date:	07/01/2019

What is the Project About?

This project aims to evaluate the effect of edible coating treatment on the organoleptic properties of beef steak samples. Beef eye round cuts are obtained from a registered and certified Western Australian commercial abattoir. Each beef steak is coated with edible coating materials except for the controls and all the steaks are vacuum packaged. Raw samples coated with three types of coating materials will be compared. Coating materials used in this study are Gelatine, Chitosan, and Bacterial Cellulose. Only physical characteristics (e.g. colour, odour, redness, freshness) and overall acceptability of raw beef steaks will be determined in this sensory evaluation, without subjecting them to eating.

Who is doing the Research?

The project is being conducted by Dr Ranil Coorey and Mr Shamika Thushara. The results of this research project will be used by Mr. Shamika Thushara to obtain his Doctor of Philosophy in Public Health at Curtin University. The project is funded by the Australian Meat Processor Cooperation.

There will be no costs to you and you will not be paid for participating in this project, except for a token gift (e.g. chocolate) with a maximum value of no more than $5.00.

Why am I being asked to take part and what will I have to do?

You will participate in a sensory evaluation of raw samples of beef steaks which are coated with different edible coatings. Firstly, we will ask you to observe raw beef samples and be provided with a sensory questionnaire wherein you will mark lines to score colour, redness, freshness, odour and overall liking, in order for the researchers to determine the acceptability of different coating applications of beef steak.

Then, you will be provided with a sensory questionnaire wherein you will mark lines to score colour, odour, redness, freshness, and overall acceptability, in order for the researchers to determine the acceptability of different coating applications of beef steak.

The sensory evaluation will be conducted at the Sensory Room of the School of Public Health in Curtin University. This room has six individual booths with a small window. You will be given the opportunity to choose booth you want to do the evaluation in. The samples, along the questionnaire, will be given to you through the window. After completing the evaluation, you will return the questionnaire through the same window.

Are there any benefits to being in the research project?

There may be no direct benefit to you from participating in this research. However, your inputs will allow us to add the existing body of knowledge regarding the quality control and improvement of export quality Australian beef. Results of this project may also help Australian beef industry to obtain more export orders contributing to the sustainability of the industry in Australia.

Are there any risks, side-effects, discomforts or inconveniences from being in the research project?

The samples you will test contain food grade edible coating materials which consist of Gelatine, Chitosan, and Bacterial Cellulose. However, you will not be required to consume the meat samples, only observation of physical attributes will be conducted. Beef is a red meat which can cause allergic reactions in some individuals. If you have any allergic reaction to red meat, please inform the student researcher so that you may be exempted as a participant in this research. Also, if you are allergic to any other food, kindly inform the researcher.

The sensory evaluation will be done in a laboratory room inside Curtin University. Participants will be asked to travel to Curtin University at a scheduled date and time to perform the sensory evaluation.

Who will have access to my information?

All the information collected from you will be non-identifiable. This includes information you provide on the sensory questionnaire during the actual sensory evaluation. No one, not even the research team will be able to identify you to this information. The collated information may be used in scientific peer-reviewed publications.

All electronic information will be stored within the secure network at Curtin University, protected with a password and properly backed-up. All hard copy will be stored in a secure location at Curtin University. All these will be kept at Curtin University for seven (7) years after the research has ended and then, it will be destroyed. The research team will have access to the information we collect in this research. The Curtin Ethics Office may access the data for audit purposes.

Will you tell me the results of the research?

We are not able to send you any results from this research as we do not collect any personal information to be able to contact you. However, the collated results of this research may be presented at conferences or published in professional journals. You will not be identified in any results that are published or presented.

Do I have to take part in the research project?

Taking part in this research project is absolutely voluntary. You do not have to take part if you do not want to. If you decide to participate but later on change your decision, you can withdraw from the project at any time. You are not required to provide us with the reasons of your withdrawal. Kindly let us know if you want to withdraw so we can make sure you are aware of any thing that needs to be done so you can withdraw safely. If you choose not to take part in this project or decide to participate but withdraw, it will not affect your relationship with the University, staff or colleagues in any manner.

If you choose to leave the study, we will continue to use any information collected. We will be unable to destroy your information because it will be collected in an anonymous way.

What happens next and who can I contact about the research?

Any further information, please contact:

Shamika Thushara Gorokgaha Gedarawatte
Email Address: s.gorokgah@postgrad.curtin.edu.au

Or

Dr Ranil Coorey (Research Supervisor)
Contact Number: +61 8 9266 1043
Email Address: R.Coorey@exchange.curtin.edu.au

If you decide to take part in this research, we will ask you to sign the consent form. Signing the consent form means you understand what you have read and what has been discussed. It also indicates that you agree to be in the research project and that you are not allergic to any food. Please take your time and ask questions you have, before you decide what to do. You will be given a copy of this information and the consent form to keep.

Curtin University Human Research Ethics Committee (HREC) has approved this study (HREC number HRE2019-0038). Should you wish to discuss the study with someone not directly involved, in particular any matters concerning the conduct of the study or your rights as a participant, or you wish to make a confidential complaint, you may contact the Ethics Officer on (08) 9266 9223 or the Manager, Research Integrity on (08) 9266 7093 or email hrec@curtin.edu.au.

Appendix B Sensory evaluation-Participant consent form

Beef Sensory Evaluation

Curtin University

CONSENT FORM

HREC Project Number:	HRE2019-0038
Project Title:	Development of an Edible Coating to Limit the Effect of Drip Loss on the Shelf Life of Beef Primal Cuts
Chief Investigator:	Dr Ranil Coorey
Student researcher:	Shamika Thushara Gorokgaha Gedarawatte
Version Number:	2.0
Version Date:	07/01/2019

- I have read the information statement and I understand its contents.
- I believe I understand the purpose, extent and possible risks of my involvement in this project.
- I do not have any food allergies or intolerances.
- I am not pregnant or breast feeding.
- I understand that participants will not be identified in any manner nor the organisation itself by name, address or any such method.
- I understand that participation is voluntary and that I can withdraw at any time with no prejudice or negative impacts
- I have had the opportunity to ask questions and I am satisfied with the answers I have received.
- I understand that this project has been approved by Curtin University Human Research Ethics Committee and will be carried out in line with the National Statement on Ethical Conduct in Human Research (2007) – updated March 2014.
- I voluntarily consent to take part in this research project.
- I understand I will receive a copy of this Information Statement and Consent Form.
- I hereby acknowledge the above and give my voluntary consent for participation in this project.

Participant Name	
Participant Signature	
Date	

Declaration by researcher: I have supplied an Information Letter and Consent Form to the participant who has signed above, and believe that they understand the purpose, extent and possible risks of their involvement in this project.

Researcher Name	
Researcher Signature	
Date	

Note: All parties signing the Consent Form must date their own signature.

Curtin University Human Research Ethics Committee (HREC) has approved this study (HREC number HRE2019-0038). Should you wish to discuss the study with someone not directly involved, in particular any matters concerning the conduct of the study or your rights as a participant, or you wish to make a confidential complaint, you may contact the Ethics Officer on (08) 9266 9223 or the Manager, Research Integrity on (08) 9266 7093 or email hrec@curtin.edu.au.

Appendix C Sensory panel screening questionnaire

Curtin University

Beef Sensory Evaluation

SENSORY PANEL SCREENING QUESTIONNAIRE

A. PERSONAL INFORMATION

Name : _____

Contact number or e-mail address (optional; for confirmation of evaluation times only)

_____

Gender : Male ____ Female ____ Other: _____

B. HEALTH DETAILS

1. Are you over the age range of 18? Yes ___ No ___
2. Do you eat beef at least once in every fortnight? Yes ___ No ___
3. Do you purchase raw beef and cook or prepare it for eating in every fortnight? Yes ___ No ___
4. Are you allergic to red meat? Yes ___ No ___
5. Are you allergic to any other food item? Yes ___ No ___
6. Do you have frequent head colds? Yes ___ No ___
7. For females, are you currently pregnant? Yes ___ No ___
8. For females, are you currently breast feeding? Yes ___ No ___
9. Are you a smoker? Yes ___ No ___
   If yes, how many cigarettes per day would you smoke? _____
10. Do you take any medications that affect your senses, especially your sense of smell or taste? Yes ___ No ___
11. Do you have any issues that currently affect your ability to see or smell? Yes ___ No ___

For questions or more information, you may contact any of the following:

Mr. Shamika Thushara Gorokgaha Gedarawatte (Student researcher)
Email address : s.gorokgah@postgrad.curtin.edu.au

Dr. Ranil Coorey (Chief Investigator)
Contact Number : +61 8 9266 1043
Email address : R.Coorey@exchange.curtin.edu.au

Ethics officer or Manager of Research Integrity
Contact Number : +61 8 9266 9223 or +61 8 9266 7093
Email address : ORD-ethicshs@curtin.edu.au

Curtin University Human Research Ethics Committee (HREC) has approved this study (HREC number HRE2019-0038). Should you wish to discuss the study with someone not directly involved, in particular, any matters concerning the conduct of the study or your rights as a participant, or you wish to make a confidential complaint, you may contact the Ethics Officer on (08) 9266 9223 or the Manager, Research Integrity on (08) 9266 7093 or email hrec@curtin.edu.au.

Appendix D Sensory evaluation questionnaire

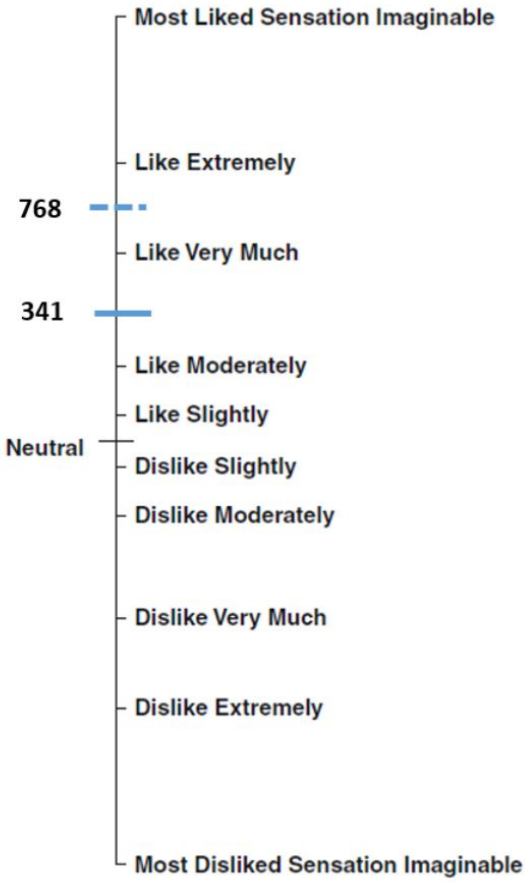
Beef Sensory Evaluation

Curtin University

SENSORY EVALUATION QUESTIONNAIRE for RAW BEEF SAMPLES

Instructions:

1. When you are ready, push the button in front of you and the samples will be presented to you.
2. Write the session date, and session time on the top portion of your questionnaire.
3. Answer the questions while observing the samples. Do not eat the sample
4. Mark a horizontal line on the scale which corresponds to your degree of liking with mentioning the three digit sample number provided as follows;



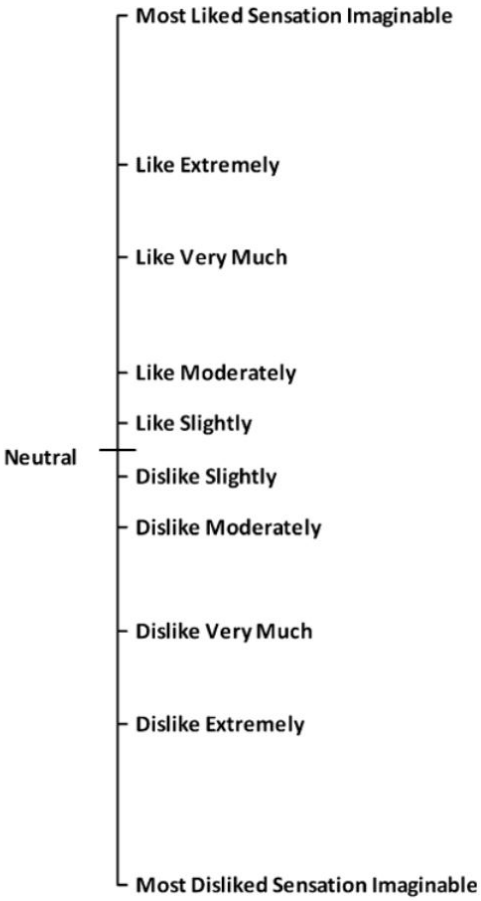
5. After completing the evaluation, push the button and slide the questionnaires through the window along with the samples.
6. Exit the sensory evaluation room.

Curtin University

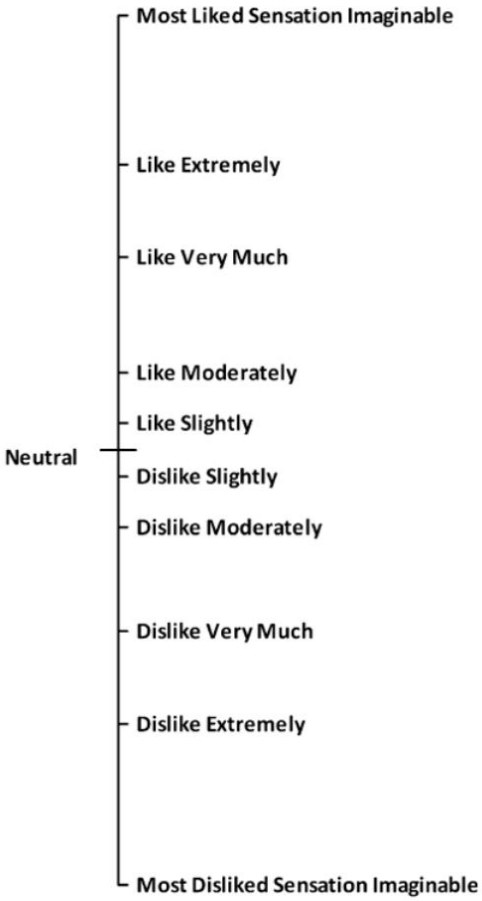
Session Date : _____ Session Time : _____

Questions:

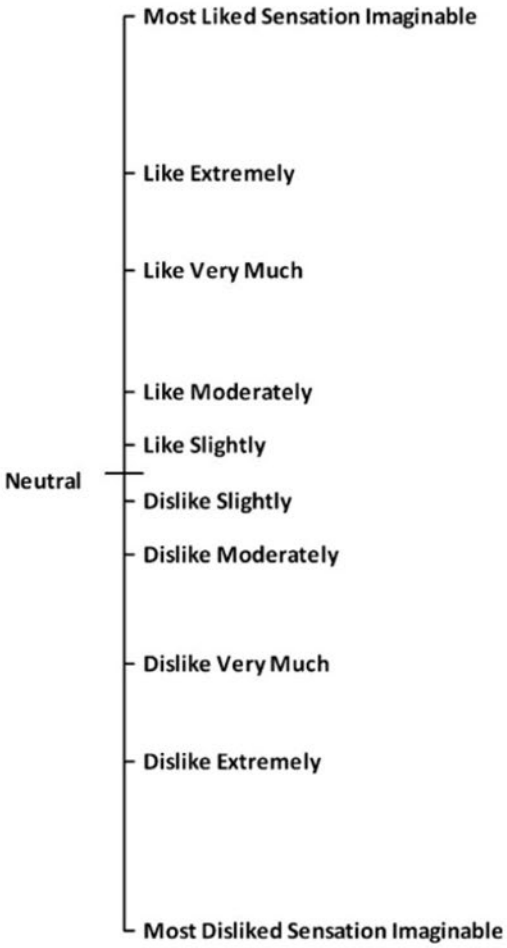
1. How would you rate the ***COLOUR & BRIGHTNESS*** of the samples?



2. How would you rate the ***FIRMNESS & TEXTURE*** of the samples?



3. How would you rate the ***ODOUR*** of the samples?



4. How would you rate the ***OVERALL ACCEPTABILITY*** of the samples?