Development of lactic-fermented vegetable and meat combinations that exploit endogenous vegetable nitrate to cure the meat

By

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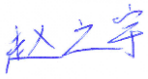
Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Signature: 

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Abstract

The main aim of this research was to investigate the development of lactic-fermented vegetable and meat combinations that exploit endogenous vegetable nitrate to cure the meat. In most cases, BFL-F02 was used as starter culture. The best results were obtained with raw cabbage and cooked beef. For the ratio of cabbage:meat, the pH value increased when the ratio was decreased. In most experiments, glucose was not added because it made no difference to the final pH. The results of pH and colour values with different starter culture made little difference to outcomes. In 4°C stoarge, the mixture will lose less fluid than the one at ambient temperature. The colour change with 20 g of meat and 10 g of cabbage was equal to adding 3 mg of sodium nitrite. During 56 days' storage, the beef colour was changed from brown to pink and back to brown again at ambient temperature but the cured beef maintained a residual pink colour when stored in the refrigerator. In the sensory test, Chinese members preferred the sweetest flavours but the other participants thought less or non-sweet samples were best.

Chapter 1: Introduction

Prior unpublished work over the past six years at AUT University has focused on developing fermented Greenshell mussel products, one of which is shown in Figure 1. This product and others made from mussels depended on what is called cook-then-ferment technology. Without cooking the microflora of the mussel outcompetes the added lactic culture resulting in putrid mess. Destroying the endogenous microflora by controlled cooking solves the problem. The work was simultaneously extended to foods like chicken (which has an aggressive microflora) and squid, and more recently to fermented cabbage and beef combinations. This last product was developed in response to a request from Mr Cam Mathias of Merit Meats, who was looking for product opportunities suitable for affluent Asian markets, where lactic-fermented foods are popular. This concept is in the manner of kimchi or sauerkraut, except that beef pieces were to be added.

Figure 1 Perna, a comminuted and fermented mussel product made from cooked mussels (Dsa, 2013).

Many undergraduate students were assigned to these cook-then-ferment projects. An undergraduate research project by Qiqi Lyu (Qiqi, 2016) first involved cooking breast chicken pieces for standard times in a microwave oven. Subsequent incubations with a lactic culture included nitrite as an additive to prevent the growth of clostridia, many of which are pathogens.

Fermentation always resulted in a satisfactorily low pH[1]. Because the chicken had been cooked it was thoughtlessly expected that chicken would not adopt the characteristic pink colour of cured meat from nitrite. That was not the case (Figure 2), but the observation was ignored until later when another undergraduate student, Dong Yusong, undertook work with fermented cabbage and beef. Nitrite was not included in the fermentation mixture, but the cooked beef nonetheless adopted the cured meat colour.

Figure 2 Fermented chicken products with nitrite. Note the faint pink colour (and the presence of gas in the bag – see the footnote for comments on gas formation).

Curing was attributable to nitrate present in the cabbage, which leached out and was subsequently reduced to nitrite by a microbe(s) in the culture mixture. The microbe responsible was almost certainly *Staphylococcus carnosus*, which is capable of reducing nitrate to nitrite (and beyond even to ammonia) (Neubauer & Götz, 1996). This discovery opened up the possibility of producing a fermented beef and cabbage product with a 'clean' label, that is to say one where the ingredients are perceived to be 'all natural'. That product is the subject of this thesis.

[1] Subsequent storage at ambient temperature, the bags lost vacuum due to gas formation. This indicated the presence of other microorganisms, either from the cooked chicken, or equipment and benches etc. or from of other cooked ingredients like lemon zest. It turned out that the gas was most likely CO_2 from alcoholic fermentation, because the smell and taste of the fermented, stored chicken was far from offensive.

Figure 3 An acceptable model product prepared from cabbage and beef. The cured appearance of the meat is obvious.

This thesis sets out to explore:

- The best way of cooking vegetables, usually cabbage, and beef before fermentation
- The effect of cabbage: meat ratio on curing
- Raw versus cooked cabbage
- The best glucose concentration to use
- If sucrose is required for sweetening, how will different starter cultures affect final pH and other product qualities?
- Product stability with extended storage
- Product safety

Before answering these questions what follows is a review of the main factors of interest in this study, lactic preservation of foods and nitrite curing. The review briefly includes aspects as to how societies view lactic fermentations and meat curing.

Literature review

Societies' view on lactic fermentation and meat curing

Currently, consumers in many Western countries have become concerned with the presence nitrate and nitrite, which are used to preserve meats. The process is calling curing and is usually obvious by the pink colour that meat develops. Studies have shown that many consumers to prefer – or at least claim to prefer – buying so-called organic and natural food above fermented and cured food because of safety concerns about chemical additives used in fermentation, like salt and phosphates, and curing (see for example,(Sebranek & Bacus, 2007). They fear the claimed links to cancer. (Bedale et al., 2016), they also found that most Americans claim to buy foods with a so-called 'clean' label, which means they perceive the ingredients as being 'all natural' and therefore non-threatening and healthy.

Consumers around the world fear additives and preservatives in their foods, especially nitrite or nitrate because they lack an understanding of these why these compounds are added to foods. Consumer confusion and fear regarding nitrate and nitrite is continuing to shape the food industry. However, 'uncured' meats, but which actually contain nitrate and nitrite derived from vegetable extracts and a starter culture, have proliferated in the marketplace in recent years (Sebranek et al., 2012).

What follows is a review of lactic fermentation and curing.

Lactic acid preservation of food

Lactic acid fermentation

Lactic acid fermentation of foods in Western culinary cultures is largely restricted to milk and meats – yoghurt/cheese and salami styles – with limited fermentation of vegetables, notably fermented cabbage called sauerkraut in German. Eastern culinary cultures ferment these products, but also many others. For example, fermented fish is particularly common in Southeast Asia (Sakai et al., 1983), where it is a major way of preserving this highly perishable food, and is aided

by the high ambient temperature in those countries. Lactic fermentation is less popular in the wealthier Western countries in part because of ready access to refrigeration.

Lactic acid bacteria

Lactic acid bacteria, which include *Lactococcus*, *Pediococcus*, *Lactobacillus*, *Streptococcus*, *Aerococcus*, *Camobacterium*, *Leuconostoc*, *Vagococcus*, *Enterococcus*, *Weissella*, and *Tetragenococcus*, are commonly used in lactic fermentation because lactic acid bacteria are characterized by a high tolerance to acidity (Stiles & Holzapfel, 1997). They are generally regarded as safe (GRAS) because of their long history of safe consumption and wide application in the production of fermented foods (Pennacchia et al., 2004). Lactic acid bacteria are characterized as fastidious microorganisms on account of complex nutritional requirements.

The preservative effect of lactic acid bacteria arises from at least two sources. First the pH after lactic fermentation is typically below 4.5, a pH that many other bacteria cannot tolerate. Second, these bacteria produce bacteriocins. Bacteriocins are ribosomally synthesized antimicrobial peptides that usually display a high degree of target specificity against strains of bacteria closely related and/or broad range antimicrobial activity.

Bacteriocins

The ability of lactic acid bacteria to produce the specific proteinaceous substances, bacteriocins which can inhibit the growth of pathogens always attract people's attention because it could enhance the shelf life of food (Soomro et al., 2002). Table 1 shows some bacteriocins which are characterized.

Nisin is a polycyclic antibacterial peptide produced by the bacterium *Lactococcus lactis*. Nisin (Figure 4) is a colourless, tasteless powder that is typically added to food at a rate of 0.25 to 37.5 mg kg^{-1} (Ryan, 2016). That is used as a food preservative. In the food industry, nisin is obtained from the culturing of *Lactococcus lactis* on substrates such as milk or dextrose. It is not chemically synthesized.

While amost bacteriocins inhibit only closely related species, nisin is a rare example of a broad-spectrum bacteriocin effective against many gram-positive organisms, including lactic acid bacteria

(commonly associated with spoilage), *Listeria monocytogenes* (a known pathogen), *Staphylococcus aureus, Bacillus cereus, Clostridium botulinum*, etc. It is also particularly effective against spores. Gram-negative bacteria are protected by their outer membrane but may become susceptible to nisin action after a heat shock or when this is coupled with the chelator EDTA. Nisin is soluble in water and can be effective at levels nearing the parts-per-billion range.

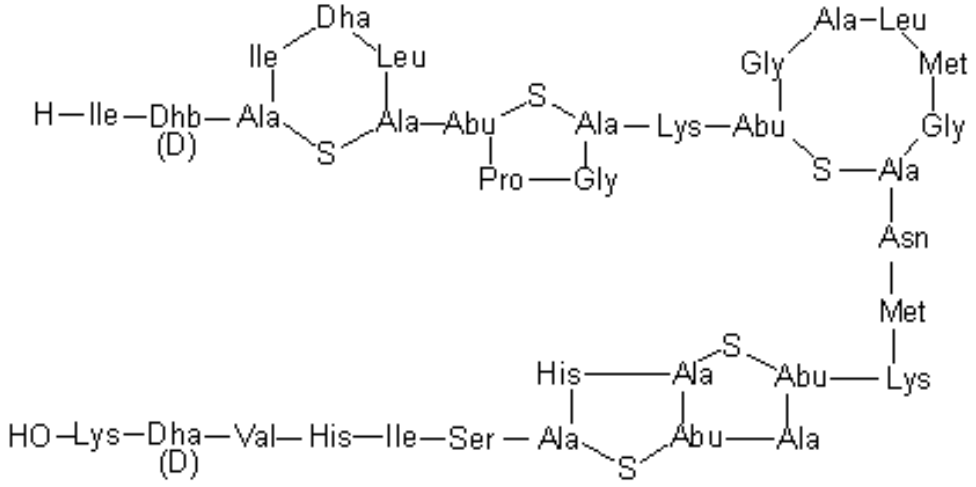

Figure 4 The amino acid sequence of nisin. According to (Mistry & Kennedy, 2003), the first 21 amino acids are mostly hydrophobic and could form a region able to bind to the membrane lipids of susceptible microorganisms.

Direct addition of nisin in vacuum-packed sliced cooked ham could significantly reduce the total LAB count (2 to 3 log cycles) in meat products when compared with control (without nisin) during chilled storage for 60 days

Inoculation of bacteriocin-producing LABs as a starter or protective cultures is a suitable strategy to ensure the safety of fermented meats. As a starter culture, the inoculation mainly contributes to produce acids and bacteriocins and hydrolyse proteins in meat thus dramatically changing the properties and organoleptic characteristics of meat products. As a protective culture, the objective use of bacteriocin-producing LABs is to inhibit the growth of unwanted bacteria without the cause of sensorial changes in meat or meat products.

Table 1 Properties of some well characterized bacteriocins.

Bacteriocin	Producer organism	Properties
Nisin	*Lactococcus lactis subsp. Lactis* ATCC 11454	Lantibiotic, broad spectrum, chromosome/plasmid mediated, bactericidal, produced late in the growth cycle
Pediocin A	*Pediococcus pentosaceus* FBB61and L-7230	Broad spectrum, plasmid mediated
Pediocin AcH	*Pediococcus acidilactici* H	Broad spectrum, plasmid mediated
Leucocin	*Leuconostoc gelidum* UAL 187	Broad spectrum, plasmid mediated, bacteriostatic, produced early in the growth cycle
Helveticin J	*Lactobacillus helveticus* 481	Narrow spectrum, chromosomally mediated, bactericidal
Carnobacteriocin	*Carnobacterium piscicola* LV17	Narrow spectrum, plasmid mediated, produced early in the growth cycle

Meat and meat products have played an important role in human diet because they can supply the enough nutrition for health. However, the unwanted microorganisms would grow on meat and lead to health risk easily. The utilization of bacteriocins produced by lactic acid bacteria as a natural preservative has received a considerable attention. Bacteriocins can be applied in meats and meat products in such major approaches including direct inoculation of bacteriocin producing Lactic acid bacteriocins cells into meat and meat products as either starter or protective cultures and using bacteriocins produced by lactic acid bacteria as a food additive (Woraprayote et al., 2016)

Table 2 Applications of bacteriocin-producing LAB in meat and meat products

Application approach	Bacteriocin-producing strain	Product	Feature	Reference
Direct inoculation (starter culture)	*Lactobacillus curvatus* DF126	Ostrich meat salami	Anti-*Listeria activity*	Dicks et al. (2004)
	Lactobacillus plantarum 423	Salami from ostrich, beef, mutton, Blesbok and Springbok	Anti-*Listeria activity*	Dicks et al. (2004); Todorov et al. (2007)
	Lactobacillus curvatus DF38	Salami from beef, horse, mutton, Blesbok and Springbok	Anti-*Listeria activity*	Todorov et al. (2007)
	Pediococcus pentosaceus BCC 3772	Nham pork sausage	Anti-*Listeria* activity; No significant changes in sensory and consumer acceptability.	Kingcha et al. (2012)
	Lactococcus lactis supsp. *lactis* 69	Charqui, Brazilian traditional salted and dried meat	Reduced spoilage bacteria during Charqui fermentation	Biscola et al. (2014)
	Lactobacillus sakei C2	Fermented pork sausage	Anti-*Listeria* and Anti-*Enterobacteriacae* activity	Gao et al. (2015)
	Lactobacillus curvatus 54M16	Fermented sausage	Reduced the number of *Staphylococci* and *Enterobacteriaceae*	Casaburi et al. (2016)
Direct inoculation (Protective culture)	*Leuconostoc carnosum* 4010	Vacuum-packed sliced cooked meats; Surface inoculation	Anti-*Listeria activity*	Budde et al. (2003)
	Leuconostoc carnosum 4010	Gas-packed saveloy	Exhibited anti-*Listeria* activity. Spraying the protective culture onto the product surface was effective	Jacobsen et al. (2003)
	Lactobacillus curvatus CWBI-B28	Raw beef/Surface inoculation	Anti-*Listeria* activity	Dortu et al. (2008)
	Lactobacillus sakei CWBI-B1365	Raw beef/Surface inoculation	Anti-*Listeria* activity	Dortu et al. (2008)
	Lactobacillus curvatus CRL705	Vacuum-packed fresh beef/Spraying with protective culture	Inhibited the growth of *Listeria* innouca and *Brochothrix thermosphacta*	Castellano and Vignolo (2006); Castellano et al. (2010)
	Lactobacillus curvatus ACU-1 (Sakacin Q)	Cooked meat; Immersion in cell suspension	Anti-*Listeria activity*	Rivas et al. (2014)
	Lactobacillus curvatus MBSa2	Salami; Mixing with salami batter before casing and incubation	Anti-*Listeria activity*	Barbosa et al. (2015)

According to the Woraprayote et al. (2016), bacteriocins can be applied to meats and meat products as food additive to prevent the growth of the microorganisms. This approach has been shown to be effective to control pathogenic and spoilage microorganisms in meat and meat products Table 3. The technique is more suitable to real meat system when live cells of lactic acid bacteria cannot produce bacteriocins.

Table 3 Applications of LAB bacteriocin preparations in meat and meat products

Bacteriocin, producer	Product	Features	Reference
Enterocins A and B, *Enterococcus faecium* CTC492	Cooked ham, minced pork, paté, and espetec.	All exhibited anti-*Listeria* activity	Aymerich et al. (2000)
Leucocins 4010, *Leuconostoc carnosum* 4010	Gas-packed sliced cooked saveloy	Both exhibited anti-*Listeria* activity. Application of bacteriocin onto meat surface was more effective approach to control *Listeria* during storage at 5 °C.	Jacobsen et al. (2003)
Lactocin 705, *Lactobacillus curvatus* CRL705	Vacuum-packed fresh beef/ Spraying with bacteriocin solution	Inhibited the growth of *Brochothrix thermosphacta*	Castellano and Vignolo (2006)
Lactocin AL705, *Lactobacillus curvatus* CRL705	Vacuum-packed fresh beef/Spraying with bacteriocin solution	Anti-*Listeria activity*	Castellano and Vignolo (2006)
Bacteriocin, *Pediococcus acidilactici*	Raw pork/Immersion in the concentrated culture supernatant	Anti-*Listeria activity*, reduced the growth of *Clostridium perfringen*	Nieto-Lozano et al. (2006)
Pentocin 31-1, *Lactobacillus pentosus* 31-1	Tray-packed chilled pork/Immersion in bacteriocin solution	Anti-activity of *Listeria monocytogenes* and *Pseudomonas fluorescens*	Zhang et al. (2010)
Enterocin AS-48, *Enterococcus faecalis* A-48-32	Low acid fermented sausage called fuets/Mixing with bacteriocin	Reduced the growth of *Listeria monocytogenes* and *Salmonella*	Ananou et al. (2010)
Nisin, Sigma Raw	Raw meat/Immersion in nisin solution	Anti-*Listeria activity*.	Mohamed et al. (2011)
Bacteriocins MT 104 and MT 162, *Enterococcus faecium*	Meat sausage/Mixing with bacteriocin	Anti-*Listeria activity*. Addition of nisin and gamma-radiation improved the antimicrobial activity of bacteriocins	Turgis et al. (2012)
Sakacin Q, *Lactobacillus curvatus* ACU-1	Cooked meat or immersion in cell free supernatant (CFS), or freeze-dried CFS reconstituted in distilled water	Both exhibited anti-*Listeria activity*. The freeze-dried reconstituted CFS was more effective one.	(Rivas et al., 2014)
Nisin, commercial nisin	Vacuum-packed sliced cooked ham/Brine injection;	Reduced the total count of LAB	(Kalschne et al., 2014)
Nisin, NiprosinTM	Ham/Coating with antimicrobials, nisin and combination of nisin and essential oil	All exhibited anti-*Listeria* activity.	Huq et al. (2015)

Many technologies including irradiation and microencapsulation of bacteriocins together with

other food preservatives were used as an advanced process to improve the food safety for a ready-to-eat meat product. Huq et al. (2015) developed anti-listeria formulations from the combination of nisin and essential oil. This treatment could reduce the number of *Listeria monocytogenes* artificially inoculated into meat sample (~5 to 6 log CFU/g) to below the detection limit (⩽50 CFU/g) from the first day of storage. The inhibition effect lasted at least 28 days of refrigerated storage.

Fermented products and curing

Vegetable fermentation

Vegetables supply much more nutrition for human health. Most of them are low in fat and rich in dietary fibres, minerals and phytochemicals. Vegetables are also the main source of water-soluble vitamins such as vitamin A, C and E (Eitenmiller et al., 2016). However, compared to the high level of nutrition, the fresh vegetable has short shelf life because the unwanted microorganisms such as pathogens will growth rapidly. In many western countries, people always use refrigerator to storage the vegetable to prolong its shelf life, but people living in undeveloped and developing countries have no access to these modern preserving techniques, who are forced to come up with natural processed methods to store vegetables like salting and fermentation.

Among the variety of fermented vegetables around the whole world, both of the kimchi in Korea and sauerkraut in German are typical products. Kimchi is a traditional, fermented Korean food that is prepared through a series of processes, including pretreatment of oriental cabbage, brining, blending with various spices and other ingredients, and fermentation. The characteristics of kimchi differ depending on the kimchi varieties, raw materials used, process, fermentation, and preservation methods (Cheigh et al., 1994). Kimchi is attained before overgrowth of *Lactobacillus brevis* and *Lactobacillus plantarum* with an optimal product pH of 4.5. The overgrowth of *L. brevis* and *L. plantarum* diminish the product's quality due to low pH, but sauerkraut production depends on these organisms.

Acid-fermented vegetables are also important sources of vitamins and minerals. *Leuconostoc mesenteroides* has been found to be important in the initiation of the fermentation of many

vegetables - e.g. cabbages, beets, turnips, cauliflower, green beans, sliced green tomatoes, cucumber, olives and sugar beet silages. In vegetables, *Leuconostoc mesenteroides* grows more rapidly and over a wider range of temperatures and salt concentrations than any other lactic acid bacteria. *Leuconostoc mesenteroides* produces carbon dioxide and acids which quickly lower the pH, thereby inhibiting the development of undesirable microorganisms and the activity of their enzymes, which may soften the vegetables. The carbon dioxide produced replaces air and provides anaerobic conditions favorable for the stabilization of ascorbic acid and the natural color of the vegetables. The growth of this species modifies the environment, making it favorable for the growth of other lactic acid bacteria. The high acidity produced by the species and other subsequent lactic acid bacteria inhibits the growth of *Leuconostoc mesenteroides.* This microbe converts glucose to approximately 45% lactic acid, 25% carbon dioxide, and 25% acetic acid and ethyl alcohol. Fructose is partially reduced to mannitol and is then readily fermented to yield equimolar quantities of lactic acid and acetic acid. The combination of acids and alcohol is conducive to the formation of esters that impart desirable flavors (Cheigh et al., 1994).

The optimal range of salt concentration of sauerkraut is 0.7 to 3.0% while that of kimchi is 3.0 to 5.0%.

Fermented squid (endogenous microflora and high salt)

In Kim et al. (1993) research, a typically fermented seafood called jeotgal in Korean cuisine was studied. The traditional products have been prepared with the addition of about 25% salt to prevent spoilage, but domestic seasoned and fermented product have only about 8% salt. To investigate the chemical compounds and sensory evaluation, the fermented products were stored in 10°C, 20°C and 30°C respectively. Chemical components such as volatile basic nitrogen, ammonia nitrogen and total nitrogen rapidly increased during storage at 20°C and 30°C, but pH value and Hunter LAB colour values slowly decreased during storage without respect of storage temperature.

Byun et al. (2000) also found that chemical components such as amino nitrogen, volatile basic nitrogen, trimethylamine, and hypoxanthine contents increased rapidly with increasing salt concentration.

Fermented meat products

In meat, water activity is 0.96 to 0.97 and pH is 5.6 to 5.8, which provides a perfect condition for microorganisms to grow and proliferate (Farnworth, 2008). In history, human beings devised many methods to process meat for longer storage time like salting, drying and fermentation. These depended largely onn their ability to reduce the water activity (aw) by removing the available water. The curing processes involve smoking, spicing, and cooking. According to the investigate of Nummer, dehydration was the earliest form of food curing (Nummer & Brian, 2002).

Raw meats such as bacon, jerky and Chinese-style sausages owe their characteristics to salting and drying alone (Hugas, 1998). After the process of fermentation and curing, the production of fermented sausage is much similar to cheese-making; both are related to salting, drying, and lactic fermentation (Frédéric Leroy, 2006).

Fermented meat products include a wide range of sausages like salami and pepperoni, also some ham products. There are some examples of fermented meat product in Table 4.

Table 4 Examples of acid-fermented seafood, cereal, and meat mixtures

Product name	Country	Ingredients	Microorganisms	Usage
Nham	Thailand	Pork, garlic, salt, rice	*Pediococcus cerevisiae, Lactobacillus plantarum, Lactobacillus brevis*	Pork meat in banana leaves
Sai-krok-prieo	Thailand	Pork, rice, garlic, salt	*Lactobacillus plantarum, Lactobacillus salivarius, Pediococcus pentosaceus*	Sausage
Nem-chua	Vietnam	Pork, salt, cooked rice	*Pediococcus sp., Lactobacillus sp.*	Sausage
Salami	Europe	Pork	*Lactobacillus, Micrococcus*	Sausage

Meat fermentation involves a series of complex microbial reactions which lead to a change on water activity and acidity (Marta Hugas, 1997). These changes in chemical properties, along with the “good” microflora surpass the spoilage and pathogenic microorganisms, generate a more stable product than raw meat. Originally, such production heavily relies on experience, but as more has been understood about fermentation, start culture s have been used and fermentation conditions modified to produce stable products of acceptable quality. By definition, the ripening of fermented

sausages includes acid production by LAB at various rates and final acidities (Bamforth & Ward, 2014)

In principle, fermentation can apply to meat from any animal. Chopped meat and fat prior to mixing with the other ingredients is best performed at low temperatures (about −4°C) to avoid contaminating of the meat particles with fat (Pennacchia et al., 2004). Curing salts are a mixture of a couple of different salts. They ensure good texture, flavour, and colour in the product directing the fermentation process. Sodium chloride also inhibits the growth of the spoilage bacteria including relatively salt-sensitive gram-negative organisms while allows acid formation by the halotolerant lactic acid bacteria. It affects flavour by performing as an antioxidant and contributes to the safety of the product by discouraging the growth of a range of pathogenic organisms, most particularly *Salmonella*, *Clostridium botulinum*, and *Listeria monocytogenes*. But the nitrite is not safety, if adding much to the food, people will concern the risk of it (Mehta et al., 2012).

History of nitrite curing

By around 200 BC, Romans recognized that some salt could impart a reddish colour to meat during preservation (Keeton, 2011). In the early 1900s, the function of nitrite had been identified within the process of curing meat. Potassium nitrate was used as a curing agent in meat for many centuries. Despite a long history of use, nitrite was nearly banned from use in foods in the 1970s due to health concerns related to the potential for carcinogenic nitrosamine formation. Finally, the discovery in the 1980s that nitric oxide is a key metabolic signaling molecule affecting a huge number of physiological processes led to a profound need to reconsider the effects and importance of nitrate and nitrite in the body. Media mentions of nitrite and nitrosamine decreased (Cassens, 1990), and an uneasy truce was thus established for most consumers in their relationship with nitrite and cured meats.

In the last two decades, the clean-label and the anti-nitrite movements have come together to spawn the development of ‘uncured’ bacon and other processed meats. The USDA does not allow the addition of synthetic chemicals, including sodium nitrite or sodium erythorbate, to be added to meat products that are labelled as ‘natural’ or ‘organic’ (Sebranek et al., 2012).

Very recently, the International Agency for Research on Cancer (IARC) pronounced that consumption of processed meats is likely to be carcinogenic (for colorectal cancer and by association with stomach cancer) to humans, citing N-nitroso compounds (NOC) among others as the components within meat that mechanistically could support a carcinogenesis mechanism (Bouvard et al., 2015). It is too early to tell yet how this announcement will impact processed meat consumption.

A summary of some of the key events in nitrate and nitrite history with respect to processed meats that have shaped consumers' views of these compounds or provided knowledge about the effects of these compounds are shown in Table 5.

Table 5 Key events and dates for nitrate and nitrate in processed meats (Bedale et al., 2016).

Date	Event	Reference
<200 BC	Some salts could impart a reddish colour to meats during preservation	Keeton (2011)
1891	Nitrite is first identified within cured meats	Binkerd and Kolari (1975)
1899	Nitrite is actually responsible for the colour of cured meats	Binkerd and Kolari (1975)
1914	Nitrite is further reduced to nitrous acid and nitric oxide, which reacts with myoglobin in meat to give cured meat its characteristic colour	Keeton (2011)
1923	USDA investigates the use of nitrite rather than nitrate as a curing agent	Kerr et al. (1926)
1925	USDA approves a maximal level of nitrite is 200 mg/kg in the meat product.	Cassens (1990)
1962	WHO recommends a limit of dietary nitrate intake of 3.7 mg or 5 mg/kg body weight sodium nitrate	Katan (2009)
1950-60s	Nitrosamine recognized as a carcinogen, and the chemistry of its formation from nitrite and secondary amines were studied.	Cassens (1990)
1970	Cooking could be a source of secondary amines, which together with ingested nitrites could potentially form nitrosamines	Lijinsky and Epstein (1970)
1972	Centre for Science in the Public Interest petitions USDA to ban or greatly reduce the use of nitrites in cured meats	Cassens (1990)
1973	First epidemiological reports that processed meats are associated with colorectal cancer	Zaldivar and Robinson (1973)
1972-75	Discovery that the nitrosamines such as nitrosopyrrolidine are formed when bacon is fried	Fazio et al. (1973)
1978	USDA lowers the amount of sodium nitrite from 200 to 120 ppm, and requires that the antioxidant ascorbate or erythorbate be included	Register (1978)
1979-80	Report that nitrite itself is a carcinogen is published and receives much public attention; this is evaluated and refuted by U.S. FDA	Newberne (1979)

1981	National Academy of Sciences report recommends reducing intake of dietary nitrate and nitrite because they can form N-nitroso compounds that are carcinogenic	Sciences (1981)
1980s	Nitrate and nitrite are shown to be formed endogenously in the body	
1986	Alternative ways of curing bacon which use lower nitrite levels are developed and allowed by USDA	Cassens (1990)
1987	Nitrosamines can form endogenously from dietary nitrate.	Tricker and Preussmann (1991)
1994	Los Angeles Times publishes a report highlighting epidemiological studies suggesting maternal consumption of hot dogs is related to brain cancers in children many years later. Reports of leukemia being related to children consuming cured meats are also published, which reignites public controversy	Bunin et al. (1994)
The late 1990s	'Uncured' processed meat products are developed.	Sebranek et al. (2012)
1998	The state of California proposes listing sodium nitrite as developmental and reproductive toxicant under a law called Proposition 65.	Assessment (1999)
2000	National Toxicology Program releases results of rodent toxicology and carcinogenicity studies, showing no evidence of carcinogenicity except for equivocal evidence in the forestomach of female mice	
2000	California Proposition 65 listing of nitrite as a developmental and reproductive toxicant is rejected by a scientific review committee	
2003	WHO recommends moderation in the consumption of preserved meats	WHO (2003)
2006	IARC concludes that ingested nitrate or nitrate under conditions is probably carcinogenic to humans (Group 2 A)	Grosse et al. (2006)
2015	IARC declares processed meats to be a Group 1 carcinogen. Red meat consumption was classified as probably carcinogenic to humans (Group 2 A)	Bouvard et al. (2015)

Benefits of nitrite

Nitrite both prevents spore germination and vegetative cell growth of *Clostridium botulinum* in meats (Archer, 2002). Nitrite also shows activity against other pathogens in meat as well, including *Listeria monocytogenes*. Beneficial effects on blood pressure have been consistently observed (Larsen et al., 2006).

Nitrite is found in colostrum and in breast milk in the initial days after a baby is delivered which the baby consume the 1mg/kg per day that is more than 10-fold the ADI for nitrite (Hord et al., 2011). This nitrite source has been proposed to play a role in protecting infants against hypoxic/ ischemic injury by serving as a source of nitric oxide, or it may protect the gastrointestinal tract from bacterial pathogens (Jones et al., 2015).

Perceived and real problems with nitrate and nitrite

Many of the health concerns related to dietary nitrite or nitrate consumption have been reviewed and a brief summary of these concerns is presented. Cancer is the most significant health risk that has historically been associated with nitrate and nitrite. While these compounds are not themselves carcinogenic, they have the potential to react with other compounds within food during cooking or in the digestive tract to form carcinogens.

A metaanalysis found no significant association between nitrate exposure and risk of thyroid cancer, hyper-or hypothyroidism, although thyroid cancer was associated with higher nitrite exposures (Bahadoran et al., 2015). Another study suggested a possible link between age-related macular degeneration and elevated nitrate-nitrogen in rural private drinking water (Klein et al., 2013)

The facts

The discovery of the profound physiological importance of nitric oxide led to the realization that dietary nitrate contributes significantly to the nitrogen reservoir for nitric oxide formation. Numerous clinical studies have also demonstrated beneficial effects of dietary nitrate consumption, especially in vascular and metabolic health. However, the latest wave of consumer sentiment against food additives, the clean-label movement, has renewed consumer fear and avoidance of preservatives, including nitrite. Education is necessary but may not be sufficient to resolve this disconnect in consumer perception.

Concept of vegetable extracts and results

The use of nitrate for curing meat is not a new concept because it is widely recognized that ancient meat curing processes for hundreds of years ago utilized natural nitrate in the form of saltpeter (potassium nitrate) and depended on inherent nitrate-reducing bacteria in the meat to convert the nitrate to nitrite (Cassens, 1990). However, modern technology improved upon the age-old process by developing a concentrated vegetable extract from celery (*Apium graveolens* var. dulce) that contained a high nitrate concentration at about 3% and a purified, concentrated strain of efficient nitrate-reducing bacteria. Celery powder has been used for many years in meat product

formulations as a flavouring agent, and starter cultures, including nitrate-reducing strains, have been used for fermenting sausage since the 1950s (Bacus, 1984). To shorten the product incubation time, some processors began to incubate the celery juice with culture before adding the mixture to the meat product (Krause et al., 2011) and, consequently, suppliers of the celery concentrate began to provide this service by "pre-converting" the nitrate to nitrite (Sebranek et al., 2012).

Colour changes of meat

Inspection of traditional fermented food, curing salts are usually added for taste, colour, texture, safety and stability (Adams & Moss, 2007). In the process of curing cabbage and beef, the oxymyoglobin is oxidised and form the metmyoglobin. After the protein denaturation, it would produce denatured metmyoglobin. With adding of nitric oxygen, the denatured metmyoglobin would be reduced and form the nitrosohemochrome which shows light pink colour. The process is shown in Figure 5. The nitrite also would develop a special flavour during the process of fermentation (Russell & Gould, 2003). The detail will be discussed in Chapter 8.

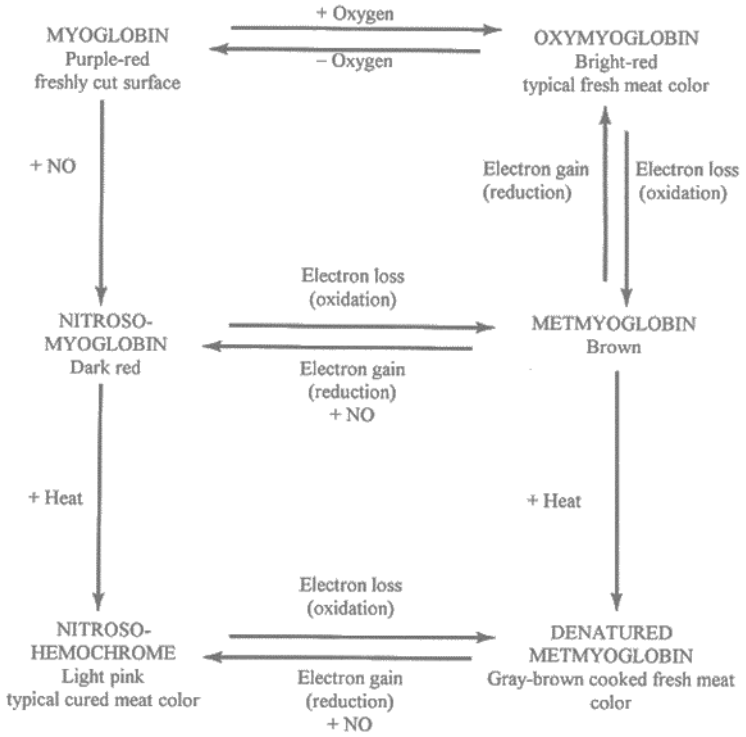

Figure 5 Meat pigment changes during cooking and curing (Cassens, 1990)

Questions to be answered in this thesis

1. **What is the best way of cooking vegetables, usually cabbage, and beef before fermentation?**
2. **What effect on curing of ratio cabbage:meat?**
3. **Raw versus cooked cabbage**
4. **What is the best glucose concentration to use?**
5. **If sucrose is required for sweetening, how will different starter cultures affect final pH and other product qualities?**
6. **Can fermented products be held at ambient temperature for extended times without loss of quality?**
7. **How safe are these products?**

Chapter 2: Generic Materials and Methods

Description

Kimchi is traditionally made from East Asian cabbage varieties, but these are only sporadically available in New Zealand. Therefore, the standard Chinese large white cabbage was used routinely, as was a single cut of beef, rumpus, dominated by *gluteus medius*.

In Figure 6, the general outline is list. But in the individual experiment, there will have differences on some part of detail.

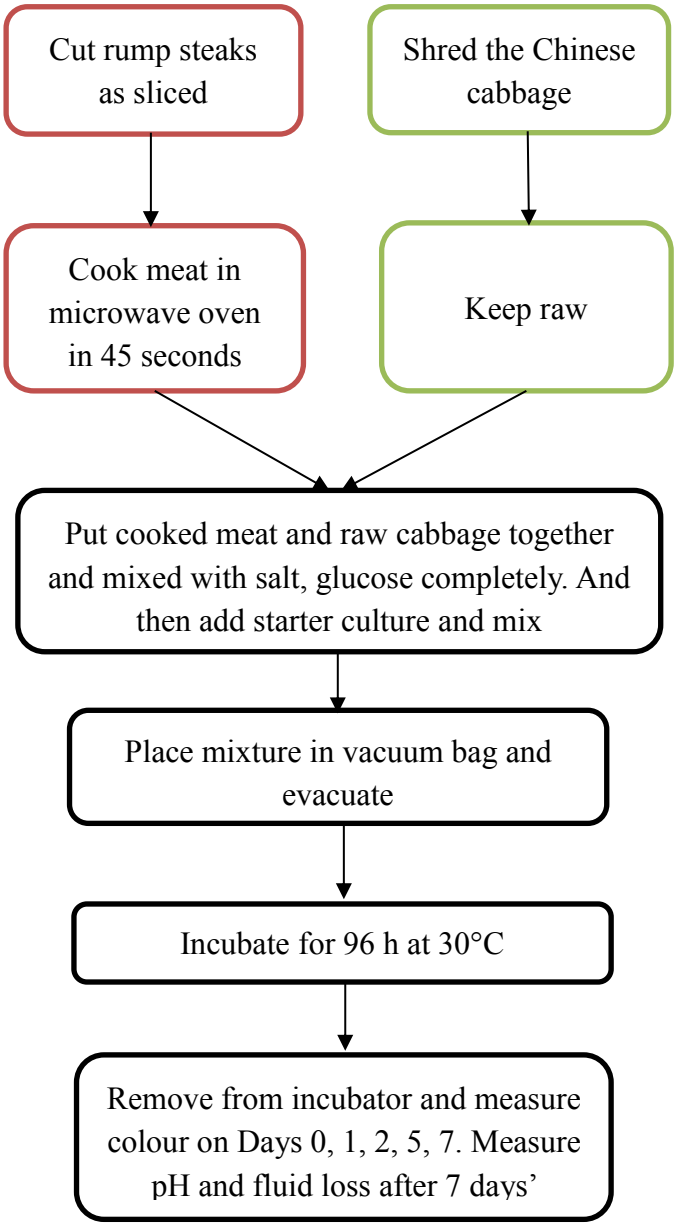

Figure 6 The generic steps of production

Meat and cabbage

Rump steak was bought from Countdown supermarket (Auckland) and Chinese cabbage from a local Asian market. The standard weight ratio of meat to cabbage was 5:1. Size reduction was achieved with a domestic kitchen knife. The cabbage would be cut into small slice and the raw meat would be cut into strips approximately 3 mm thick, 50 mm long and 20 mm wide shown in Figure 7 under conditions as hygienic as possible considering the varied use of the food laboratory.

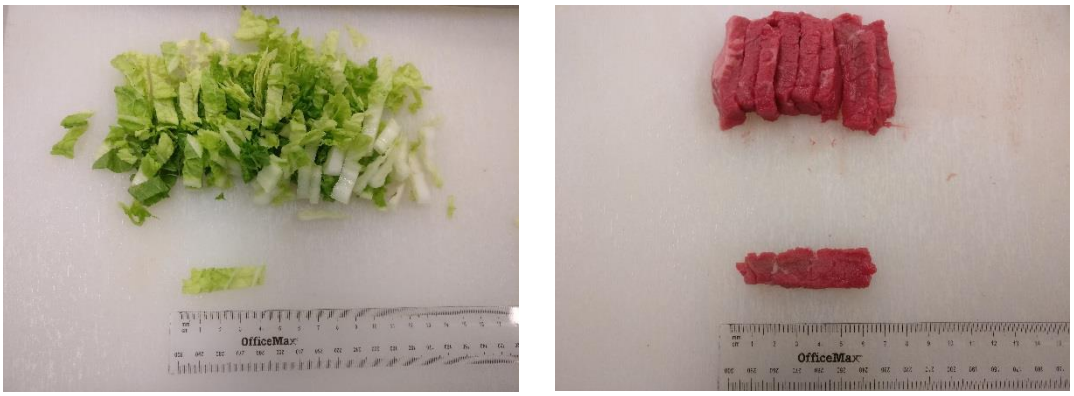

Figure 7 The size of meat and cabbage used in experiments

Production equipment

The microwave oven was SHARP Carousel R222TW with full power of 800 W. Where cooking was required, 20 g meat was cooked for 45 sec and showed an obvious brown colour. Cut raw cabbage was held for no more than 1 h in a refrigerator after cut.

Conventional knives, mixing bowls and other domestic tools were used to prepare the mixtures. The vacuum bags were barrier vacuum bags, 220 x 250 mm made by Iconpack, Australia and supplied by Duninghams (Auckland). No gas transmission data were available, but vacuums were normally maintained, and any gas accumulation was almost certainly caused by contamination microorganisms (described in later chapters).

The vacuum packer was DZ-400/T (Figure 8). The standard evacuation time was 15 sec with a sealing time of 1.5 sec. The incubation oven was a LabServ Oven maintained at 30°C for a maximum standard 96 h. Chilled storage was at 4°C in a domestic refrigerator.

Figure 8 Vacuum packer

Food additives

Iodised table salt, anhydrous D-(+)-glucose, and table sugar (sucrose) were sourced from the food laboratory. Sodium nitrite was analytical reagent grade. Salt was always added at the rate of 2.0 g per 120 g meat and cabbage mixture, translating to a final concentration of 1.67% by weight. Glucose was not always added. However, a lactic-fermenting culture always was.

These cultures, were supplied by Chr-Hansen and their broad properties are described in Table 6. Typically, these cultures contain blends of lactic acid bacteria, Staphylococcus species and less commonly, some different yeast species. Except where the performance of the cultures was compared (Chapter 6), all works were done with *BFL-F02* which consist of *Pediococcus pentosaceus* and *Staphylococcus carnosus ssp.* Chr-Hansen describes this culture as which the high concentration of *Pediococcus pentosaceus* gives a controlled and moderate pH-drop. The used *Staphylococcus carnosus ssp.* gives a milder and more “Mediterranean” flavour. The acidification gives a mild lactic acid taste. Another culture of particular interest was *T-SC-150* which consist of *Lactobacillus sakei* and *Staphylococcus carnosus.* Neither bacterium can ferment sucrose and this may be of particular value for reasons discussed in Chapter 6. Cultures were stored at -10 °C and brought to room temperature for as short a times as possible.

Table 6 Starter cultures used in individual experiments and their properties

Culture name	Bacteria included	Characteristics
BFL-F02	*Pediococcus pentosaceus*, *Staphylococcus carnosus* ssp.	The high concentration of *Pediococcus pentosaceus* gives a moderate pH-drop. The acidification gives a mild lactic acid taste. The used *Staphylococcus carnosus* ssp. gives a milder flavour.
SM-194	*Pediococcus pentosaceus*, *Lactobacillus sakei*, *Staphylococcus xylosus*, *Staphylococcus carnosus*, *Debaryomyces hansenii*	Multi-application culture that combines all positive features of the different strains. *Lactobacillus sakei* suppress the growth of a lot of indigenous bacteria. *Pediococcus pentosaceus* with its mild lactic acid taste and the accelerated pH-drop at higher temperatures. The combination of two different *Staphylococci* for more intensive colour formation and mild aroma development. And the yeast *Debaryomyces hansenii* on top to obtain a more "Mediterranean" flavour.
F-LC	*Pediococcus acidilactici*, *Lactobacillus curvatus*, *Staphylococcus xylosus*	Culture for acidification and prevention of *Listeria*. Applicable at a wide temperature range. *Pediococcus acidilactici* and *Lactobacillus curvatus* give a moderate pH-drop with a mild acidification flavour. *Staphylococcus xylosus* gives good colour formation and stability and mild flavour. Application in: Fermented sausages
BFL-F04	*Lactobacillus sakei*, *Staphylococcus carnosus* ssp.	The sucrose positive *Lactobacillus sakei* suppress the growth of a lot of indigenous bacteria. The combination of the two new developed *Staphylococci* gives a good colour formation and an intensive, but mild aroma. This special combination of the strains shows a fast pH-drop and leads to a firm texture.
T-SC-150	*Lactobacillus sakei*, *Staphylococcus carnosus*	Gives a product German salami flavour. The acidification leads to a lactic acid taste. The used *Lactobacillus sakei* suppress the growth of a lot of indigenous bacteria. The used *Staphylococcus carnosus* gives good colour stability and a mild aroma.

Product assessment

Colour is the first quality attribute of food evaluated by consumers, and is therefore an important component of food quality relevant to market acceptance (Wu & Sun, 2013).

Photographs were frequently taken with a mobile phone camera. The NIX colour sensor (Figure 9) is a novel hand-held colour meter for measuring the colour of surface on plastics, fabrics, leathers or any complex surface like woods, and wirelessly routes accurate colour information to smartphones or tablets. Its design blocks out all ambient light and uses its own calibrated light source to provide a high accuracy and precision. It requires no user calibration.

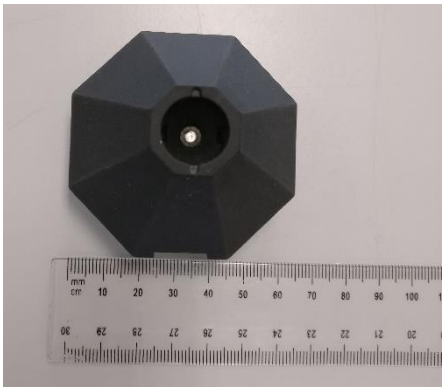

Figure 9 NIX colour sensor

The illuminant D50 was used, which represents natural white light (Judd et al., 1964), having an emission temperature of 5003K. (The commonly used D65 illuminant better represents noon light.)

The observer angle used in was 2°[2] (CIE 1934). This means the observer would be able to use only the fovea region of the retina, known to be the most sensitive to colour. The important advantage of the NIX meter is that it measures with an aperture no bigger than 3 mm. This means that colour can be measured in spots within a heterogeneous object, which is clearly the case for mixtures of cabbage and meat.

To use the meter an App called 'Nix pro colour sensor' has to be downloaded to a smart phone. Data is recorded on the phone. Colour can be recorded in five formats, the most commonly used of which is the L, a, b colour space (Figure 10). In this figure L, a, and b are expressed as L*, a* and b*, which is the standard nomenclature for 10° Observer. The 2° Observer was used in this research, so L, a and b are the correct terms.

[2] In 1964, the CIE defined an additional standard observer, this time based upon a 10° field of view; this is referred to as the 10° Observer.

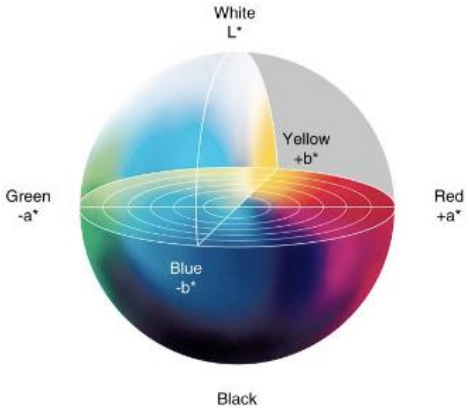

Figure 10 Colour scale (Dobos)

L is scale of 0 (black, no light reflected) to 100 (white, all light reflected); a is green (-a) to red (+a); b is blue (-b) to yellow (+b). According to Wikipedia (https://en.wikipedia.org/wiki/Lab_color_space), "The scaling and limits of the a and b axes will depend on the specific implementation of Lab colour, as described below, but they often run in the range of ± 100 or −128 to +127 (signed 8-bit integer)".

Because of the vacuum bag package lying between the NIX aperture and the food under study, the value of L, a and b could not be read directly, because light was reflected from the bag. To overcome this problem an evacuated barrier bag containing a matt black piece of cardboard was used to record the light reflectance from a single layer of the bag. This value was subtracted from each replicated measure of the food before statistical analysis.

The pH meter was a MeterLab PHM 201 portable pH meter produced by HACH with a combination electrode, routinely calibrated with pH 4 and pH 7 reference buffers. pH was always measured in the fluid that accumulated in the fermentation mixture.

Data analysis

All the data was input into Microsoft Excel and analysed by XLSTAT which is developed by the Addinsoft. XLSTAT is statistical software that integrates seamlessly into Excel. The most common routine used was analysis of variance (ANOVA).

Conclusion

The methods described in this Chapter 2 are generic to all subsequent work. The work to be described next is in the order shown in Table 7.

Table 7 Sequence of experiments to develop the commercial model of kimchi and meat

Experiment	Description	Chapter
Fluid loss of cabbage and meat	Measure fluid loss in meat and cabbage respectively in different cooking time and time to add salt and glucose.	3
Effect of salt and glucose on pH and colour of meat and cabbage	Mix cabbage cooked in 45 sec and raw meat and measure pH and colour.	3
Colour, pH and fluid loss of cabbage and meat under different condition	Measure and analyse the effect of food cooked or not and salt and glucose added or not.	3
Effects of added glucose and cabbage cooking on properties where salt was added and the meat was cooked	Measure and analyse the effect of cabbage cooking and glucose added and their interaction.	3
Comparing the difference between glucose added and cabbage cooking with adding the starter culture	To explore the difference of effect of cabbage cooking and glucose added with adding the starter culture	3
The effect of cabbage cooking and different incubation temperatures	To investigate the effect of cabbage cooking and at different temperature (23° and 30°C) and their interaction.	4
The effect of post incubation storage at two temperatures, 4°C and 23°C for 7 days on pH	To investigate the change of pH value in 7 days' storage.	4
The effect of post incubation storage at two temperatures, 4°C and 23°C for 56 days	To investigate the change of pH value and colour in 2 months' storage.	4
Importance of cabbage on meat colour changes	Compare the result of sample mixing with a small amount of cabbage and glucose or not to investigate the colour change of meat.	5
The effect of different amount of cabbage on pH and meat colour	To investigate the effect of different amounts of cabbage (0, 20, 40, 60, 80 and 100g) on change of colour and pH.	5
Effectiveness of small amounts of nitrite and of cabbage on meat colour changes	To investigate the effect of different amount of nitrite and cabbage on colour change and pH and compare them	5
Effect of different amounts of cabbage on the colour outcomes with culture T-SC-150	Use T-SC-150 to instead of BFL-F02 and measure colour and pH which is sucrose negative.	6
Effect of different starter cultures	Use 5 different culture (BFL-F02, SM-194, F-LC, BFL-F04 and T-SC-150) to compare the difference between them.	6
Sensory test	The mixture with adding different amount of sucrose (0%, 1%, 2%, 3% and 5%) and taste them.	7
Concluding discussion	Discuss the reason for the colour change, the effect of state of matter of cabbage, daily intake of nitrite and commercial prospects for this product.	8

Chapter 3: Establishing basic methods

Fluid loss of cooked cabbage and cooked meat

Introduction

A product swamped by the fluid is an undesirable attribute of the proposed product, so the aim of this experiment was to explore the effect of salt added separately to meat and cabbage, before and after cooking for various times.

Methods

Table 8 shows the experimental design where each treatment was represented by four replicate and therefore independent trials. For both cabbage and meat, the salt and glucose were added at the ratio of 2% and 1% by weight, to 100 g of cabbage (2 g, 1 g) and to 20 g of meat (0.4 g, 0.2 g). The mean mass of cabbage was controlled at 100.4 ± 0.2 g and for meat was controlled at 20.3 ± 0.3 g. A single control for meat and cabbage was included where no salt or glucose was added. Cooking time was either 0, 45 or 90 sec. After cooling, which took only a few minutes, the treatments including any fluid were vacuum packed with no culture added, and left at ambient temperature overnight. Fluid losses were recorded, and other observations were made.

Table 8 Fluid loss of cabbage and meat

Cooking time (sec)	Salt and glucose added to	Fluid loss ± SD (% of initial weight)
	Cabbage	
0	No cooking	19.9 ± 3.3[1]
45	Before cooking	15.1 ± 0.8
90	Before cooking	20.4 ± 2.2
45	After cooking	13.5 ± 0.8
90	After cooking	14.4 ± 0.8
90	Not added	10.7
	Meat	
0	No cooking	3.3 ± 0.4[1]
45	Before cooking	32.1 ± 2.2
90	Before cooking	40.0 ± 3.0
45	After cooking	30.7 ± 2.2
90	After cooking	42.4 ± 2.0
90	Not added	39.5

[1] The 0 sec treatments had 8 replicates rather than 4

Result and discussion

With no cooking (0 sec) Table 8 shows that the losses from cabbage were 19.9%. With one exception (90 sec, before cooking) all treatments were lower than 19.9%, the lowest being 13.5% for 45 sec, salt and glucose added after cooking.

Fluid losses for meat with no cooking were 3.3%, which was an attractive result. With cooking, the lowest loss was 30.7% for 45 sec, salt and glucose added after cooking.

Some colour observations were made. The raw meat treatment (no cooking) changed colour from bright red to light red, and it is proposed that with the reduced oxygen concentration there was less oxymyoglobin formed and thus a lighter colour. Cooked cabbage maintained some green colour in contrast to the raw cabbage which became more yellow, typical of senescence in cabbage. The short cooking time could help chlorophyll to against the acidic damage (Blais, 2012). The enzyme system responsible for yellowing by chlorophyll degradation in response to physical damage (knife, vacuum) is likely to have been denatured.

In conclusion, cabbage cooked for 45 seconds with salt and glucose added after cooking was

the best choice because fluid losses were least. Meat was best kept raw (but as will be shown later in this chapter it is better to cook the meat).

Effect of salt and glucose on pH and colour of meat and cabbage

Introduction

In the previous experiment, the colour of meat and cabbage changed after storage overnight. So, the aim of this experiment is to investigate the effect of salt and glucose added on pH and colour of raw meat (0 sec cooking) and cooked cabbage (45 sec) combined in vacuum bags. (A cooking time of 45 sec was established above.) No fermentation culture was added.

Methods

Table 9 shows the experimental design where the single salt/glucose treatment was represented by four replicates and a single control without salt and glucose added. For each replicate, 100 g of cabbage was separately cooked, and the combined cabbage and fluid were mixed 20 g of raw meat plus 2 g of salt and 1 g of glucose, resulting in final concentrations of about [3]1.68% and 0.83% of salt and glucose respectively. After evacuation, the colour was measured at Days 0, 1, 5 and 7. Storage was at ambient temperature. pH was measured only at Day 7 because there was only one opportunity to open the vacuum bags.

Table 9 The design to explore the effect of adding salt plus glucose or not.

	Mean weight cabbage (g)	Cabbage cooking time (sec)	Weight of raw meat (g)	Weight of salt (g)	Weight of glucose (g)
Treatment	100.5 ± 0.3	45	20.2 ± 0.3	2	1
Control	100.6	45	20.7	0	0

Result and discussion

Table 10 is the result of pH value of 4 samples with adding salt and glucose and 1 control without salt and glucose. Compared with pH of the control which reaches to 3.9, the mean pH value of the treated replicates was 3.38, and largely unvarying. The low pH of the salt/glucose treatment strongly suggests that a lactic fermentation developed, with any number of possibilities for the origin of the microbes responsible.

[3] In hindsight the final salt and glucose concentrations should have been 2% and 1% to match the previous experiment.

Table 10 pH of the control and 4 replicates, salt and glucose added	
Treatment	pH
Salt/glucose	3.42
Salt/glucose	3.37
Salt/glucose	3.38
Salt/glucose	3.35
Control	3.89

After 7 days' storage, the result shows that *a* value in meat and cabbage was becoming high. Compared with the meat control, meat added salt and glucose had higher a value which means the red colour is deeper than the colour in control shown in Figure 11. In Figure 12 for cabbage, it also shows the *a* value in cabbage on Day 7 is higher than the value on Day 0, in this case meaning the green chlorophyll colour was lost and yellowness increased as shown in *b* values. But there was little difference between the added salt and glucose treatment and the control.

Figure 11 *L*, *a* and *b* values of meat colour with salt and glucose

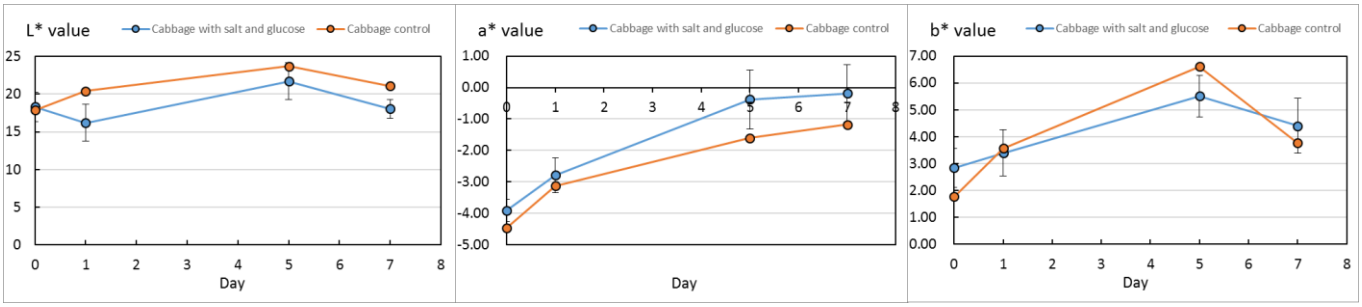

Figure 12 *L*, *a* and *b* value of cabbage colour with salt and glucose

It was concluded that salt and glucose had little effect on colour under these conditions.

Colour, pH and fluid loss of cabbage and meat under different condition

Introduction

The previous experiment showed that the raw meat and cooked cabbage colour stored under vacuum was largely unaffected by salt and glucose. The aim of this experiment is to investigate the effect of salt and glucose added (2 g and 1 g) or not and cabbage and meat cooking (45 sec) or not (0 sec) separately on colour change, pH and fluid loss under vacuum. As above, no culture was added.

Methods

Table 11 shows the experimental design where each treatment was represented by three replicates. The ingredients were organised into 12 treatments based on different combination of cabbage and meat, cooking or not, with added salt and glucose or not respectively. For each replicate, 100 g of cabbage was mixed with 20 g of meat with 2 g of salt and 1 g of glucose, or not, in a vacuum bag. After evacuation, the 12 treatments were left at room temperature and the colour measured on Days 0, 1, 5 and 7. These measurements were complemented by photography. pH was measured only at Day 7 because there was only one opportunity to open the vacuum bags. Photographs were taken at Day 0 and Day 7.

Table 11 Design for cabbage and meat cooking, with salt and glucose independently added. Each treatment had three replicates.

Weight of cabbage (g)	Cabbage	Weight of meat (g)	Meat	Salt (g)	Glucose (g)
100	Raw	20	Raw	0	0
100	Raw	20	Raw	2	0
100	Raw	20	Raw	0	1
100	Cooked	20	Raw	0	0
100	Cooked	20	Raw	2	0
100	Cooked	20	Raw	0	1
100	Raw	20	Cooked	0	0
100	Raw	20	Cooked	2	0
100	Raw	20	Cooked	0	1
100	Cooked	20	Cooked	0	0
100	Cooked	20	Cooked	2	0
100	Cooked	20	Cooked	0	1

Result and discussion

The results (Table 12) show that salt and glucose had important effects on fluid loss and pH at Day 7. This day was routinely selected because it represents what a consumer would buy. (Changes on earlier days are only of academic interest, but are examined in many later experiments.)

Added salt always increased fluid loss, whereas glucose reduced fluid loss. The low pH values, typical of fermented cabbage, were unexpected because a lactic acid culture had not been added to the mixtures. However, it seems highly likely that a lactic fermentation occurred due to contamination of lactobacilli from cabbage or meat (cooked or raw), kitchen equipment or surfaces, or from multiple sources.

The effect of cooking was not obvious from inspection of Table 12.

Table 12 Results for the effects of cabbage and meat cooking, salt and glucose on fluid loss and pH. Data were all from Day 7.

Cabbage	Meat	Salt	Glucose	Fluid loss ± SD (%)	pH ± SD
Raw	Raw	0	0	22.7 ± 1.2	4.01 ± 0.02
Raw	Raw	2	0	25.5 ± 0.7	3.59 ± 0.04
Raw	Raw	0	1	17.1 ± 0.4	3.91 ± 0.03
Cooked	Raw	0	0	16.8 ± 1.8	3.85 ± 0.04
Cooked	Raw	2	0	19.9 ± 0.9	3.61 ± 0.06
Cooked	Raw	0	1	12.4 ± 0.8	3.92 ± 0.05
Raw	Cooked	0	0	14.9 ± 1.3	4.06 ± 0.09
Raw	Cooked	2	0	25.5 ± 0.7	3.74 ± 0.02
Raw	Cooked	0	1	12.2 ± 0.4	4.00 ± 0.01
Cooked	Cooked	0	0	17.6 ± 1.7	4.02 ± 0.17
Cooked	Cooked	2	0	22.0 ± 0.9	3.72 ± 0.04
Cooked	Cooked	0	1	17.2 ± 2.1	4.13 ± 0.14

The analysis of variance for fluid and pH, where overall means were also calculated, is shown in Table 13, confirming the salt and glucose effects. Cooking cabbage and meat had little effect on fluid loss and pH.

Table 13 Analysis of variance in twelve treatments, measured on Day 7.

	Fluid loss (%)	P value	pH	P value
Salt				
−	16.4	***[1]	3.90	***
+	23.2		3.67	
Glucose				
−	20.6	***	3.82	*
+	14.7		3.98	
Cabbage				
Raw	19.6	NS	3.89	NS
Cooked	17.7		3.87	
Meat				
Raw	19.1	NS	3.82	*
Cooked	18.2		3.95	

[1] NS, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

The colour results equivalent to treatment shown in Table 12 for cabbage and meat colours are shown in Appendix 1 and is not discussed.

Analysis of variance for cabbage colour at Day 7 (Appendix 2) showed that salt, glucose, cabbage cooking and meat cooking had few if any important effects on colour value. Certainly, highly significant differences were shown, but inspection shows these effects could not be important. For example, cooking meat changed the value of cabbage from 1.5 to 0.0, the latter being perfectly neutral between red and green. The statistical significance was *** ($P < 0.001$), but with the values so close to zero, statistical significance becomes somewhat meaningless. However, the addition of salt reduced *L* value (28.3 to 24.7, *), and that may be visible to the eye.

Figure 13 Comparison of raw (top) and cooked (bottom) meat at Day 0 (left) and Day 7 (right). Cabbage was raw for both. No glucose or salt was added. A pink meat colour developed by Day 7 for raw and cooked meat.

Analysis of variance for meat colour Appendix 3 showed that neither salt, glucose nor cooking of cabbage affected meat colour at Day 7. In contrast, meat underwent very interesting colour changes from Day 0 to Day 7. The four images in Figure 13 show the colour changes in raw meat and cooked meat between these two days. These suggest that redness increases from Day 0 to Day 7. This was confirmed by *L*, *a* and *b* values (Table 14).

Table 14 Raw and cooked meat colour on Day 0 and Day 7

Day 0	*L*	*a*	*b*
Raw	6.9 ± 2.7	11.2 ± 1.2	7.1 ± 0.8
Cook	18.0 ± 3.0	6.3 ± 0.7	11.3 ± 1.0
Day 7	*L*	*a*	*b*
Raw	13.5 ± 3.2	18.7 ± 1.5	8.4 ± 1.0
Cook	20.7 ± 2.6	14.6 ± 0.9	11.8 ± 0.7

Cooking raw meat turned it brown due to metmyoglobin formation on Day 0. This was

expected. Thus redness decreased from 11.2 to 6.3, and yellowness increased, 7.1 to 11.3. By Day 7, redness was restored to 14.6 and yellowness was unchanged. Lightness increases slightly (18.0 to 20.7). Thus the meat became slightly pinker, reminiscent of nitrite cured meat. (These changes were also tracked on Days 1 and 5 [data not shown].) This remarkable result is explored more deeply in later experiments.

The main conclusion is that salt strongly increased the fluid loss and glucose would reduce it at Day 7. The salt, glucose, and cabbage cooking did not have an important effect on colour change. Cooked meat changed meat colour from brown to slightly pink.

In terms of product development, although the salt increases the fluid loss, the salt is essential because the taste of the product is important. At this time the decision was made to always cook the meat because it developed a more attractive bright pink colour than the raw equivalent, likely to be supported by the higher L value (20.7 compared with 13.5).

Effects of added glucose and cabbage cooking on properties where salt was added and meat was cooked

Introduction

The previous experiment showed that cooked meat had a more attractive bright pink colour. Salt has to be added by default for products of this type. The aim of the present experiment was to explore the effect and interaction of glucose added or not, and cabbage cooked or not, combined with the default cooked meat and salt, all evacuated and incubated at ambient temperature. As before, no culture was added.

Methods

The ingredient and their amounts of treatment are shown in Table 15. The experimental design was presented as four replicates for each treatment. For each replicate, 20 g of meat was separately cooked for 45 sec and mixed 100 g of cabbage cooking for 45 sec and 0 sec respectively with 2 g of salt and glucose (1 g and 0 g) in vacuum bag. After evacuation, the 4 treatments were left into room temperature and measure colour on Day 0, 2, 4 and 7. pH was measured only at Day 7.

Table 15 Design for glucose addition and cabbage cooking, where meat was cooked and salt was added. There were four replicates per treatment.

Weight of cabbage (g)	Cabbage	Weight of meat (g)	Meat	Salt (g)	Glucose (g)
100	Raw	20	Cooked	2	0
100	Raw	20	Cooked	2	1
100	Cooked	20	Cooked	2	0
100	Cooked	20	Cooked	2	1

Result and discussion

The result (Table 16) show that glucose had little effect on fluid loss and pH at Day 7. No large difference occurred in replicates with added glucose or not.

The effect of cooking cabbage was obvious in Table 16, where raw cabbage resulted in greater fluid loss, and this was confirmed by analysis of variance.

Table 16 Effect of cabbage cooking and glucose addition and on fluid loss and pH on Day 7. The meat was always cooked and salt was always added.

Cabbage	Glucose (g)	Fluid loss ± SD (%)	pH ± SD
Raw	0	34.5 ± 2.3	3.98 ± 0.06
Raw	1	34.1 ± 3.4	3.82 ± 0.04
Cooked	0	31.8 ± 4.8	4.32 ± 0.06
Cooked	1	28.5 ± 4.1	4.00 ± 0.15

Table 17 shows that glucose had no effect on fluid loss or pH, but that cabbage cooking had an important effect on fluid loss (34.3 reduced to 30.1%, ***). The effect of cooking cabbage on change of pH was minor but was lower where the cabbage was raw (4.16 versus 3.89) suggesting that the cabbage microflora was a source of lactic acid bacteria.

For the interaction of glucose and cabbage cooking, it showed an important effect on pH (3.81 to 4.31, ***). The cooked cabbage without glucose added had the highest pH value (4.31) and raw cabbage with glucose added had the lowest pH (3.81). The interaction between glucose and cabbage cooking had no significant effect on fluid loss.

Table 17 Analysis of variance of the four treatments.

	Fluid loss (%)	P value	pH	P value
Glucose				
–	33.2	NS	4.10	NS
+	31.3		3.90	
Cabbage				
Raw	34.3	***	3.89	*
Cooked	30.1		4.16	
Interaction				
– x Raw	34.5	NS	3.98	***
– x Cooked	31.8		4.31	
+ x Raw	34.1		3.81	
+ x Cooked	28.5		4.00	

The colour equivalent to Table 16 for cabbage and meat colour is shown Appendix 4 and is not discussed because although there were significant differences up to $P < 0.01$, they would be difficult or impossible to visualise. Some values were close to zero, where statistical models can yield meaningless significance values. Appendix 5 and Appendix 6 show the analyses of variance, but are not discussed further.

Of more importance was the meat colour change, shown for Days 0 and 7 (Table 18). As shown in the previous experiment, the colour changed from brown to pink. The redness was increased in all replicates. (These changes were also tracked on Days 2 and 4 [data not shown].) From Day 0 to Day 7, the redness of cooked meat increased from 6.4 to 18.5 (***) but *L* and *b* were statistically unchanged.

Table 18 Cooked meat colour on Day 0 and Day 7. The only significant difference was for a values (***).

Day 0	*L*	*a*	*b*
Cooked	19.2 ± 2.2	6.4 ± 0.7	11.6 ± 0.9
Day 7	*L*	*a*	*b*
Cooked	21.8 ± 1.3	18.5 ± 0.9	12.6 ± 0.6

To conclude, the question as to whether glucose should be added or not and cabbage should be cooked or not was not resolved for colour changes and pH, although cooking cabbage resulted in less fluid loss. The effects were generally minor.

At this point in development it was decided to introduce a defined fermentation culture (BFL-F02, Chr.-Hansen), so the next experiment is simply a repeat of the present, but with culture added. The question was: how does that affect the outcomes?

Comparing difference between glucose added and cabbage cooking with adding a starter culture

Introduction

The aim of this experiment was to investigate the effect of starter culture on the same condition with the previous experiment.

Methods

Table 19 shows the experimental design where each treatment was represented by four replicates. For each replicate, 20 g of meat was separately cooked for 45 sec and mixed 100 g of cabbage cooking for 45 sec and 0 sec respectively with 2 g of salt, glucose (1 g and 0 g) and 0.04 g starter culture (BFL-F02)[4] in a vacuum bag. The amount of starter culture (0.04 g) was a constant amount. After evacuation, the 4 treatments were left into room temperature and measure colour on Day 0, 2, 4 and 7. pH was measured only at Day 7.

Table 19 Treatment of glucose added and cabbage cooking with adding starter culture.

Weight of cabbage (g)	Cabbage	Weight of meat (g)	Meat	Salt (g)	Glucose (g)	Starter culture (g)
100	Raw	20	Cooked	2	0	0.04
100	Raw	20	Cooked	2	1	0.04
100	Cooked	20	Cooked	2	0	0.04
100	Cooked	20	Cooked	2	1	0.04

Result and discussion

Table 20 showed that the cabbage cooking and glucose added had no effect on fluid loss and pH value under adding starter culture in room temperature. However, compared with the previous experiment, the pH value is lower in this experiment. It seems that a lactic fermentation occurred after starter culture added.

[4] The starter culture was dispersed in a small volume of water before added in mixture.

Table 20 Treatment of glucose added and cabbage cooking with adding a starter culture

Starter culture (g)	Glucose (g)	Cabbage	Fluid loss ± SD (%)	pH ± SD
0.04	0	Raw	32.5 ± 0.9	3.54 ± 0.04
0.04	1	Raw	33.2 ± 1.5	3.60 ± 0.04
0.04	0	Cooked	29.7 ± 4.5	3.66 ± 0.03
0.04	1	Cooked	32.7 ± 1.6	3.67 ± 0.06

The analysis of variance for fluid and pH is shown in Table 21, confirming the glucose and cabbage cooking effects and their interaction. Cooking cabbage and glucose added had little effect on fluid loss and pH though the statistical significance of pH was ** ($P < 0.01$). But with the values so close (3.57 to 3.67), it becomes unimportant. This table also show that fluid in treatments without glucose were lower than the one added glucose although it showed not important.

Table 21 Analysis of variance of four treatments, with four replicates each.

	Fluid loss (%)	P value	pH	P value
Glucose				
–	31.1	NS	3.60	
+	33.0		3.64	NS
Cabbage				
Raw	32.9	NS	3.57	
Cooked	31.2		3.67	**
Interaction				
– x Raw	32.5		3.54	
– x Cooked	29.7		3.66	
+ x Raw	33.2	NS	3.60	**
+ x Cooked	32.7		3.67	

The colour equivalent to Table 20 for cabbage and meat colour is shown Appendix 7 and is not discussed because although there were significant differences up to $P < 0.01$, they would be difficult or impossible to visualise. (As before, some values were close to zero, where statistical models can yield meaningless significance values.) Appendix 8 and Appendix 9 show the analyses of variance, but are not discussed further.

The meat colour change, is shown for Days 0 and 7 (Table 22). As shown previously the colour changed from brown to pink. The redness was increased in all replicates. (These changes were

also tracked on Days 3 and 5 [data not shown].) From Day 0 to Day 7, the redness of cooked meat increased from 5.6 to 14.4 (***) but *L* and *b* were statistically unchanged.

Table 22 Cooked meat colour on Day 0 and Day 7. The only significant difference was for a values (***).

Day 0	*L*	*a*	*b*
Cooked	17.6 ± 1.9	5.6 ± 0.6	11.4 ± 0.7
Day 7	*L*	*a*	*b*
Cooked	18.5 ± 1.2	14.4 ± 0.9	11.3 ± 0.5

To conclude, adding glucose had no important effects on fluid loss and pH, and in most following experiments glucose was not added, and where it is added, the reasons for addition are discussed. Although cabbage cooking resulted in minor increases in fluid loss, whether to include it or not remained an open question because raw cabbage would always introduce a microflora, but at the same time cooking cabbage on a commercial scale adds cost.

To this point, all incubations were performed at ambient temperature and only in the present experiment has culture been added. Lactic acid fermentations are usually done at typically 30°C, so in the next chapter this temperature was routinely adopted and culture was always added.

Chapter 4: Effects of temperature and storage time on product attributes

The effect of cabbage cooking and different incubation temperatures

Introduction

At ambient temperature, starter culture had no effect on colour change, pH and fluid loss. The aim of this experiment is to explore the effect of cabbage cooking at different incubation temperatures.

Methods

The ingredient and their amounts of treatment are shown in Table 23. Every treatment had four replicates. For each replicate, 20 g of meat was separately cooked for 45 sec and mixed 100 g of cabbage cooking for 45 sec and 0 sec respectively with 2 g of salt and 0.04 g starter culture (BFL-F02) in a vacuum bag. After evacuation, two treatments were left at ambient temperature and two at 30°C for 96 hours. Subsequent storage was at ambient temperature. Colour was measured on Days 0, 1, 2, 4, 5 and 7. pH was measured only at Day 7.

Table 23 Treatment of effect of temperature and cabbage cooking

Temperature (°C)	Weight of cabbage (g)	Cabbage cooking	Weight of meat (g)	Meat cooking	Salt (g)	Starter culture (g)
30	100	Raw	20	Cooked	2	0.04
23	100	Raw	20	Cooked	2	0.04
30	100	Cooked	20	Cooked	2	0.04
23	100	Cooked	20	Cooked	2	0.04

Result and discussion

Table 24 shows that different temperature and cabbage cooking had little effect on fluid loss and pH under different temperature with starter culture added at Day 7. The low pH value seems that a lactic fermentation occurred because of the starter culture.

Table 24 Treatment of effect of temperature and cabbage cooking

Temperature (℃)	Cabbage cooking	Fluid loss ± SD (%)	pH ± SD
23	Raw	33.0 ± 1.8	3.50 ± 0.02
30	Raw	31.2 ± 1.4	3.47 ± 0.03
23	Cooked	32.0 ± 5.6	3.57 ± 0.04
30	Cooked	30.6 ± 2.4	3.55 ± 0.05

The analysis of variance for fluid and pH was shown in Table 25. It showed the incubation and cabbage cooking had little effect on fluid loss and pH value. A significant difference with cabbage cooking on pH were shown, but inspection shows these effects could not be important because their means were so close.

Table 25 Analysis of four treatments, with four replicates each.

	Fluid loss (%)	P value	pH	P value
Temperature (°C)				
23	32.5	NS	3.54	NS
30	30.9		3.51	
Cabbage				
Raw	32.1	NS	3.49	**
Cooked	31.3		3.56	
Interaction				
23 * Raw	33.0	NS	3.50	NS
23 * Cooked	32.0		3.57	
30 * Raw	31.2		3.47	
30 * Cooked	30.6		3.55	

The colour equivalent to Table 24 for cabbage is shown in Appendix 10 and is unremarkable, except that *L* value was increased by cooking, and the results are abstracted in Table 26.

Table 26 Colour means and their analysis of variance in cabbage

Cabbage	*L*		*a*		*B*	
Raw	15.4	***	2.0	***	26.3	NS
Cooked	26.7		-0.3		25.2	

However, these differences may be difficult to visualise. Assessment of cabbage colour was complicated by the unavoidable colour variation between different parts of the cabbage plant. Some parts are close to white and other parts are varying shades of green.

Appendix 11 for cabbage colour and Appendix 12 for meat colour show the analyses of variance, but are not discussed.

Of continuing interest was the meat colour change, shown for Days 0 and 7 (Table 27). As shown previously the colour changed from brown to pink. The redness was increased in all replicates.

Table 27	Cooked meat colour on Day 0 and Day 7		
Day 0	*L*	*a*	*b*
	21.3 ± 2.7	5.8 ± 0.5	12.4 ± 0.8
Day 7	*L*	*a*	*b*
	21.9 ± 1.4	16.8 ± 0.7	12.4 ± 0.5

To conclude, the question as to whether cabbage should be cooked or not and replicates should be incubated or not was not resolved for colour changes, pH and fluid loss although cooking cabbage resulted in a subtle effect on L value in cabbage.

In the next experiment, the aim was to investigate the effect of temperature and time, two storage temperature would be used (4 and 23°C) and pH value should be measured after incubation (Day 4, 5, 6 and 7).

The effect of post incubation storage at two temperatures, 4°C and 23°C for 7 days on pH

Introduction

The pH on Day 7 was cleared but the pH after incubation was unknown. The aim of this experiment is to investigate the change of pH value after 96 hours incubation.

Methods

Table 28 shows the experimental design where each treatment was represented by eight replicates. For each replicate, 20 g of meat was separately cooked for 45 sec and mixed 100 g of raw cabbage with 2 g of salt and 0.04 g starter culture (BFL-F02) in a vacuum bag. After evacuation, treatments were left into an oven which kept 30°C in 96 hours. On Day 4 when replicates were taken out, measuring the colour of all samples and opening two replicates from each treatment to measure the pH and fluid loss. The other replicates were put into the ambient temperature and refrigerator respectively. Repeat the same step on Day 5, 6 and 7.

Table 28 Treatment of low temperature and ambient temperature

Temperature (°C)	Weight of cabbage (g)	Cabbage	Weight of meat (g)	Meat	Salt (g)	Starter culture (g)
4	100	Raw	20	Cooked	2	0.04
23	100	Raw	20	Cooked	2	0.04

Result and discussion

Table 29 showed that different storage temperature and time had no effect on pH value with starter culture added. The storage time had little effect on pH at 4°C. At 23°C, with the increasing of storage time, the pH was decreased from 3.8 to 3.7 and the statistical significance of pH was * ($P < 0.05$).

Table 29 pH change from Day 4 to Day 7		
Day	pH ± SD (4°C)	pH ± SD (23°C)
4	3.79 ± 0.02	3.83 ± 0.04
5	3.85 ± 0.08	3.86 ± 0.01
6	3.75 ± 0.01	3.68 ± 0.06
7	3.85 ± 0.04	3.70 ± 0.04
Effect of storage day	NS	*

The colour equivalent to Table 29 for cabbage and meat colour is shown Appendix 13 and is not discussed because the more important thing is pH measurement. Appendix 14 and Appendix 15 show the analyses of variance, but are not discussed further.

The meat colour change, shown for Days 0 and 7 (Table 30). As shown previously the colour changed from brown to pink. The redness was increased in all replicates. (These changes were also tracked on Days 4, 5 and 6 [data not shown].) From Day 0 to Day 7, the redness of cooked meat increased from 6.5 to 15.4 and 6.1 to 15.5 respectively (***) but *L* and *b* were statistically unchanged under 4°C and 23°C.

Table 30 Cooked meat colour on Day 0 and Day 7. The only significant difference was for a values (***) between Day 0 and Day 7.			
Day 0	*L*	*a*	*b*
4°C	20.1 ± 0.8	6.5 ± 0.4	11.4 ± 0.5
23°C	18.1± 2.2	6.1 ± 0.7	11.4 ± 0.5
Day 7	*L*	*a*	*b*
4°C	18.9 ± 1.2	15.4 ± 0.2	10.6 ± 0.7
23°C	19.8 ± 0.1	15.5 ± 0.8	11.2 ± 0.3

To conclude, the pH value was unchanged and the storage time had little effect on the change of pH at 4°C after fermentation. However, the pH of replicates stored at ambient temperature (23°C) was decreased with increasing storage time but not obviously.

In the next experiment, the step was to investigate the effect of storage time on pH value and colour change. It is simply a repeat of the present, but with the storage time increased to 2 months.

The effect of post incubation storage at two temperatures, 4°C and 23°C for 56 days

Introduction

The previous experiment showed that the pH of treatments decreased with increasing the storage time to seven days but the effect was minor. Therefore, the aim of this experiment was to investigate the effect of post incubation storage to two months at two temperatures, 4°C and 23°C, both established immediately after the 96 h incubation.

Methods

Table 31 shows the experimental design. (The six replicates at Day 56 included reserve replicates remaining at the end.) For each replicate, 20 g of meat was separately cooked for 45 sec and mixed 100 g of raw cabbage with 2 g of salt and 0.04 g starter culture (BFL-F02) in a vacuum bag. After evacuation, treatments were incubated at 30°C for 96 hours. Colour was measured on every replicate before opening for pH measurement. Fluid loss was measured only on Day 56.

Table 31 Number of replicates for the storage temperature experiment

Day	4°C	23°C
0	2 (23°C)	
4	2 (30°C)	
5	2	2
7	2	2
14	2	2
28	2	2
56	6	6

Result and discussion

Table 32 shows that the pH values at each of the two temperatures did not vary much with time, except that there was an apparent final increase at Day 56 for 4°C storage. The reason for this is not known, but it was supported by six replicates. At all times between Days 5 and 56 the pH of the ambient storage treatment was lower, suggesting ongoing fermentation that is not surprising.

Table 32 pH changes and statistics from Day 0 to Day 56

Day	pH ± SD (4°C)	pH ± SD (23°C)	
0	5.44 ± 0.06		
4	3.66 ± 0.06		Effect of storage temperature
5	3.66 ± 0.03	3.56 ± 0.01	*
7	3.76 ± 0.01	3.56 ± 0.07	NS
14	3.70 ± 0.02	3.58 ± 0.04	NS
28	3.66 ± 0.01	3.54 ± 0.00	**
56	3.86 ± 0.02	3.56 ± 0.06	***
Effect of storage time between Days 5 and 56	***	NS	

Table 33 shows that the fluid loss was affected by the storage temperature. The ambient storage treatment lost more fluid than the treatment at 4°C, but the difference might not be obvious in practical application. The relative contributions of pH and temperature to the difference are not known.

Table 33 Fluid loss and analysis of variance at Day 56 at two storage temperatures.

Fluid loss ± SD (%) (4°C)	Fluid loss ± SD (%) (23°C)
24.5 ± 0.7	26.7 ± 0.6
Effect of temperature	***

The colour equivalent to Table 32 cabbage is shown in Appendix 16 and is unremarkable, except that *L* value was higher at 4°C, and these results are abstracted in Table 34. However, these differences may be difficult to visualise.

Table 34 Cabbage colour means and their analysis of variance at Day 56.

Temperature (°C)	*L*		*a*		*b*	
4	17.5	***	-1.6	NS	13.6	NS
23	13.0		-1.0		14.6	

Appendix 17 for cabbage colour and Appendix 18 for meat colour show the analyses of variance, but are not discussed.

However, of more interest was the meat colour change, shown for Days 0, 7 and 56 (Table 35).

Table 35 Cooked meat colour on Days 0, 7 and 56.			
Day 0	*L*	*a*	*b*
4°C	20.4 ± 2.5	6.4 ± 0.5	11.4 ± 0.7
23°C	20.9 ± 2.3	6.5 ± 0.7	12.0 ± 0.5
Day 7			
4°C	22.4 ± 1.1	17.7 ± 0.5	12.1 ± 0.2
23°C	20.8 ± 1.9	18.6 ± 0.8	12.6 ± 0.6
Day 56			
4°C	20.7 ± 1.4	11.5 ± 0.5	11.6 ± 0.6
23°C	19.9 ± 1.9	11.0 ± 4.9	12.8 ± 0.5
Effect of time	NS	***	NS
Effect of temperature		NS	

As shown previously the colour changed from brown to pink from Day 0 to Day 7 and the redness was increased in all replicates. But by Day 56, an important change occurred: the meat colour changed from pink to pale brown with some residual pink. At 4°C, all replicates maintained residual pinkness. There was unchanged bright pink colour at ambient temperature but only in two of the six replicates. The other four turned pale brown (Figure 14). The final means at Day 56 were very close (11.0 ± 4.9 and 11.5 ± 0.5) but the standard deviation at ambient temperature treatment was large. The change from pinkness to brown is due to the loss of the pink nitrosohaemochrome characteristic of nitrite cured meat when the haem iron is oxidised from the ferrous to the ferric form of metmyoglobin because of exposure to oxygen (Russell & Gould, 2003).

As to why some replicates showed variable reversion to browning, it is possible that air exclusion at evaluation might have varied, and if some bags were in close contact with others, oxygen transmission might have been slowed. Unquestionably however, the reversion to metmyoglobin characteristic of cooked meat would be due to oxygen transmission.

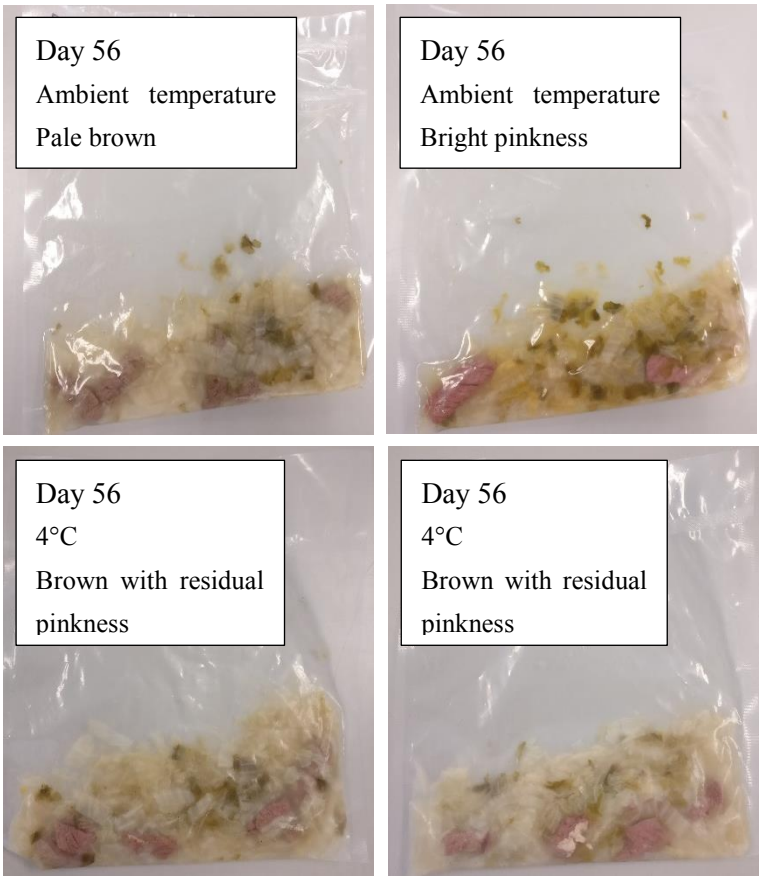

Figure 14 Comparison of colour between two storage temperatures. At ambient temperature, four replicates were pale brown and two were bright pink. At 4°C, all replicates were brown with some residual pinkness.

To conclude, the replicates stored at ambient temperature had a lower pH than at 4°C. The ambient temperature treatment lost slightly more fluid than at 4°C. And meat colour reverted to the pale brown of metmyoglobin by Day 56.

This and prior experiments strongly suggested that nitrate/nitrite from cabbage was responsible for NO generation resulting in meat curing under vacuum. In the next chapter this phenomenon is explored in more detail.

Chapter 5: Effect of different amount of cabbage and nitrite

Importance of cabbage on meat colour changes

Introduction

The working hypothesis at this point was that nitrate from cabbage was being reduced to nitrite that under reducing conditions cured the meat. The obvious experiment was to see if cabbage was essential for the colour change.

Methods

Table 36 shows the experimental design where each treatment was represented by four replicates. For each replicate, 20 g of meat was separately cooked for 45 sec and mixed different amounts of cabbage (0, 20 and 40 g) with different amount of salt (0.33, 0.67 and 1 g) and 0.04 g starter culture (BFL-F02) in a vacuum bag. The different quantities of salt were required to keep the final salt concentrations approximately the same. Of the two treatments with no cabbage, one had added 2 g glucose (2 g). After evacuation, incubation was 30°C for 96 hours. Meat colour was measured on Days 0, 1, 2, 4, 5 and 7. pH was measured only at Day 7. Cabbage colour was not measured.

Table 36 The effect of cabbage quantity of meat colour.

Weight of cabbage (g)	Cabbage	Weight of meat (g)	Meat	Salt (g)	Glucose (g)	Starter culture (g)
40	Raw	20	Cooked	1.00	0	0.04
20	Raw	20	Cooked	0.67	0	0.04
0	Raw	20	Cooked	0.33	2	0.04
0	Raw	20	Cooked	0.33	0	0.04

Result and discussion

Table 37 shows that the amount of cabbage had a large effect on the pH value. For the treatment without added cabbage, glucose added had a significant effect on reducing pH but was still higher than for the replicates with cabbage. This was surprising because 20 g of raw cabbage would never yield 2 g of glucose. However, the cabbage may be contributing some unknown cofactors to the fermentation.

Table 37 pH of replicates with different amount of cabbage on Day 7.

Cabbage (g)	Meat (g)	Glucose (g)	pH ± SD
40	20	0	4.01 ± 0.03
20	20	0	4.29 ± 0.06
0	20	2	4.67 ± 0.05
0	20	0	5.26 ± 0.10
Effect of different amount of cabbage and glucose			***

The meat colour changed, shown for Days 0 and 7 (Table 38). As shown previously the colour changed from brown to pink from Day 0 to Day 7 and the redness was increased in replicates mixed with 40 and 20 g cabbage. Their *a** values were close. But the colour of the two treatments without cabbage were largely unchanged at Day 7 whether glucose was added or not.

Table 38 Cooked meat colour on Days 0 and 7. The only significant difference was for a* values (***) between Days 0 and 7, due only to the plus cabbage treatments.

Amount of cabbage (g)	Glucose (g)	*L*	*a*	*b*
Day 0				
40	0	16.5 ± 2.5	6.2 ± 0.7	11.0 ± 0.5
20	0	17.5 ± 0.6	6.3 ± 0.3	11.4 ± 0.3
0	2	12.9 ± 1.5	6.9 ± 0.5	10.8 ± 0.5
0	0	15.9 ± 1.0	6.7 ± 0.2	11.2 ± 0.5
Day 7				
40	0	18.3 ± 0.6	16.5 ± 0.5	11.3 ± 0.5
20	0	19.0 ± 2.1	15.9 ± 0.7	10.9 ± 0.3
0	2	14.2 ± 4.5	6.7 ± 0.3	9.2 ± 0.4
0	0	17.6 ± 1.9	7.2 ± 0.6	11.3 ± 1.5
Effect of different amount of cabbage on colour value		NS	***	NS

One treatment – 0 g cabbage and 2 g added glucose – produced gas in the vacuum bag. This was interpreted as a contaminating alcoholic fermentation and was not considered important (Figure 15). More importantly, this figure shows that the brown meat at Day 0 was still brown at Day 7.

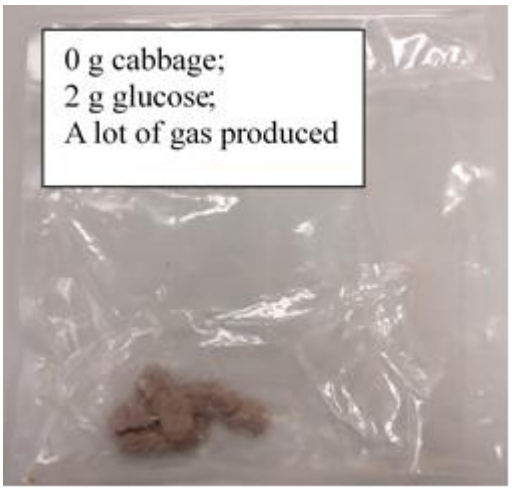

Figure 15 The replicate added no cabbage and 2 g glucose. A lot of gas produced in a vacuum bag. The meat was brown.

The important outcome of this experiment was that cabbage was essential for the meat colour change. This effect was researched more fully in the next two experiments.

The effect of different amount of cabbage on pH and meat colour

Introduction

The aim of this experiment was to explore the effect of different amount of cabbage from 0 to 100 g on pH and meat colour.

Methods

Table 39 shows the experimental design where each treatment was represented by four replicates. For each replicate, 20 g of meat was separately cooked for 45 sec, and then mixed with different amounts of raw cabbage (0, 20, 40, 60, 80 and 100 g) with matching amounts of salt (0.33, 0.67, 1, 1.33, 1.67 and 2g) plus 0.04 g starter culture (BFL-F02) in a vacuum bag. The amount of starter culture (0.04 g) was a constant. After evacuation, treatments were incubated at 30°C for 96 hours. Meat colour was measured on Days 0, 1, 2, 4, 5 and 7. pH was measured only at Day 7. Cabbage colour was no measured.

Table 39 Experimental design for different amount of cabbage.

Weight of cabbage (g)	Weight of meat (g)	Salt (g)	Starter culture (g)
100	20	2.00	0.04
80	20	1.67	0.04
60	20	1.33	0.04
40	20	1.00	0.04
20	20	0.67	0.04
0	20	0.33	0.04

Result and discussion

Table 40 shows that different amount of cabbage affected pH value greatly at Day 7 and in this analysis the pairwise comparisons are included, but standard deviations are not shown for clarity. With decreasing amount of cabbage, the pH value increased from 3.40 to 5.11, and the overall statistical significant was *** ($P < 0.01$).

Table 40 pH of replicates with different amount of cabbage on Day 7.

Cabbage (g)	Meat (g)	pH ± SD	Pairwise comparison[1]
100	20	3.40 ± 0.05	a
80	20	3.50 ± 0.05	ab
60	20	3.63 ± 0.06	bc
40	20	3.72 ± 0.02	c
20	20	3.96 ± 0.02	d
0	20	5.11 ± 0.14	e
Effect of amount of cabbage		***	

[1] Tukey multiple range test where different letters mean at least * differences ($P < 0.05$).

Table 41 shows the colour changed from brown to pink from Day 0 to Day 7 and the redness was increased in all replicates even if the amount of cabbage was reduced to as little as 20 g. However, the colour of the treatment without cabbage was unchanged at Day 7. The meat remained brown.

Table 41 Cooked meat colour from different amounts of cabbage to fixed amount of meat.

Amount of cabbage (g)	*L*	*a*	Pairwise comparison[1] for *a*	*b*
Day 0				
100	22.9 ± 1.2	5.8 ± 0.2	a	13.0 ± 0.4
80	23.2 ± 2.0	5.6 ± 0.3	a	12.4 ± 0.2
60	19.7 ± 2.8	6.4 ± 0.4	a	12.6 ± 0.4
40	18.7 ± 2.4	6.0 ± 0.3	a	12.4 ± 0.2
20	18.7 ± 0.8	6.7 ± 0.3	a	12.7 ± 0.3
0	16.9 ± 1.8	5.9 ± 0.3	a	11.9 ± 0.2
Effect of different amount of cabbage on colour value		NS		
Day 7				
100	22.0 ± 1.0	16.9 ± 0.6	bc	12.8 ± 0.4
80	23.4 ± 1.8	16.7 ± 0.5	b	12.3 ± 0.6
60	21.9 ± 1.4	17.6 ± 1.7	bc	11.9 ± 0.3
40	21.7 ± 1.9	17.6 ± 0.5	bc	11.7 ± 0.3
20	21.5 ± 1.1	18.3 ± 0.5	c	11.9 ± 0.4
0	15.3 ± 4.6	6.8 ± 0.6	a	12.8 ± 1.3
Effect of different amount of cabbage on colour value	NS	***		NS
Effect of time	NS	***	***	NS

[1] The Tukey comparison extended over all *a* values, where different letters mean at least * differences ($P < 0.05$).

To conclude, the different amount of cabbage had an important effect on pH value, which increased with decreasing amount of cabbage, suggesting that the carbohydrate source became limiting. But it had no effect on the colour change between 20 and 100 g.

These colour results suggested that nitrate diffusing from remarkably little cabbage, under the reductive environment of an anaerobic fermentation, was sufficient to generate enough nitrite to cure the meat. This raised the question: how much nitrite was being produced in the fermentative environment? Rather than develop a dedicated assay for nitrite in this matrix, it was decided to answer the question by calibration: how much nitrite is needed to match the colour change from cabbage-derived nitrite?

Effectiveness of small amounts of nitrite and of cabbage on meat colour changes

Introduction

The aim of this experiment was to correlate added sodium nitrite with added cabbage effects on meat colour.

Methods

Table 42 (of nitrite) and Table 43 (of cabbage) show the experimental design where each treatment was represented by four replicates.

In 1925, USDA approves the use of sodium nitrite for curing of meat to a maximal level of 200 mg/kg (and now changed to 120 mg/kg) in the finished meat (Cassens, 1990). Thus if the weight of meat were 20 g, the maximum amount of sodium nitrite would be 4 mg. Thus, for each treatment, 20 g of meat was separately cooked for 45 sec and then mixed with 0.33 g of salt, different amounts of sodium nitrite (0, 0.2, 0.5, 1 and 4 mg) and 0.4 g glucose (the fermentable sugar) plus 0.04 g starter culture (BFL-F02).

In treatments mixed with different amount of cabbage (0, 5, 10, 20 and 40 g), 20 g of meat was separately cooked for 45 sec and mixed with raw cabbage with matching amounts of salt (0.33, 0.42, 0.50, 0.67 and 1 g) and glucose (0.4, 0.5, 0.6, 0.8 and 1.2 g) plus 0.04 g starter culture (BFL-F02).

After evacuation, all treatments were incubated at 30°C for 96 hours. Meat colour was measured on Days 0, 1, 2, 4, 5 and 7. pH was measured only at Day 7.

Table 42 Experimental design for sodium nitrite addition.

Weight of meat (g)	Salt (g)	Glucose (g)	Sodium nitrite (mg)	Starter culture (g)
20	0.33	0.4	4.0	0.04
20	0.33	0.4	1.0	0.04
20	0.33	0.4	0.5	0.04
20	0.33	0.4	0.2	0.04
20	0.33	0.4	0.0	0.04

Table 43 Experimental design for cabbage addition.

Weight of cabbage (g)	Weight of meat (g)	Salt (g)	Glucose (g)	Starter culture (g)
40	20	1.00	1.2	0.04
20	20	0.67	0.8	0.04
10	20	0.50	0.6	0.04
5	20	0.42	0.5	0.04
0	20	0.33	0.4	0.04

Result and discussion

Table 44 shows that different amount of sodium nitrite had significant but unimportant effects on pH, whereas Table 45 shows that the pH value increased when the cabbage amount decreased. However, there was enough added glucose to lower the pH to stable levels (pH < about 4.5).

Table 44 pH of treatments with different amount of sodium nitrite on Day 7.

Sodium nitrite (mg)	Meat (g)	pH ± SD	Pairwise comparison
4	20	4.52 ± 0.02	a
1	20	4.53 ± 0.03	ab
0.5	20	4.59 ± 0.03	b
0.2	20	4.57 ± 0.03	ab
0	20	4.56 ± 0.03	ab
Effect of amount of nitrite		*	

Table 45 pH of replicates with a small amount of cabbage on Day 7.

Cabbage (g)	Meat (g)	pH ± SD	Pairwise comparison[1]
40	20	3.69 ± 0.05	a
20	20	3.96 ± 0.03	b
10	20	4.13 ± 0.08	c
5	20	4.26 ± 0.05	d
0	20	4.41 ± 0.03	e
Effect of amount of cabbage (and glucose)		***	

[1] Tukey multiple range test where different letters mean at least * differences (P < 0.05).

Table 46 shows the amount of nitrite had a large effect on meat colour change. Except for the treatments with 0 and 0.2 mg nitrite, the meat colour changed from brown to pink in treatments

with between 1 and 4 mg of sodium nitrite. The treatment with 0.5 mg nitrite changed colour only on a small part of the meat surface. For the treatment with 4 mg of sodium nitrite, the colour changed even before evacuation. This part will be discussed in concluding Chapter 8.

Table 46 Cooked meat colour with different amounts of sodium nitrite.

Amount of sodium nitrite (mg)	*L*	*a*	Pairwise comparison[1] for *a*	*b*
Day 0				
4	14.6 ± 3.5	9.9 ± 1.4	a	9.8 ± 0.5
1	17.6 ± 3.4	8.0 ± 0.4	ab	10.5 ± 0.5
0.5	16.0 ± 2.2	7.5 ± 0.3	ab	10.1 ± 0.5
0.2	16.7 ± 1.8	7.0 ± 0.4	ab	11.1 ± 0.5
0	16.2 ± 1.9	7.0 ± 0.7	ab	10.7 ± 0.5
Day 7				
4	20.7 ± 2.5	18.9 ± 0.7	c	10.8 ± 0.3
1	19.5 ± 0.7	15.0 ± 2.2	d	9.8 ± 0.7
0.5	17.9 ± 2.3	9.8 ± 2.7	a	9.5 ± 0.9
0.2	19.7 ± 2.7	6.5 ± 0.6	b	10.2 ± 0.2
0	19.4 ± 1.7	6.1 ± 0.5	b	10.5 ± 0.8
Effect of amount of nitrite on colour value	NS	***		NS

[1] The Tukey comparison extended over all *a* values.

As shown in Table 47, the amount of cabbage affected meat colour greatly. In the treatments with 10, 20 and 40 g cabbage, colour changed from brown to pink. The treatment with 5 g cabbage changed colour but only a small part. The treatment without cabbage was unchanged.

Table 47 Cooked meat colour with different amounts of cabbage.

Amount of cabbage (g)	*L*	*a*	Pairwise comparison for *a*[1]	*b*
Day 0				
40	18.8 ± 3.8	6.8 ± 0.7	a	12.2 ± 1.2
20	18.9 ± 2.8	7.0 ± 0.5	a	11.8 ± 0.7
10	19.5 ± 1.8	7.3 ± 0.4	a	12.2 ± 0.2
5	20.5 ± 2.2	6.8 ± 0.7	a	11.6 ± 1.1
0	19.3 ± 2.8	7.2 ± 0.4	a	11.9 ± 0.6
Day 7				
40	21.9 ± 1.0	19.2 ± 0.3	b	12.2 ± 0.4
20	21.8 ± 0.8	19.0 ± 0.6	b	11.9 ± 0.6
10	20.6 ± 1.4	18.0 ± 0.6	b	11.6 ± 0.7
5	21.0 ± 1.7	12.3 ± 2.2	c	10.9 ± 0.6
0	20.4 ± 2.2	7.2 ± 0.8	a	11.0 ± 0.4
Effect of amount of cabbage on colour value	NS	***		NS
Effect of time	NS	***	***	NS

[1] The Tukey comparison extended over all *a* values.

In correlating sodium nitrite concentration with nitrite[5] from cabbage it was useful to plot the *a* data from Table 46 and Table 47, where the x axis has two scales, sodium nitrite expressed in milligrams and cabbage in grams (Figure 16). The equivalence point for 10 g of cabbage was approximately 3.3 mg of sodium nitrite.

[5] This is free nitrite whatever the counter cation.

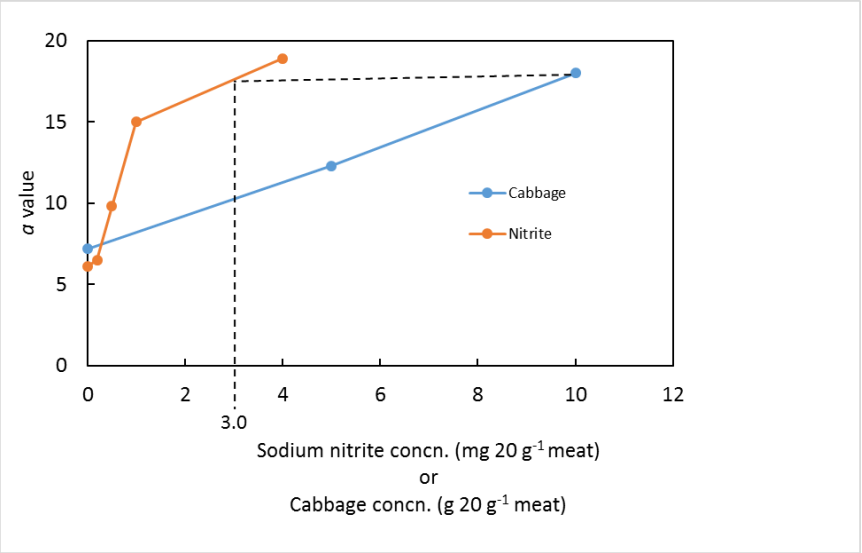

Figure 16 Comparison of a value between sodium nitrite concentration and nitrite from cabbage.

In the previous experiment (page 50) showed that the cooked meat would not change colour from brown to pink without added cabbage, so clearly the nitrite was derived from cabbage. In the regulations set down by Food Standards Australia New Zealand (FSANZ 2017), all nitrite and nitrate salt was calculated as sodium salt, so that will be the basis of calculation.

The concentration of 3 mg of sodium nitrite added to 20 g of meat was equivalent to 150 mg kg-1, which is below the limit set by FSANZ. On the basis of colour, the nitrite from 10 g of cabbage (see Table 47) was equivalent to 3 mg of sodium nitrite added to 20 g of meat. All that sodium nitrite came from only 10 g of cabbage. Therefore, the concentration of Na nitrite derived from cabbage must have been 300 mg kg-1 of cabbage, diluting to a final concentration of 100 mg kg-1. (The mix contained 10 g of cabbage and 20 g of meat, so 10/30 = 1/3). This does not match the 150 mg kg-1 for added sodium nitrite, but it must be realised that the equivalence values are approximate.

This raised the question: is there about 300 mg kg-1 of sodium nitrite in cabbage. There is a literature on this (Zhong et al., 2002). Zhong et al. showed that typical concentrations of nitrate and nitrite in Chinese cabbage were 2120 and 0.183 mg kg-1, respectively, when expressed as the free ions. These values translate to 2910 and 0.275 mg kg-1 when expressed as the sodium salts. Clearly, there is a huge discrepancy between 300 mg kg-1 and 0.275 mg kg-1. However, if nitrate

were to be reduced under the fermentation conditions there would be a sufficiently large pool of nitrate as a substrate. It appears that reduction of nitrate to nitrite is the source cause of the colour change.

What this means in terms of commercial preparations of celery juice used as a nitrite curing substitute in the USA and in terms of human health is discussed in the final Chapter 8. In the meantime, the effect of different cultures on fermentation and a pilot sensory experiment are to be described in the next two chapters.

Chapter 6: Effect of different starter cultures

Introduction

The School of Science at AUT had been donated five starter cultures from Chr. Hansen Pty., Melbourne, BFL-F02, SM-194, F-LC, BFL-F04 and T-SC-150. The first, BLF-F02 has been routinely used in previous chapters. In this chapter, the overall fermentation performance of these five cultures will be investigated.

A variety of cultures from several manufacturers are available, which in most cases include the microorganisms that predominate in the traditional fermented meat products. Thus, a uniform fermentation with the right homo-fermentative lactic acid bacteria is secured, and the flavour development, colour formation and colour stability are improved by adding an additional flora of species from different species (Chr-Hansen, no date). These are claimed to generate particular regional flavours (Table 48), and one or more have been shown to markedly supress the growth of *Listeria*, a food-borne bacterial pathogen, but originating soil, water and some animals, including poultry and cattle. It can be present in raw milk and foods made from raw milk. It can also grow in food processing environments, and can contaminate a variety of processed meats. Unlike many other microbe *Listeria* can grow at refrigeration temperature. *Listeria* is destroyed by pasteurization or cooking.

Table 48 Starter culture used in individual experiment and their properties (Chr-Hansen, no date).

Culture name	Bacteria included	Characteristics
BFL-F02	*Pediococcus pentosaceus*, *Staphylococcus carnosus* ssp.	The high concentration of *Pediococcus pentosaceus* gives a moderate pH-drop. The acidification gives a mild lactic acid taste. The used *Staphylococcus carnosus* ssp. gives a milder flavour.
SM-194	*Pediococcus pentosaceus*, *Lactobacillus sakei*, *Staphylococcus xylosus*, *Staphylococcus carnosus*, *Debaryomyces hansenii*	Multi-application culture that combines all positive features of the different strains. *Lactobacillus sakei* suppress the growth of a lot of indigenous bacteria. *Pediococcus pentosaceus* with its mild lactic acid taste and the accelerated pH-drop at higher temperatures. The combination of two different *Staphylococci* for more intensive colour formation and mild aroma development. And the yeast *Debaryomyces hansenii* on top to obtain a more "Mediterranean" flavour.
F-LC	*Pediococcus acidilactici*, *Lactobacillus curvatus*, *Staphylococcus xylosus*	Culture for acidification and prevention of *Listeria*. Applicable at a wide temperature range. *Pediococcus acidilactici* and *Lactobacillus curvatus* give a moderate pH-drop with a mild acidification flavour. *Staphylococcus xylosus* gives good colour formation and stability and mild flavour. Application in: Fermented sausages
BFL-F04	*Lactobacillus sakei*, *Staphylococcus carnosus* ssp.	The sucrose positive *Lactobacillus sakei* suppress the growth of a lot of indigenous bacteria. The combination of the two new developed *Staphylococci* gives a good colour formation and an intensive, but mild aroma. This special combination of the strains shows a fast pH-drop and leads to a firm texture.
T-SC-150	*Lactobacillus sakei*, *Staphylococcus carnosus*	Gives a product German salami flavour. The acidification leads to a lactic acid taste. The used *Lactobacillus sakei* suppress the growth of a lot of indigenous bacteria. The used *Staphylococcus carnosus* gives good colour stability and a mild aroma.

Due to time constraints, the work reported here does not extend to testing *Listeria* inhibition, but in the Discussion an experimental approach to testing the efficacy of these cultures against *Listeria* in this cabbage/meat matrix are described. The aim of this experiment was to explore the effect of five culture on colour change and final pH value.

Methods

Table 49 shows the experimental design. For each of 4 replicates, 20 g of meat was separately cooked for 45 sec and, in a vacuum bag, mixed 100 g of raw cabbage with 2 g of salt and 0.04 g of starter culture, either BFL-F02, SM-194, F-LC, BFL-F04 or T-SC-150. After evacuation, treatments were incubated at 30°C for 96 hours. Meat colour was measured on Days 0, 1, 2, 4, 5 and 7. pH and fluid loss were measured only at Day 7.

Table 49 Experimental design for five starter cultures.

Weight of cabbage (g)	Weight of meat (g)	Salt (g)	Starter culture	Weight of culture (g)
100	20	2	BFL-F02	0.04
100	20	2	SM-194	0.04
100	20	2	F-LC	0.04
100	20	2	BFL-F04	0.04
100	20	2	T-SC-150	0.04

Result and discussion

Table 50 shows that different starter cultures had barely significant effect on fluid loss and a greater statistical effect pH value (***) at Day 7. However, the pH difference is probably not important because all treatments were highly acidic.

Table 50 Treatment of effect of temperature and cabbage cooking

Starter culture	Fluid loss ± SD (%)	Pairwise comparison for fluid loss	pH ± SD	Pairwise comparison for pH[1]
BFL-F02	32.6 ± 3.6	a	3.49 ± 0.01	ab
SM-194	30.0 ± 4.1	ab	3.52 ± 0.03	bc
F-LC	30.7 ± 1.4	ab	3.57 ± 0.02	cd
BFL-F04	28.1 ± 1.2	ab	3.45 ± 0.03	a
T-SC-150	26.1 ± 3.2	b	3.58 ± 0.03	d
Effect of starter culture	P = 0.06		***	

[1] Tukey multiple range test where different letters mean at least * differences (P < 0.05).

Table 51 shows the colour changed from brown to pink from Day 0 to Day 7 and the redness was increased in all replicates. But there was no difference between culture treatments. In spite of the similarities, it was not known how different cultures would perform in terms of meat colour when different amounts of cabbage were used. Recall that for BFL-F02 (page 53) 20 g of cabbage per 20 g of meat was just enough to generate the colour change. It was not possible to test this with all cultures, so one was selected, T-SC-150, which was the culture that generated the lowest fluid loss (Table 50).

Table 51 Cooked meat colour. The only significant difference was for a values (***) between Days 0 and 7. Culture had no effect.

Starter culture	*L*	*a*	Pairwise comparison for *a*[1]	*b*
Day 0				
BFL-F02	22.1 ± 1.0	7.0 ± 0.5	a	12.9 ± 0.3
SM-194	21.3 ± 1.0	6.9 ± 0.2	a	12.7 ± 0.4
F-LC	19.4 ± 3.4	6.4 ± 0.6	a	12.9 ± 0.6
BFL-F04	20.3 ± 2.2	6.1 ± 0.8	a	12.5 ± 0.7
T-SC-150	19.0 ± 1.5	6.6 ± 0.9	a	13.0 ± 0.4
Day 7				
BFL-F02	21.6 ± 2.4	18.3 ± 0.2	b	12.9 ± 0.5
SM-194	22.3 ± 1.8	19.0 ± 0.8	b	12.9 ± 0.7
F-LC	21.2 ± 0.7	19.2 ± 1.5	b	12.6 ± 0.4
BFL-F04	23.0 ± 1.4	19.0 ± 0.6	b	12.8 ± 0.7
T-SC-150	21.3 ± 1.9	18.8 ± 1.7	b	12.5 ± 0.9
Effect of time on colour value.	NS	***	***	NS
Effect of different culture	NS	NS		NS

[1] The Tukey comparison extended over all *a* values.

Effect of different amounts of cabbage on the colour outcomes with culture T-SC-150

In the previous experiment, there was a significant difference between the fluid loss due to the standard culture used previously, BFL-F02, and T-SC-150. T-SC-150 generated a lower fluid loss and this could be commercially important. It was therefore proposed to test how T-SC-150 would perform when different amounts of cabbage were added. This parallels the experiment reported on page 53 where BFL-F02 was tested.

Methods

Table 52 shows the experimental design where each treatment was represented by four replicates. For each replicate, 20 g of meat was separately cooked for 45 sec and mixed different amounts of cabbage (20, 40, 60, 80 and 100g) and, proportionately, different amounts of salt (0.67, 1, 1.33, 1.67 and 2g), plus 0.04 g starter culture (T-SC-150) all in vacuum bags. The amount of starter culture (0.04 g) was a constant. After evacuation, treatments were incubated at 30°C for 96 hours. Meat colour was measures on Days 0, 1, 3, 4, 5 and 7. pH was measured only at Day 7.

Table 52 Experimental design for T-SC-150 with different amount of cabbage

Weight of cabbage (g)	Weight of meat (g)	Salt (g)	Starter culture (g)
100	20	2.00	0.04
80	20	1.67	0.04
60	20	1.33	0.04
40	20	1.00	0.04
20	20	0.67	0.04

Result and discussion

Table 53 shows that different amounts of cabbage affected pH greatly at Day 7. With decreasing cabbage, the pH value increased from 3.37 to 3.86 and was statistically significant ($P < 0.001$). Although statistically significant, the pH outcomes for culture T-SC-150 were little different from BFL-F02 outcomes. It is highly unlikely that pH 3.86 and 3.96 (20 g of cabbage) would cause a recognisable flavour change.

Table 53 pH value with different cultures (BFL-F02 and T-SC-150) and different amounts of cabbage at Day 7.

	T-SC-150		BFL-F02	
Cabbage (g)	pH ± SD	Pairwise comparisons[1]	pH ± SD	Pairwise comparisons[1]
100	3.37 ± 0.02	a	3.40 ± 0.05	a
80	3.41 ± 0.03	a	3.50 ± 0.05	b
60	3.50 ± 0.05	b	3.63 ± 0.06	c
40	3.61 ± 0.01	c	3.72 ± 0.02	d
20	3.86 ± 0.03	e	3.96 ± 0.02	f
Effect of amount of cabbage	***		***	

[1] Tukey multiple range test where different letters within or between columns mean at least * differences ($P < 0.05$).

Table 54 shows the colour changed from brown to pink from Day 0 to Day 7 and the redness was increased in all replicates even if the amount of cabbage were reduced. Although the statistical significant of *b* value was large, it was meaningless because their means were very close.

Table 54 Cooked meat colour arising from T-SC-150. The only significant difference was for a values (***) between Days 0 and 7.

Amount of cabbage (g)	*L*	*a*	Pairwise comparison for *a*[1]	*b*
Day 0				
100	17.3 ± 1.4	6.2 ± 0.4	a	12.0 ± 0.7
80	16.3 ± 1.2	7.6 ± 1.7	a	11.8 ± 1.3
60	16.4 ± 3.3	6.2 ± 1.2	a	10.5 ± 1.5
40	15.7 ± 0.8	6.9 ± 0.5	a	11.4 ± 1.0
20	15.0 ± 1.6	7.0 ± 0.7	a	10.5 ± 0.7
Day 7				
100	18.2 ± 1.9	16.6 ± 1.0	b	12.2 ± 0.2
80	19.3 ± 0.9	17.4 ± 1.0	b	12.3 ± 0.5
60	20.5 ± 1.7	17.1 ± 0.5	b	11.5 ± 0.5
40	19.0 ± 1.6	17.8 ± 0.9	b	11.6 ± 0.2
20	19.8 ± 0.6	16.5 ± 0.5	b	10.8 ± 0.4
Effect of time	NS	***	***	***
Effect of different amount of cabbage on colour value	NS	NS		NS

[1] The Tukey comparison extended over all *a* values.

Table 55 shows that although all meat changed colour to pink, the *a* value of meat colour was unaffected by culture.

Table 55 Cooked meat colour with different culture (BFL-F02 and T-SC-150) at Day 7.

Amount of cabbage (g)	*L*	*a*	Pairwise comparison for *a*[1]	*b*
BFL-F02				
100	22.0 ± 1.0	16.9 ± 0.6	a	12.8 ± 0.4
80	23.4 ± 1.8	16.7 ± 0.5	a	12.3 ± 0.6
60	21.9 ± 1.4	17.6 ± 1.7	a	11.9 ± 0.3
40	21.7 ± 1.9	17.6 ± 0.5	a	11.7 ± 0.3
20	21.5 ± 1.1	18.3 ± 0.5	a	11.9 ± 0.4
T-SC-150				
100	18.2 ± 1.9	16.6 ± 1.0	a	12.2 ± 0.2
80	19.3 ± 0.9	17.4 ± 1.0	a	12.3 ± 0.5
60	20.5 ± 1.7	17.1 ± 0.5	a	11.5 ± 0.5
40	19.0 ± 1.6	17.8 ± 0.9	a	11.6 ± 0.2
20	19.8 ± 0.6	16.5 ± 0.5	a	10.8 ± 0.4
Effect of different amount of cabbage on colour value	NS	NS	NS	NS

[1] The Tukey comparison extended over all *a* values.

In conclude, the result of this experiment with T-SC-150 were very similar to those of the previous experiment where culture BFL-F02 was used. Different cultures had no significant effect on colour change and pH value with different amount of cabbage.

In next chapter, a sensory experiment with BFL-F02 will be described.

Chapter 7: Informal sensory test

Introduction

One primary aim of this research was to explore the meat curing phenomenon caused by the combination of nitrate stemming from cabbage in a reductive (anaerobic) environment. Another aim was product development of behalf of Merit Meats. In the event of a one-year Masters project there was no time available for formal sensory testing. However, a very limited sensory trial was undertaken in the manner of a focus group with open discussion. For this work, sucrose was added to the mix to counter the obvious sourness for pH value around 3.5. The question asked of the six participants was: what is a suitable addition rate for sucrose?

Methods

Table 56 shows the experimental design. For each replicate, 20 g of meat was separately cooked for 45 sec and mixed 100 g of raw cabbage with 2 g of salt and different amount of sucrose (0, 1.2, 2.4, 3.6 and 6 g) in a vacuum bag. After evacuation, treatments were incubated at 30°C for 96 hours and stored chilled for three days.

A small focus group comprised five people, two young ethnic Chinese, one young ethnic, and two older pakeha New Zealanders. Samples of the five treatments were marked A to E where A[6] contained no sucrose (0 g), B (1.2 g), C (2.4 g), D (3.6 g) and E (6 g) (Figure 17). The participants tasted the samples in no particular order using chopsticks to sample. The participants were asked to identify their preferred treatment, and the results were discussed in open forum.

Table 56 Sensory test on different amount of sucrose in fermented meat and cabbage treatments

Weight of cabbage (g)	Weight of meat (g)	Salt (g)	Sucrose (g)	Weight of culture (g)
100	20	2	0.0	0.04
100	20	2	1.2	0.04
100	20	2	2.4	0.04
100	20	2	3.6	0.04
100	20	2	6.0	0.04

[6] In hindsight the letters A to E should not have followed the increasing sweetness sequence.

Result and discussion

Sample E (6 g sucrose added) was the sweetest among five treatments while A (0 g) was only salty and sour. All participants found that the salt and sour taste was associated with cabbage, whereas the meat was tasteless in all treatments. With increasing of the amount of sucrose, the sourness decreased.

The two Chinese participants chose E (6 g) as their favourite. This was not surprising because salty sweet flavours are culturally acceptable for cold dishes in China (not so for hot except in Shanghainese cuisine). The other participants thought the A (0 g) and D (3.6 g) samples were best. But they indicated that there was no large difference between B (1.2 g), C (2.4 g) and D (3.6 g) and these samples gave them similar taste. Comparing A (0 g), D (3.6 g) and E (6 g), the difference was obvious. For all treatments, the participants opined the amount of meat should be increased.

Figure 17 The sensory test

Chapter 8: Concluding discussion

The reasons for colour change

In this work, the colour of the Chinese cabbage changes from green to olive brown because the chlorophyll in green leaves is unstable. When leafy vegetables are heated or are in an acidic condition, the chlorophyll loses the magnesium ion (Mg^{2+}) at the centre of its porphyrin ring to become pheophytin, which presents as an olive brown colour. The magnesium ion is replaced with the hydrogen ion ($2H^{+}$) (Takamiya et al., 2000). During the execution of the experiments, the mixture is no heated beyond 30°C, but the pH falls to approximately 3.6. So, the magnesium ion is lost by low pH condition as shown in Figure 18.

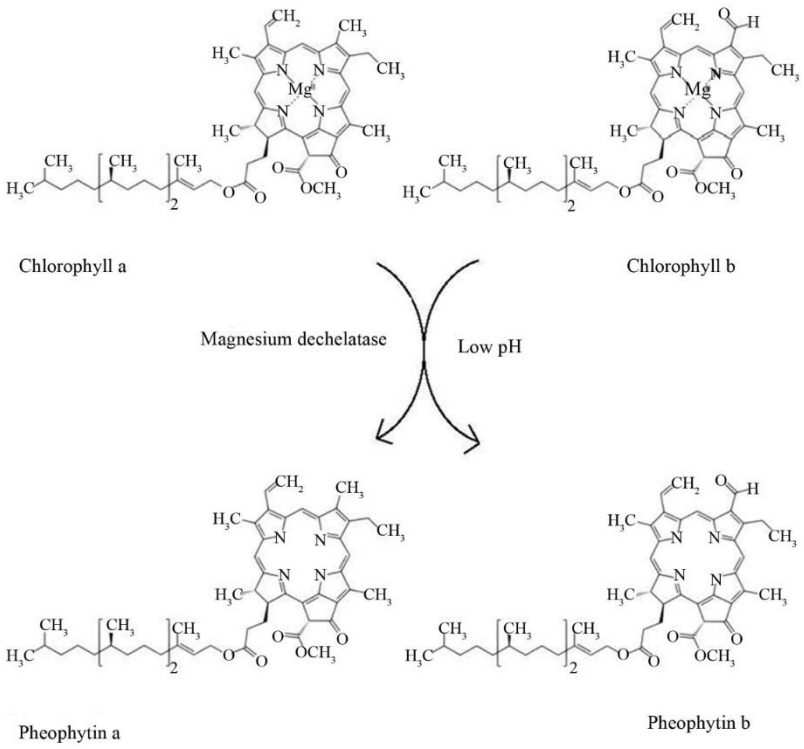

Figure 18 Structures of chlorophylls a and b and their pheophytins (Hsu et al., 2013).

The cooked meat colour changes from brown to light pink. When red is protected from air, say in a vacuum bag, it is purple-red due to myoglobin. On exposure to air (or pure oxygen) over 10s of minutes and hours, the myoglobin bids molecular oxygen to form oxymyoglobin which presents as a bright-red colour. At this point myoglobin is not oxidised because the iron ion at the centre of its

porphyrin ring remains in the 2+ oxidation state. However, with time an electron is lost from the iron ion, converting oxymyoglobin to metmyoglobin (3+ state) which presents as an unattractive brown colour. This process is accelerated by cooking, and furthermore leads to denaturation of the myoglobin protein. This is particularly evident in microwave cooking, where denatured metmyoglobin is an unattractive grey-brown.

During the fermentation with cabbage, a large amount of nitrate (5+ oxidation state) is released from the cabbage and is reduced nitrite (3+) by nitrate-reducing starter culture. The nitrite is further reduced into the nitric oxide (NO) (2+), and reduces the Fe^{3+} of metmyoglobin to Fe^{2+} in the porphyrin ring. The final product is the light nitrosohaemochrome (light-pink) under the anaerobic condition of lactic acid fermentation (Cassens, 1990). (Added culture is not essential for this process because nitrate-reducing bacteria intrinsic to raw meat, processing equipment and other salami ingredients are present.)

The detail of the reaction of Methods is shown in Figure 19.

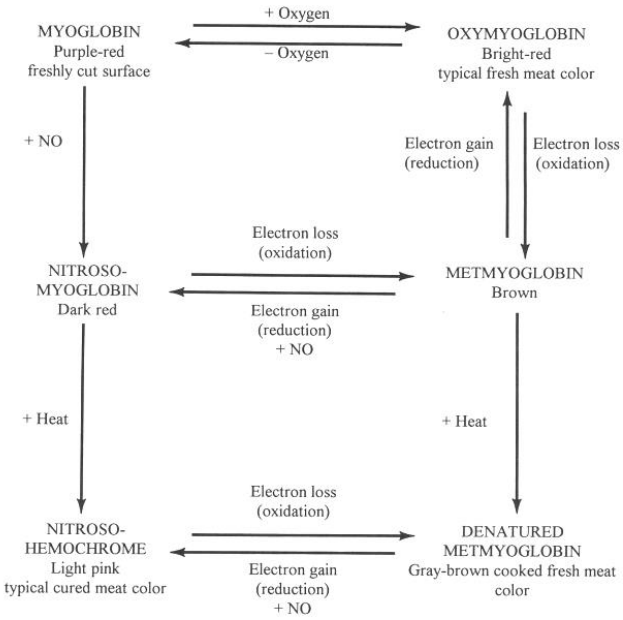

Figure 19 A schematic showing the changes that occur in red meat from exposure to oxygen and nitric oxide. (Cassens, 1990).

The effect of state of matter of cabbage (solid or liquid)

The World Health Organisation has recently declared that processed meats are a health hazard, increasing the risk of cancer. This matter is discussed in the next section, but whatever the truth of this, some consumers are likely to become concerned about eating cured meats because of the added nitrite. Producers have to responded with an array of 'natural' processed meats options sporting the catchy phrases such as 'no synthetic preservatives' or 'no nitrites added'. Celery has a very high concentration of natural nitrate, and treating celery juice with an anaerobic bacterial culture produces nitrite. Celery juice there being used commercially in the USA at least, where its addition leads to the claim of "no nitrite added" in processed meat (Schwarcz, 2017). (Bizarrely, the USDA does not allow a cured meat claim for such products (Sebranek & Bacus, 2007).)

In the previous experiment, to imitate the celery juice used as a nitrite curing substitute in the USA, the 100 g of cabbage was made as pulp by blender to instead of sliced cabbage. But the liquid cabbage could not be packed by the vacuum packer in a vacuum bag. Only one sample was near success but still lost some liquid. However, after evacuation in the incubation oven, the cabbage pulp became yellow and meat became light pink colour too. It proved that the meat colour would be changed even if the cabbage presented in a more homogenised form.

The daily intake of nitrite

In 1978, USDA lowered the amount of sodium nitrite which can be used in food from 200 to 120 ppm (or as potassium nitrite, 148 ppm). According to Bryan and Ivy (2015), vegetables contribute more than 85% of the daily dietary intake of nitrate, and endogenous synthesis (Gangolli et al., 1994) is an important contributor to human's overall exposure of nitrate. Hord et al. (2009) estimated that approximately 80% of dietary nitrate is derived from vegetable consumption. Less than 5% of the ingested nitrate and nitrite comes from cured meat, with the majority being from vegetables and saliva. The results of the present study indirectly confirm these conclusions. The oral bacteria which found on the surface of the tongue partially convert nitrate to nitrite at around 5% of the total nitrate (Bryan & Ivy, 2015). Assuming an individual ate 300 g of leafy vegetable a day, this would release about 600 mg of Na nitrate (Zhong et al., 2002), generating about 30 mg of Na nitrite per day from oral bacteria alone because humans, unlike prokaryotes, lack the enzymatic

machinery to convert nitrate to nitrite and the oral bacteria is the only known way to reduce the nitrate to nitrite (Bryan & Ivy, 2015). (There may be other undescribed reductive sites in the digestive system.) In contrast, the quantity of nitrite from a daily consumption of 200 g of cured meat (at the FSANZ nitrite limit) might be 5 mg of Na nitrite.

Vegetables are clearly a major source of nitrate/nitrite and raised the question as to why cured meats have been singled out as 'dangerous'? A major 2015 review by the International Agency for Research on Cancer (IARC) concluded processed meat could be classified as 'carcinogenic to humans' given the evidence that it causes colorectal cancer. However, IARC is a research organization that evaluates the evidence available on the causes of cancer but does not make health recommendations as such. National governments and WHO are responsible for developing nutritional guidelines. This evaluation by IARC reinforces a 2002 recommendation from WHO that person who eats meat should moderate the consumption of processed meat to reduce the risk of colorectal cancer. Some other dietary guidelines also recommend limiting consumption of red meat or processed meat, but these are focused mainly on reducing the intake of fat and sodium, which are risk factors for cardiovascular disease and obesity. Individuals who are concerned about cancer could consider reducing their consumption of red meat or processed meat until updated guidelines related specifically to cancer have been developed (WHO, 2015).

The results reported here, the routine use of celery juice as a curing agent in the USA, and the Bryan and Ivy (2015) reported in this section casts severe doubt on the logic behind IARC reasoning. However, in its defence it must be realised that cured meats are often smoked and there is good evidence that excessive consumption of smoked meats. The benzpyren, also known as benzo[a]pyrene, is a negative by-product derived from the manufacturing process of smoked meats which is the result of incomplete combustion at temperatures between 300 °C and 600 °C. It is listed as a Group 1 carcinogen by the IARC. (http://monographs.iarc.fr/ENG/Classification/latest_classif.php)

Its diol epoxide metabolites react and bind to DNA, resulting in mutations and eventually cancer (Figure 20).

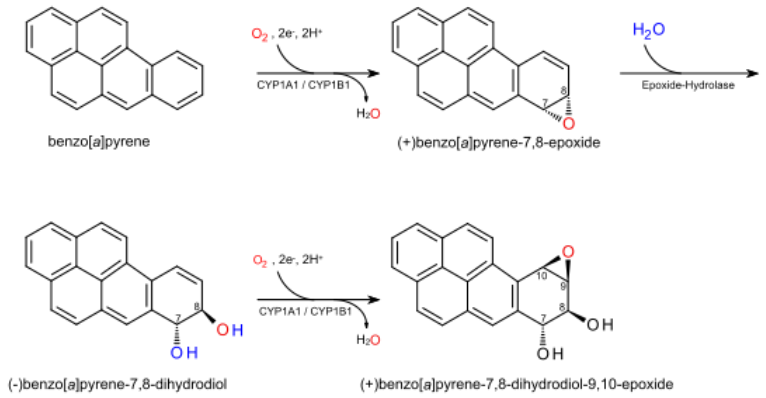

Figure 20 Metabolism of benzo[a]pyrene yielding the carcinogenic benzo[a]pyren-7,8-dihydrodiol-9,10-epoxide.(Rostkowska et al., 1998)

Nitrosamines occur mostly in meat and dairy products. They are formed by the reduction of nitrate. Nitrate is reduced to nitrite by the reducing bacteria and then the nitrite is converted into nitrous acid in acidic environment, and nitrous acids react with secondary amines to form nitrosamine compounds (Figure 21). Compared with benzpyrene, certain levels of nitrosamines taken in the body with any food ingredient are less likely to cause cancer alone but they increase the risk of developing cancer.

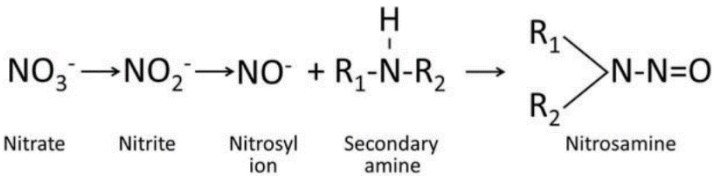

Figure 21 Formation of nitrosamine from nitrate.

However, to single out nitrite as a villain appears to be unreasonable. Moreover, in cured foods that contain the commonly used reductant ascorbic acid (vitamin C), nitrosamines do not occur (Tannenbaum, 1989)

Commercial prospects for this product

Meat is sold in very many product forms, such as chilled raw cuts, frozen raw cuts, cooked canned meat, dry preserved and smoked meats, and cured meat where nitrite is the key ingredient that sets it apart from other forms. Because of its special flavour caused by nitrite curing and the ability to inhibit pathogens like clostridia, cured meat is popular among consumer throughout the world. Its safety and longevity can be further enhanced by the process of lactic fermentation. In respect of consumer fears of cured meat, regulations might prevent the use of a 'naturally cured' claim on the meat/cabbage product, but at the very least a statement about 'no added nitrite or nitrate' could be included in promotional literature.

Where might this product be sold? The likely target markets are east and northeast Asia, where fermented foods, e.g. kimchi, are widely accepted, with the added advantage that the product is sourced from New Zealand. It is also very stable, and requires minimal refrigeration. A consensus from the informal sensory trial (Chapter 7) was that the meat:cabbage ratio should be increased. Cost is obviously a major factor here and this decision would require a good deal of thought. Likewise, the degree of sweetness, and the addition of specific flavours needs consideration.

In what context would the product be consumed? Given its stability, requiring little if any refrigeration, it could be a convenience product consumed on-the-go with wood chopsticks or a plastic fork. Or it may be used as a banquet food shared from a rotating serving table.

As for packaging, the contents may or may not be visible when the pack is unopened. For either format a barrier bag with a lower oxygen transmission rate than used here should be used. (Recall that after several weeks the pink reverted to grey-brown.) Metallised bags are particularly oxygen impermeable and if these were used the contents would not be visible. Artwork would convey the product description.

This product is ready for further development.

Question and answer

1. **What is the best way of cooking vegetables, usually cabbage, and beef before fermentation?**

   The cabbage should be kept raw because there was no large difference between raw and cooked cabbage but cabbage cooking would increase the cost.

   The beef should be cooked because it developed a more attractive bright pink colour than the raw equivalent.

2. **What effect on curing of ratio cabbage:meat?**

   With decreasing ratio of cabbage:meat, the pH value increased.

3. **Raw versus cooked cabbage**

   The cooked cabbage resulted in minor increases in fluid loss, whether to include it or not remained an open question because raw cabbage would always introduce a microflora, but at the same time cooking cabbage on a commercial scale adds cost.

4. **What is the best glucose concentration to use?**

   In most following experiments glucose was not added because adding glucose had no important effects on fluid loss and pH.

5. **If sucrose is required for sweetening, how will different starter cultures affect final pH and other product qualities?**

   Some starter cultures are sucrose-negative and the others are sucrose-positive. In sucrose-negative culture, sucrose is only used to be sweetness and will not change final pH. The sucrose-positive culture will react with sucrose and release the lactic acid to lower the pH.

6. **Can fermented products be held at ambient temperature for extended times without loss of quality?**

   No. When the mixture was stored in vacuum bag at room temperature, the beef would

change the colour from brown to pink and back to brown again. The change from pinkness to brown is due to the loss of the pink nitrosohaemochrome characteristic of nitrite cured meat when the haem iron is oxidised from the ferrous to the ferric form of metmyoglobin because of gradual exposure to oxygen. It is possible that air exclusion at evaluation might have varied, and if some bags were in close contact with others, oxygen transmission might have been slowed. (Discussed in Chapter 4)

7. How safe are these products?

The pH of the product is lower than 4. In the acidic condition, most harmful microorganisms would be killed and foods are safe to eat.

Assuming the quantity of nitrite from a daily consumption of 200 g of cured meat (at the FSANZ nitrite limit) might be 5 mg of Na nitrite. It is safe to eat.

Appendices

Appendix 1 Twelve different treatments, with three replicates each.

Cabbage	Meat	Salt	Glucose	Cabbage *L* ± SD	Cabbage *a* ± SD	Cabbage *b* ± SD	Meat *L* ± SD	Meat *a* ± SD	Meat *b* ± SD
Raw	Raw	0	0	25.7 ± 4.6	1.9 ± 0.2	17.4 ±2.0	15.0 ± 1.8	17.7 ± 1.5	7.9 ± 0.6
Raw	Raw	2	0	20.4 ± 2.1	0.4 ± 0.3	12.9 ± 1.3	18.5 ± 1.3	19.6 ± 0.7	9.7 ± 0.8
Raw	Raw	0	1	27.5 ± 10.3	2.1 ± 0.4	11.9 ± 4.6	10.9 ± 1.9	18.5 ± 1.7	8.1 ± 0.8
Cooked	Raw	0	0	26.5 ± 3.5	1.7 ± 0.8	8.4 ± 1.4	12.1 ± 1.2	17.4 ± 1.1	8.2 ± 1.5
Cooked	Raw	2	0	29.4 ± 3.7	1.0 ± 1.0	8.0 ± 1.8	13.5 ± 3.4	20.7 ± 0.3	8.9 ± 0.7
Cooked	Raw	0	1	32.4 ± 1.5	1.7 ± 0.9	11.0 ± 3.1	10.8 ± 0.9	18.2 ± 0.9	7.8 ± 0.4
Raw	Cooked	0	0	26.2 ± 3.6	-1.1 ± 0.1	17.8 ± 4.3	24.0 ± 2.6	14.2 ± 0.7	12.4 ± 0.2
Raw	Cooked	2	0	22.7 ± 2.7	-0.7 ± 0.3	12.9 ± 1.2	19.8 ± 0.9	15.5 ± 0.7	11.7 ± 0.2
Raw	Cooked	0	1	29.7 ± 0.5	0.8 ± 0.7	14.6 ± 1.8	22.1 ± 1.8	14.8 ± 0.2	11.3 ± 0.3
Cooked	Cooked	0	0	31.4 ± 3.6	-0.3 ± 1.0	11.4 ± 2.2	18.9 ± 1.8	14.6 ± 0.5	12.1 ± 0.6
Cooked	Cooked	2	0	26.3 ± 1.5	0.1 ± 0.8	12.0 ± 3.0	19.5 ± 4.1	13.3 ± 0.4	11.0 ± 0.9
Cooked	Cooked	0	1	26.9 ± 2.5	1.2 ± 0.7	15.1 ± 2.8	20.0 ± 0.8	15.3 ± 0.5	12.3 ± 0.4

Appendix 2 Analysis of colour change in cabbage.

	L	P value	*a*	P value	*b*	P value
Salt						
-	28.3	*	1.0	NS	13.4	NS
+	24.7		0.2		11.4	
Glucose						
-	26.1	NS	0.4	**	12.6	NS
+	29.1		1.4		13.1	
Cabbage						
Raw	25.4	*	0.6	NS	14.6	**
Cooked	28.8		0.9		11.0	
Meat						
Raw	27.0	NS	1.5	***	11.6	NS
Cooked	27.2		0.0		13.9	

Appendix 3 Analysis of colour change in meat.

	L	P value	*a*	P value	*b*	P value
Salt						
-	16.7	NS	16.3	NS	10.0	NS
+	17.8		17.3		10.3	
Glucose						
-	17.7	NS	16.6	NS	10.2	NS
+	15.9		16.7		9.9	
Cabbage						
Raw	18.4	NS	16.7	NS	10.2	NS
Cooked	15.8		16.6		10.1	
Meat						
Raw	13.5	***	18.7	***	8.4	***
Cooked	20.7		14.6		11.8	

Appendix 4 Four different treatments, with four replicates each. (cabbage cooking and glucose added or not)

Cabbage	Meat	Salt	Glucose	Cabbage *L* ± SD	Cabbage *a* ± SD	Cabbage *b* ± SD	Meat *L* ± SD	Meat *a* ± SD	Meat *b* ± SD
Raw	Cooked	2	0	19.5 ± 2.4	0.5 ± 0.8	20.3 ± 2.0	22.1 ± 1.0	18.3 ± 1.4	12.5 ± 0.5
Raw	Cooked	2	1	18.6 ± 2.1	0.3 ± 0.5	22.7 ± 2.2	22.1 ± 1.0	18.3 ± 0.6	12.9 ± 0.8
Cooked	Cooked	2	0	21.9 ± 0.9	0.3 ± 0.6	19.4 ± 1.9	22.7 ± 1.2	18.0 ± 0.5	12.8 ± 0.3
Cooked	Cooked	2	1	24.1 ± 2.5	0.5 ± 0.4	22.4 ± 2.1	20.4 ± 0.8	19.3 ± 0.7	12.3 ± 0.8

Appendix 5 Analysis of colour change in cabbage.

	L	P value	a	P value	b	P value
Glucose						
-	20.7	NS	0.4	NS	19.8	*
+	21.4		0.4		22.6	
Cabbage						
Raw	19.0	**	0.4	**	21.5	NS
Cooked	23.0		0.4		20.9	
Interaction						
– * Raw	19.5	*	0.5	NS	20.3	NS
– * Cooked	21.9		0.3		19.4	
+ * Raw	18.6		0.3		22.7	
+ * Cooked	24.0		0.5		22.4	

Appendix 6 Analysis of colour change in meat.

	L	P value	*a*	P value	*b*	P value
Glucose						
-	22.4	NS	18.2	NS	12.6	NS
+	21.2		18.8		12.6	
Cabbage						
Raw	23.0	NS	18.3	NS	12.7	NS
Cooked	22.5		18.7		12.5	

Interaction						
– * Raw	22.1		18.3		12.5	
– * Cooked	22.7	*	18.0	NS	12.8	NS
+ * Raw	22.1		18.3		12.9	
+ * Cooked	20.4		19.3		12.3	

Appendix 7 Four different treatments, with four replicates each. (cabbage cooking and glucose added or not with culture added)

Cabbage	Meat	Salt	Glucose	Starter culture	Cabbage *L* ± SD	Cabbage *a* ± SD	Cabbage *b* ± SD	Meat *L* ± SD	Meat *a* ± SD	Meat *b* ± SD
Raw	Cooked	2	0	0.04	24.5 ± 3.0	-0.4 ± 0.6	25.3 ± 3.2	19.5 ± 0.5	15.4 ± 0.6	11.8 ± 0.2
Raw	Cooked	2	1	0.04	20.7 ± 3.3	0.0 ± 0.2	22.7 ± 3.1	17.2 ± 1.0	14.4 ± 1.0	11.3 ± 0.7
Cooked	Cooked	2	0	0.04	29.5 ± 4.0	-0.3 ± 0.4	22.5 ± 1.7	18.3 ± 0.8	13.9 ± 0.6	11.4 ± 0.5
Cooked	Cooked	2	1	0.04	28.4 ± 3.3	-0.6 ± 0.4	21.5 ± 5.8	19.2 ± 1.1	13.9 ± 0.8	10.9 ± 0.4

Appendix 8 Analysis of colour change in cabbage

	L	P value	*a*	P value	*b*	P value
Glucose						
–	27	NS	-0.3	NS	23.9	NS
+	24.5		-0.3		22.1	
Cabbage						
Raw	22.6	**	-0.2	NS	24.0	NS
Cooked	28.9		-0.5		22.0	
Interaction						
– * Raw	24.5		-0.4		25.3	
– * Cooked	29.5	*	-0.3	NS	22.5	NS
+ * Raw	20.7		0.0		22.7	
+ * Cooked	28.3		-0.6		21.5	

Appendix 9 Analysis of colour change in meat

	L	P value	*a*	P value	*b*	P value
Glucose						
–	18.9	NS	14.6	NS	11.6	NS
+	18.2		14.1		11.1	
Cabbage						
Raw	18.3	NS	14.9	NS	11.5	NS
Cooked	18.7		13.9		11.1	
Interaction						
– * Raw	19.5		15.4		11.8	
– * Cooked	18.3	*	13.9	*	11.4	NS
+ * Raw	17.2		14.4		11.3	
+ * Cooked	19.3		13.9		10.9	

Appendix 10 Four different treatments, with four replicates each. (cabbage cooking and different incubation temperature)

Cabbage	Meat	Salt	Starter culture	Temperature (℃)	Cabbage *L* ± SD	Cabbage *a* ± SD	Cabbage *b* ± SD	Meat *L* ± SD	Meat *a* ± SD	Meat *b* ± SD
Raw	Cooked	2	0.04	23	15.3± 1.9	2.6 ± 1.6	25.5 ± 1.0	22.7 ± 0.9	17.4 ± 0.5	12.8 ± 0.2
Raw	Cooked	2	0.04	30	28.8 ± 5.5	-0.3 ± 0.7	26.9 ± 2.7	21.8 ± 1.3	16.4 ±1.0	11.9 ± 0.4
Cooked	Cooked	2	0.04	23	15.5 ± 1.0	1.3 ± 0.4	27.2 ± 2.6	21.2 ± 1.7	17.1 ± 0.1	12.4 ± 0.4
Cooked	Cooked	2	0.04	30	25.1 ± 3.7	-0.4 ± 0.3	23.4 ± 1.8	22.1 ± 1.6	16.4 ± 0.6	12.2 ± 0.3

Appendix 11 Analysis of colour change in cabbage

	L	P value	*a*	P value	*b*	P value
Incubation						
-	22.1	NS	1.2	NS	26.2	NS
+	20.3		0.5		25.3	
Cabbage						
Raw	15.4	***	2.0	***	26.3	NS
Cooked	26.7		-0.3		25.2	
Interaction						
- * Raw	15.3	NS	2.6	NS	25.5	*
- * Cooked	28.9		-0.3		26.9	
+ * Raw	15.5		1.3		27.2	
+ * Cooked	25.1		-0.4		23.4	

Appendix 12 Analysis of colour change in meat

	L	P value	*a*	P value	*b*	P value
Incubation						
-	22.3	NS	16.9	NS	12.4	NS
+	21.6		16.8		12.3	
Cabbage						
Raw	22.0	NS	17.2	*	12.6	**
Cooked	22.0		16.4		12.1	
Interaction						
- * Raw	22.7	NS	17.4	NS	12.8	*
- * Cooked	21.8		16.4		11.9	
+ * Raw	21.2		17.1		12.4	
+ * Cooked	22.1		16.4		12.2	

Appendix 13 Two different treatments, with eight replicates each. (different storage temperature)

Cabbage	Meat	Salt	Starter culture	Temperature (℃)	Cabbage *L* ± SD	Cabbage *a* ± SD	Cabbage *b* ± SD	Meat *L* ± SD	Meat *a* ± SD	Meat *b* ± SD
Raw	Cooked	2	0.04	4	25.0 ± 1.3	1.7 ± 0.0	23.1 ± 0.6	18.9 ± 1.2	15.4 ± 0.2	10.6 ± 0.7
Raw	Cooked	2	0.04	23	23.7 ± 1.3	1.0 ± 0.4	30.9 ± 2.1	19.8 ± 0.1	15.5 ± 0.8	11.2 ± 0.3

Appendix 14 Analysis of colour change in cabbage

	L	P value	*a*	P value	*b*	P value
Temperature (°C)						
4	25.1		1.6		23.1	
23	23.6	NS	0.9	NS	30.8	*

Appendix 15 Analysis of colour change in meat

	L	P value	*a*	P value	*b*	P value
Temperature (°C)						
4	18.8		15.4		10.6	
23	19.8	NS	15.5	NS	11.2	NS

Appendix 16 Two different treatments, with fifteen replicates each. (different storage temperature and 56 days' storage)

Cabbage	Meat	Salt	Starter culture	Temperature (℃)	Cabbage *L* ± SD	Cabbage *a* ± SD	Cabbage *b* ± SD	Meat *L* ± SD	Meat *a* ± SD	Meat *b* ± SD
Raw	Cooked	2	0.04	4	17.5 ± 1.0	-1.6 ± 0.4	13.6 ± 3.0	19.9 ± 1.9	11.0 ± 4.9	12.8 ± 0.5
Raw	Cooked	2	0.04	23	12.9 ± 1.6	-1.0 ± 0.6	14.6 ± 2.9	20.7 ± 1.4	11.5 ± 0.5	11.6 ± 0.6

Appendix 17 Analysis of colour change in cabbage on Day 7 and Day 56

	L	P value	*a*	P value	*b*	P value
Day 7						
Temperature (°C)						
4	16.6		0.5		21.3	
23	15.5	NS	0.8	NS	21.2	NS
Day 56						
Temperature (°C)						
4	17.5		-1.6		13.6	
23	13.0	***	-1.0	NS	14.6	NS

Appendix 18 Analysis of colour change in meat on Day 7 and Day 56

	L	P value	*a*	P value	*b*	P value
Day 7						
Temperature (°C)						
4	22.4		17.7		12.1	
23	20.8	*	18.6	**	12.6	*

Original presentation of stats

Stats 1 ANOVA result of cabbage and meat cooked or not

	Salt		Glucose		Cooking			
					Cabbage		Meat	
	0	1	0	1	Raw	Cooked	Raw	Cooked
Fluid loss %	16.4	23.2	20.6	14.7	19.6	17.7	19.1	18.2
	P < 0.0001		P < 0.0001		P = 0.195		P = 0.578	
	large effect		large effect		no effect		no effect	
pH	3.9	3.67	3.82	3.98	3.89	3.87	3.82	3.95
	P < 0.0001		P = 0.011		P = 0.86		P = 0.033	
	large effect		no effect		no effect		no effect	
Cabbage: L*	28.3	24.7	26.1	29.1	25.4	28.8	27	27.2
	P = 0.03		P = 0.067		P = 0.026		P = 0.885	
	no effect		no effect		no effect		no effect	
a*	1	0.2	0.4	1.4	0.6	0.9	1.5	0
	P = 0.053		P = 0.008		P = 0.456		P < 0.0001	
	no effect		no effect		no effect		large effect	
b*	13.4	11.4	12.6	13.1	14.6	11	11.6	13.9
	P = 0.13		P = 0.687		P = 0.002		P = 0.058	
	no effect		no effect		no effect		no effect	
Saturation					14.6	11	11.7	13.9
Hue angle					1.5	1.5	1.4	1.6
Meat: L*	16.7	17.8	17.7	15.9	18.4	15.8	13.5	20.7
	P = 0.514		P = 0.301		P = 0.098		P < 0.0001	
	no effect		no effect		no effect		more light	
a*	16.3	17.3	16.6	16.7	16.7	16.6	18.7	14.6
	P = 0.259		P = 0.949		P = 0.887		P < 0.0001	
	no effect		no effect		no effect		large effect	
b*	10	10.3	10.2	9.9	10.2	10.1	8.4	11.8
	P = 0.618		P = 0.601		P = 0.841		P < 0.0001	
	no effect		no effect		no effect		large effect	
Saturation					19.6	19.4	21	19
Hue angle					0.5	0.5	0.4	0.7

Stats 2 Effect of glucose and cabbage cooking on sample

	Glucose		Cabbage		Glucose*Cooking			
	None	Added	Raw	Cooked	None *Raw	None *Cooked	Added *Raw	Added *Cooked
Fluid loss %	33.2	31.3	34.3	30.1	34.5	31.8	34.1	28.5
	P = 0.381		P < 0.0001			P = 0.148		
	no effect		large effect			no effect		
pH	4.1	3.9	3.89	4.16	3.98	4.31	3.82	4
	P = 0.381		P = 0.011			P < 0.0001		
	no effect		no effect			large effect		
Cabbage: L*	20.7	21.4	19	23	19.5	21.9	18.6	24.01
	P = 0.659		P = 0.067			P = 0.011		
	no effect		no effect			large effect		
a*	0.4	0.4	0.4	0.4	0.5	0.3	0.3	0.5
	P = 0.982		P = 0.008			P = 0.947		
	no effect		no effect			no effect		
b*	19.8	22.6	21.5	20.9	20.3	19.4	22.7	22.4
	P = 0.014		P = 0.687			P = 0.11		
	no effect		no effect			no effect		
Meat: L*	22.4	21.2	23	22.5	22.1	22.7	22.1	20.4
	P = 0.071		P = 0.414			P = 0.045		
	no effect		no effect			large effect		
a*	18.2	18.8	18.3	18.7	18.3	18	18.3	19.3
	P = 0.167		P = 0.409			P = 0.205		
	no effect		no effect			no effect		
b*	12.6	12.6	12.7	12.5	12.5	12.8	12.9	12.3
	P = 0.937		P = 0.619			P = 0.555		
	no effect		no effect			no effect		
Saturation			22.3	22.5				
Hue angle			0.6	0.6				

Stats 3 Effect of glucose and cabbage cooking on sample with adding starter culture

	Glucose		Cabbage		Glucose*Cooking			
	None	Added	Raw	Cooked	None *Raw	None *Cooked	Added *Raw	Added *Cooked
Fluid loss %	31.1	33	32.9	31.2	32.5	29.7	33.2	32.7
	P = 0.159		P = 0.228			P = 0.242		
	no effect		no effect			no effect		
pH	3.6	3.64	3.57	3.67	3.54	3.66	3.6	3.67
	P = 0.288		P = 0.001			P = 0.002		
	no effect		no effect			large effect		
Cabbage: L*	27	24.5	22.6	28.9	24.5	29.5	20.7	28.3
	P = 0.315		P = 0.003			P = 0.013		
	no effect		large effect			large effect		
a*	-0.32	-0.32	-0.18	-0.45	-0.4	-0.3	0	-0.6
	P = 0.993		P = 0.226			P = 0.211		
	no effect		no effect			no effect		
b*	23.9	22.1	24	22	25.3	22.5	22.7	21.5
	P = 0.343		P = 0.275			P = 0.536		
	no effect		no effect			no effect		
Saturation			24	22				
Hue angle			-1.6	-1.6				
Meat: L*	18.9	18.2	18.3	18.7	19.5	18.3	17.2	19.2
	P = 0.266		P = 0.511			P = 0.012		
	no effect		no effect			large effect		
a*	14.6	14.1	14.9	13.9	15.4	13.9	14.4	13.9
	P = 0.311		P = 0.022			P = 0.048		
	no effect		no effect			large effect		
b*	11.6	11.1	11.5	11.1	11.8	11.4	11.3	10.9
	P = 0.062		P = 0.153			P = 0.124		
	no effect		no effect			no effect		
Saturation			18.8	17.8				
Hue angle			0.7	0.7				

Stats 4 The treatments with four replicates each. (cabbage cooking and different incubation temperature)

		Incubation		Cabbage		Temperature*Cooking	
		None (23℃)	Yes (30℃)	Raw	Cooked		
Fluid loss %		32.5	30.9	32.1	31.3	0.896	
			no effect		no effect		no effect
pH		3.54	3.51	3.49	3.56	0.736	
			no effect		no effect		no effect
Cabbage:	L*	22.1	20.3	15.4	26.7	0.274	
			no effect		no effect		no effect
	a*	1.2	0.5	2	-0.3	0.207	
			no effect		no effect		no effect
	b*	26.2	25.3	26.3	25.2	0.031	
			no effect		no effect		no effect
Saturation		26.2	25.3	26.4	25.2		
Hue angle		1.5	1.6	1.5	-1.6		
Meat: L*		22.3	21.6	22	22	0.248	
			no effect		no effect		no effect
	a*	16.9	16.8	17.2	16.4	0.772	
			no effect		no effect		no effect
	b*	12.4	12.3	12.6	12.1	0.034	
			no effect		no effect		no effect
Saturation		21	20.8	21.3	20.4		
Hue angle		0.6	0.6	0.6	0.6		

Stats 5 Analysis variance of colour change and pH from Day 4 to Day 7

		Room temperature (23℃)	Refrigerator (~4℃)
		Cabbage	
Cabbage:	L*	23.6	25.1
		P=0.406	no effect
	a*	0.9	1.6
		P=0.154	no effect
	b*	30.8	23.1
		P=0.036	large effect
Saturation		30.813	23.155
		P=0.037	large effect
Hue angle		1.542	1.502
		P=0.043	large effect
Meat:	L*	19.8	18.8
		P=0.383	no effect
	a*	15.5	15.4
		P=0.979	no effect
	b*	11.2	10.6

	P=0.355	no effect			
Saturation	19.123	18.695			
	P= 0.573	no effect			
Hue angle	0.626	0.603			
	P=0.477	no effect			
	Room temperature (23℃)				
Day	4	5	6	7	
pH	3.82	3.85	3.68	3.69	
	P=0.021	large effect			
	Refrigerator (~4℃)				
Day	4	5	6	7	
pH	3.78	3.85	3.74	3.85	
	P=0.222	no effect			

Stats 6 The result measured in different time and storage temperature.

Day 7	Temperature	
	23°C	4°C
Cabbage: L*	15.5	16.6
	P=0.353	no effect
a*	0.8	0.5
	P=0.333	no effect
b*	21.2	21.3
	P=0.928	no effect
Saturation	21.215	21.306
Hue angle	1.533	1.547
Meat: L*	20.8	22.4
	P=0.019	no effect
a*	18.6	17.7
	P=0.004	large effect
b*	12.6	12.1
	P=0.025	no effect
Saturation	22.466	21.441
Hue angle	0.595	0.600
Day 56		
	23°C	4°C
Cabbage: L*	13	17.5
	P=0.000	no effect
a*	-1	-1.6
	P=0.058	no effect
b*	14.6	13.6
	P=0.591	no effect

Saturation		14.634	13.694			
		P=0.037	large effect			
Hue angle		-1.502	-1.454			
		P=0.043	large effect			
Meat: L*		19.9	20.7			
		P=0.4266	no effect			
	a*	11	11.5			
		P=0.817	no effect			
	b*	12.8	11.6			
		P=0.003	large effect			
Saturation		16.877	16.334			
Hue angle		0.861	0.790			
		23°C				
Day		4	5	7	14	28	56
pH		3.7	3.6	3.6	3.6	3.5	3.6
		P = 0.357	no effect			
		4°C				
Day		4	5	7	14	28	56
pH		3.6	3.7	3.8	3.7	3.7	3.9
		P < 0.0001	no effect			

Stats 7 Importance of cabbage adding.

		Meat	
		20g	40g
pH		4.3	4.0
		P = 0.00	large effect
Meat: L*		19.0	18.3
		P= 0.601	no effect
	a*	15.9	16.5
		P = 0.234	no effect
	b*	10.9	11.3
		P = 0.286	no effect
Saturation		19.32	19.97
		P = 0.215	no effect
Hue angle		0.601	0.600
		P = 0.934	no effect
		Glucose added	
0g cabbage		No	Yes
pH		5.3	4.7
		P < 0.0001	large effect
Meat: L*		17.6	14.2
		P = 0.209	no effect
	a*	7.2	6.7
		P = 0.177	no effect

	b*	11.3	9.2
		P= 0.033	no effect
Saturation		13.447	11.421
		P= 0.044	no effect
Hue angle		1.001	0.942
		P=0.020	no effect

Stats 8 Different amount of cabbage used in experiment

		Meat 20g	40g	60g	80g	100g
pH		3.96	3.72	3.63	3.5	3.4
		P < 0.0001	Large effect			
Meat:	L*	21.6	21.7	21.9	23.4	22
		P = 0.433	no effect			
	a*	18.3	17.6	17.6	16.7	16.9
		P = 0.139	no effect			
	b*	11.9	11.7	11.9	12.3	12.8
		P = 0.007	no effect			
Saturation		21.8	21.1	21.2	20.7	21.2
Hue angle		0.6	0.6	0.6	0.6	0.6

Stats 9 Different amount of nitrite used in experiment

		Sodium nitrite 0mg	0.2mg	0.5mg	1mg	4mg
pH		4.6	4.6	4.6	4.5	4.5
			P =0.019	no effect		
Meat:	L*		19.7	17.9	19.5	20.7
			P= 0.378	no effect		
	a*		6.5	9.8	15.0	18.9
			P <0.0001	large effect		
	b*		10.2	9.5	9.8	10.8
			P =0.047	no effect		
Saturation			12.1	13.7	17.9	21.8
			P <0.0001	large effect		
Hue angle			1.003	0.785	0.583	0.518
			P <0.0001	large effect		

Stats 10 The minimum amount of cabbage used in experiment

		Weight of Cabbage 0g	5g	10g	20g	40g
pH		4.4	4.3	4.1	4.0	3.7
			P <0.0001	large effect		
Meat:	L*		21.0	20.6	21.8	21.9

		P= 0.45	no effect		
	a*	12.3	18.0	19.0	19.2
		P <0.0001	large effect		
	b*	10.9	12.3	12.3	12.3
		P =0.062	no effect		
Saturation		16.6	21.5	22.4	22.8
		P <0.0001	large effect		
Hue angle		0.733	0.573	0.558	0.567
		P = 0.004	large effect		

Stats 11 Different amount of cabbage with T-SC-150.

		Meat				
Weight of cabbage		20g	40g	60g	80g	100g
pH		3.86	3.61	3.50	3.41	3.37
		P < 0.0001	Large effect			
Meat:	L*	19.8	19.0	20.5	19.3	18.2
		P = 0.260	no effect			
	a*	16.5	17.8	17.1	17.4	16.6
		P = 0.173	no effect			
	b*	10.8	11.6	11.5	12.3	12.2
		P = 0				
Saturation		19.693	21.246	20.656	21.337	20.641
		P = 0.022	no effect			
Hue angle		0.580	0.580	0.591	0.617	0.635
		P = 0.054	no effect			

Stats 12 The analysis variance of five different culture effect on pH, colour change and fluid loss.

		BFL-F02	*SM-192*	*F-LC*	*BFL-F04*	*T-SC-150*
pH		3.49	3.45	3.57	3.52	3.58
		P <0.0001	no large effect			
Fluid loss		39.750	34.250	37.500	36.625	31.875
		P = 0.061	no effect			
Meat: L*		21.6	23.0	21.2	22.3	21.3
		P = 0.56	no effect			
	a*	18.3	19.0	19.2	19.0	18.8
		P = 0.785	large effect			
	b*	12.9	12.8	12.6	12.9	12.5
		P = 0.85	no effect			
Saturation		22.4	22.9	23.0	23.0	22.6
		P = 0.91	large effect			
Hue angle		0.615	0.593	0.582	0.595	0.587
		P = 0.468	large effect			
Cabbage:	L*	17.6	18.6	16.3	20.4	17.2
		P = 0.564	no effect			
	a*	-0.2	0.9	0.6	0.1	0.3

		P = 0.166	large effect			
	b*	20.5	25.1	23.1	19.3	23.5
		P = 0.005	no effect			
Saturation		20.5	25.1	23.1	19.3	23.5
		P = 0.005	large effect			
Hue angle		0.011	1.538	1.544	0.779	0.772
		P = 0.428	no effect			

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