Improving Freeze Thaw Stability in Dog Rolls Through Selected Meat Binders

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Abstract

This Master Thesis was conducted to investigate the potential use of selected food gums, accepted, and approved legislatively both within New Zealand and abroad, to improve the Freeze thaw stability of dog roll products.

Dog rolls have evolved into popular pet food products marketed for the high-end user due to its high meat content and controlled use of additives. Given its popularity within New Zealand, it is currently only marketed locally due to having a short shelf life of up to three months at refrigerated storage. To increase market opportunities overseas, the shelf life of dog rolls must be increased, and freeze thaw stability must be maximised to allow export to market overseas under frozen storage.

Improving the freeze thaw stability of dog rolls was carried out based on commercial considerations, customer acceptability and guided by a selection criterion to satisfy requirements of developing an acceptable commercial product within New Zealand and abroad. Product performance was based on analysing current commercial products under pilot scale performance using best performing functional ingredients and assessed using TPA hardness and measuring thaw drip loss after one freeze thaw cycle. Several hydrocolloids were considered potential candidates upon which only sodium alginate and iota carrageenan combined with the synergistic effect of xanthan gum and locust bean gum eventuated in an acceptable product when formulated with a chicken and pumpkin dog roll. The same combination of ingredients was tested using a beef and beetroot base product which did not achieve the same effect as in a chicken and pumpkin base product.

The best performing combination of functional gums which improved the chicken-based dog rolls comprised kappa carrageenan (0.39 %), xanthan gum (0.07 %), LBG (0.22 %), $CaCO_3$ (0.04 %), and sodium alginate (0.23 %). Iota carrageenan performed similarly to sodium alginate when used in the same proportion in combination with the other gums and remained the favourable alternative ingredient over sodium alginate due to its lower cost while improving product performance for freeze thaw stability.

Further optimisation for beef and beetroot dog roll products was recommended by assessing raw beef meat composition or using alternative vegetable filling.

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1 Introduction

Being regarded as “man’s best friend”, dogs have become highly cherished as an important family member and companion to pet owners around the world. As such, pet owners are increasingly conscious about their dog’s diet, wellbeing and health. Such concerns have developed mutual interest in both pet owners and pet food producers to provide wholesome and beneficial pet foods that do not compromise the health and safety of domesticated dogs.

As part of the order Carnivora, today's domestic dog (*Canis familiaris*) is known to be omnivorous given its line of inheritance dating back from ancestral species of Canidae. Dentition traits existing in most breeds of domesticated dogs indicate a close resemblance to the wolf (*Canis lupus*), which hunt in packs mostly for meat but are widely adapted to vegetative foraging when prey is not abundant (Van Valkenburgh, 1989). Owing to this adaptation in feeding behaviour, dog owners and pet food manufacturers have been incorporating a mixture of both plant and animal products as feed for domestic dogs.

Dog owners are increasingly dependent on recommended diets to feed their dogs and find convenience in commercially prepared meals which are ready to eat and available with minimum preparation. Given this case, a high degree of trust is bestowed to pet food companies to provide nutritiously safe and convenient meals for the pet owner. One such product receiving high recognition are dog rolls, which have become a popular alternative to dry and canned pet foods.

In New Zealand, pet food manufacturing is governed by two (2) important legislative acts administered by the Ministry of Primary Industries (MPI).

1. The Agricultural Compounds & Veterinary Medicines Act 1996 (ACVM Act), which enforce regulations governing feeding of animals including labelling and declaration of ingredients. Pet food registrations as veterinary medicines are also regulated under this act.
2. Animal Product Act 1999 (APA) which regulate manufacturing activities from manufacturing through to permits for exports granted through the MPI under the Animal Products Regulations (Ministry for Primary Industries, 2017; New Zealand Pet Food Manufacturers Association, 2019).

Due to globalization in world trade and the large market available in the United States of America, the U.S. Food & Drug Administration takes the lead in regulatory affairs governing importation and distribution of goods into the US and to protect its citizens. While the FDA imposes jurisdiction over animal feeds, including pet foods throughout the US, the Association of American feed Control Officials (AAFCO), provides forums for regulatory officials to contribute and create model bills and regulations governing animal feeds including pet foods within states. Thus, all potential products for market within the US including those manufactured outside of the USA are expected to comply with FDA or AAFCO model regulations pertaining to pet food products. Although not compulsory, meeting FDA or AAFCO standards imparts a high regard for compliance and safety in animal and pet foods throughout US trading partners on a global scale. The FDA has taken charge to ensure that products not conforming to legislative requirements or posing as a possible source for contamination are either recalled or withdrawn from markets across the US or in regions under FDA jurisdiction.

Major product recalls involving melamine contamination in pet foods within the US in 2007 had led to establishment of a new law "Ensuring the safety of pet food" passed by congress to establish ingredient, processing and labelling standards including nutrition and ingredient labelling on pet food packaging by 2009, as well as providing informed customer awareness on ingredients (Hyman et al., 2007). Such occurrences have resulted in customers being cautious about the ingredients used in their selected pet food.

Apart from legislature, health implications based on diet sources are factors of consideration for this study. Identification of several cases of diabetes mellitus in dogs, where glucose metabolism was impaired, has led to concerns over the presence of complex carbohydrates in diets. Both the type and source of complex carbohydrates have been known to play significant roles to influence the rise in post prandial glycaemia, leading to increased blood sugar levels after feeding (Nuttall et al., 1993). Recent findings of dilated cardiomyopathy (DCM) in dogs, a condition which causes the heart walls to shrink causing low blood pressure, has been partly related to low taurine diets. Although such symptoms are not common in all dogs, statistically, Golden retrievers, Labrador retrievers, Doberman pinchers and boxers make up the common species mostly affected by DCM (Sidhu et al., 2018).

With respect to this study, the product of focus will be dog roll. This product is a semi moist, pasteurized dog food product containing meat and vegetables packed into casings and kept refrigerated until used. Processing involves pasteurization of comminuted meat and vegetables to inactivate pathogenic microorganisms followed by hot filling into casings, rapid cooling and then storage at refrigerated temperatures.

At present, this product is limited to a shelf life of 80 days at refrigerated conditions. Freezing the product results in dramatic quality loss exhibited by high thaw drip losses and textural changes. These physiochemical alterations in a frozen product impedes expansion into markets for the product offshore.

1.1 Project aim and objectives

The aim of the project was to investigate the application of alternative meat binders to improve freeze thaw stability in dog rolls. This was achieved through the following objectives:

- Identification of potential binders through a search of literature and screening based on a pre-defined criterion guided by scientific and commercial acceptability.
- Development of a process on a pilot scale to produce prototype dog rolls based on current formulations.
- Development of a protocol to test the freeze thaw stability of dog rolls including measurements for drip loss and textural changes.
- To use the developed protocols to measure freeze thaw stability of current factory samples and those replicated on a pilot scale at Massey University, Palmerston North, New Zealand.
- Development of prototypes on a pilot scale formulated with potential binders with improved freeze thaw stability.

2 Literature Review

Binders and preservatives play significant roles as additives in many manufactured food products, including pet foods. Their ability to influence product attributes and stability are valued highly. The aim of this literature review was to identify potential meat binders, natural preservatives and processing methods that show potential to create a superior, safe and shelf stable “Dog Roll” pet food product. Investigations and reviews were undertaken to deduce ingredient performance and stability upon freezing and thawing operations, including use of selected binders and preservatives in other dog food products. With the increase in published nutrition concerns regarding supplementing diets with plant based ingredients (Case et al., 2011), of which some have been found to be species sensitive (Agar, 2001), this study was undertaken to investigate current binder alternatives and natural preservatives that can satisfy all aspects conforming to a nutritionally acceptable pet food commodity based on scientific evidence.

The literature Review will focus on identifying appropriate additives which may lead to developing a product that will be freeze-thaw stable and cost effective to manufacture. The three major objectives are:

a) To identify possible binders for use in dog roll products undergoing freezing and thawing stress.
b) Investigating freeze thaw stability of these binders.
c) Identify valid experimental and testing methods to assess product development.

2.1 Meat Binders

Binders are considered additives derived from either plants or animals which function to hold together meat products by the entrapment of excess water and the binding together of separated meat tissues. Binding additives consist largely of extracted proteins which are utilized for achieving a cohesive effect on meat pieces upon application and set during heating or in a raw state. While starches and polysaccharides (gums) are considered extenders in a meat formulation, they play significant roles as binding agents in their role to bind excess water during gelatinisation. Frazer et al, (1993) classified binders according to three main constituents as either being starch, protein, or polysaccharide (gum) based.

Other binders can be categorically classified as cold set binders. These include calcium alginates, transglutaminase and fibrinogen / thrombin systems (Boles, 2011) .

For this review, each class of binders will be reviewed with an emphasis of their suitability for use in dog food products such as hot set dog rolls, canned meat products and dry dog kibble.

2.2 Animal Meat Derived Binders

Restructuring technologies allow low value trimmings to be processed into value-added products. Such products can also be formulated to meet growing consumer demands given the increasing interests in such products offering lower salt and fat contents (Boles, 2011). A key component of restructuring is the binding of trimmings together. Therefore, considering such techniques and products will help identify potential natural meat-based binding agents which may be used in creating freezer stable pet food products.

Generally, there are two methods widely applied in reconstructed meat products. The traditional salt / phosphate solutions or using cold set binding.

2.2.1 Hot Set vs Cold Set Binders

Binders regarded as cold set binders exhibit the ability to form a cohesive structure or gel without the need of heat energy for activation. Also, regarded as non-thermal gelation agents (Boles, 2011), common groups of cold set binders include transglutaminase (enzymes), calcium alginates (alginates and Ca^{2+}) and fibrinogen/ thrombin systems (blood plasma fractions) . In contrast, hot set binders require heat to set the binding network such as that of protein meat exudates (Trout et al., 1986). Cold set binders can bind reconstituted meat products that provide a structure similar to whole muscle tissue which allows manufacturers to salvage trimmings and lower cost of meats by creating value added products that can be sold as fresh or refrigerated (Beltran-Lugo et al., 2005). However, in some applications both hot set and cold set binders can be used in combination (Means et al., 1987), depending on the product characteristics and storage conditions intended for customers.

2.2.2 Meat Protein Exudates

Meat restructuring using meat derivatives such as blood plasma proteins (Lipner, 1972) or myosin extracts (Siegel et al., 1979) is an evolving process and has been developed and used extensively since the 1960s. Mass (1963), first patented the idea of mechanically working meat tissue to extract a specific exudate (myosin) which could be used as binder to hold two pieces of meat together, demonstrating that salt and phosphate solutions could be incorporated to extract the myofibrillar protein, myosin, from raw muscle which could then be used to reconstruct meat pieces.

According to Knipe (2014), tumbling involves the rigorous physical treatment to meat pieces by impacting energy via the process of lifting and falling against each other, whereas massaging (mechanically working) of meat pieces involves frictional energy created by meat pieces rubbing against each other, aided by rotating paddles. These processes of mechanical agitation of meat pieces gained increased attention during the 1970s where it where it was used to promote:

i. The production of salt soluble protein exudates (actin and myosin),
ii. enhanced tenderness,
iii. enhanced juiciness and
iv. the development of uniformly unique cured products.

Tumbling was identified as an efficient method to yield meat exudates by the loosening of muscle fibres, promoting the absorption of brine and the release of myosin proteins (Krause et al., 1978). The choice of pre-rigor versus post-rigor meats has been considered and pre-rigor meats were found to be ideal for manufacturing conventional restructured products (Farouk et al., 2005). It has also been found that freezing and thawing of raw meat prior to tumbling does not have adverse effects on the binding properties of the meat exudate, provided there is complete thawing, although excess purge on such meats can interfere with the binding process (Boles, 2011). To reduce purge and increase exudate yields, tumbling under vacuum is a more efficient method as it reduces foaming and promotes binding (Pearson et al., 1996). Such methodologies drive the complexity and feasibility of the utilization of tumbling in a manufacturing context regarding cost and maintenance.

2.2.2.1 Use of Salt and Phosphates

Salt and phosphates play vital roles in meat protein extraction systems. The presence of salt specifically enables the extraction of myofibrillar proteins that can then be used as binders in restructured meat products. Depending on the final product, typically (0.5-1.5) % salt is used for uncured products, whereas (1.5-2.5) % salt can be used for cured products, however, lower salt content is desirable to control lipid oxidation, especially in raw products (Gray et al., 1987). Cooking yields are found to be maximized with a sodium chloride concentration of (1.5-2.5) %, which also increased ionic strength (Trout et al., 1986). The use of salt improves the water binding properties of meats, especially in restructured meat products (Carballo et al., 2006), as well as reducing purge on frozen products (Raharjo et al., 1995). Therefore the main uses of salt in pet food manufacture are for controlling rancidity, obtaining yield and for use in the process of protein exudate extraction, as naturally dogs do not have an affinity to salt given that sufficient amounts are present in the meat they consume (Fregley, 1980).

Just as importantly as salt, the addition of phosphates to restructured meat products promotes solubility of meat proteins and increases water holding capacity (Offer et al., 1983). Phosphates can also act as chelating agents and contribute to the flavour stability of cooked products (Boles, 1990). However, nearing the expected maximum level of use (0.5 %), may cause the product to develop metallic off flavours (Lawrence et al., 2004).

Although the use of both salt and phosphorous accelerates the extraction process of protein exudates from meats, the process of extraction itself is not wholly dependent on these two substances and can be achieved by processing methods and mechanical massaging methods such as that patented by Gagliardi & Eugene (1988). More so, the addition of salt to any dog food product risks development of hypertension as reported by Coleman and Guyton (1969), therefore, a preferred binder should not employ the use of additional salts.

2.2.3 Blood Plasma Protein

A major role of an ideal binder is for effective absorption of moisture as well as the binding of water and fat that is released from meat during cooking. This would lead to a stable meat product, especially in ground meats (Devadason et al., 2010).

Currently, blood plasma and isolated blood proteins are both used as meat binders in the food industry, with the former (blood plasma) also functioning as an emulsifying agent. Blood plasma functions as a binder by forming an effective gel structure in meats (Hickson et al., 1980). In particular when compared to gelatin, wheat gluten, isolated soy protein and alginate/calcium, plasma powder has been reported to be more effective in its binding ability but remains inferior to egg whites in some applications (Lu et al., 1999). The selection of an ideal binder, and comparison between plasma and egg whites including milk proteins should also include considerations for allergic reactions in some dog breeds.

Blood plasma has been found to be an ideal binder for muscle to fat and fat to fat binding systems in meat products over other products normally regarded for restructured meat products (Lu et al., 1999). Such results have led a Dutch company, Sonac BV, to register a patent describing an effective binder, fibrimex®, which is a commercial cold set meat binder widely applicable to restructured and reconstituted meat products. The process to create the binder involves using thrombin to convert fibrinogen into fibrin which interacts with collagen in meats and creates a successful binding action in reconstituted meat products (Wijngaards et al., 1988). Comparing against hot set methods where massaging, tumbling and heat is applied to set the bind, the cold set method discussed reduces oxidative rancidity (Raharjo et al., 1989), use of salt, which can induce hypertension in dogs (Vogel, 1966) and doesn't incur added equipment, energy and time constraints.

The patent for the manufacturing process of fibrimex® expired in 2015 which leaves opportunity for manufacturers and researchers to pursue making their own fibrin using thrombin (Woerner, 2015).

Blood plasma use is limited by its binding strength in low pH meat products due to protein denaturation affecting gel structure which results in a weaker gel. Thereby, plasma would not be an ideal binder for fermented products or in the case of porcine plasma, in products with a pH below 5.5 (Parés et al., 1998). However, the potential to use blood plasma protein has been well documented to achieving stable binding properties (Kim et al., 2017).

2.2.4 Egg White Powder

Egg white was first reported to have superior binding properties on meat products by Siegal et al. (1979). Egg white powders were later proven to be more effective than raw egg whites in binding strength given their higher crude protein concentration (Kato et al., 1990). Further investigations by Lu and Chen (1999), proved that de-sugared egg whites and egg white powders were superior to bovine, porcine, lamb, broiler plasma powders, gelatine, wheat gluten, isolated soy protein, freeze dried broiler breast meat powder and sodium alginate/ calcium carbonate (6:1) binders in their ability to bind "muscle to muscle" components.

Egg white powders are de-sugared to remove glucose as it causes browning (caramelization) during spray drying. This process is usually achieved by using enzymes or microbes that produce enzymes to eliminate glucose. De-sugared egg white powder is also more resistant to microbial contamination (Sisak et al., 2006). Structurally, proteins are more stable after forming conjugates with polysaccharides and egg white galactomannan conjugates were found to possess excellent emulsifying properties as well as displaying high stability at 100 °C for 3 minutes (Kato et al., 1993). Such characteristics are highly desired by the product intended for this work. The recommended binder should possess excellent binding and emulsification properties and be stable at pasteurisation temperatures. A key functional property highlighted for (de-sugared) egg white powder is its high antimicrobial property which could also aid in the preservation of products.

Freeze thaw investigations of egg white proteins found that the process can modify protein structural components. This occurs due to protein denaturation, disassociation, and possible aggregation. However, freeze thaw actions greatly improve the foaming characteristics of albumen proteins and egg whites (Duan et al., 2017). Although suitable for utilisation in this study, previous animal studies concluded that dried egg whites may not be a suitable protein source for dogs as it is not fully digested by dog pancreatic juices (Imondi et al., 1973). More so, raw egg white contains avidin, an enzyme which inhibits biotin metabolism in dogs and cats resulting in biotin deficiency (Baugh et al., 1968). Thermal death time studies for the destruction of biotin binding activity of avidin have shown a much higher resistance than previously thought recording a decimal reduction time of 25 minutes at 121 °C (Durance et al., 1992). This evidence suggests that eggs would not be a suitable candidate in this study as heat processing for dog roll occurs at lower temperatures than those needed for proper inactivation.

2.2.5 Freeze Dried Fish Muscles Protein

Commercial manufacturing processes for making binders traditionally relied on freeze drying processes. However, the freezing effects of this process limits the binding ability of certain protein sources due to protein denaturation especially in fish (Matsuda, 1988). Given the potential for fish proteins to serve as effective binders owing to their strong interactions with other proteins and possession of superior gelation attributes, it was found that freeze drying of cod surimi resulted in less superior kamaboko products due to denatured muscle proteins (Lanier et al., 2000). In the past, fish protein denaturation could be overcome as reported by Matsuda et al (1979) and Matsuda (1988), by the use of sodium glutamate, sorbitol, sodium chloride, monosaccharides or disaccharides after freeze drying and during storage to inhibit fish muscle protein denaturation.

Other studies to improve use of fish protein as binders revealed that protein denaturation was significantly reduced when reducing agents were used during freeze drying operations. This ultimately resulted in strong binding properties on reconstituted pork sticks (Chung et al., 2000). Denaturation of muscle proteins during freezing is thought to be caused by development of hydrophobic and hydrophilic interactions within the muscle, especially amongst disulphide bonds (Jiang et al., 1987), causing losses of native protein networks. Incorporating certain reducing agents such as cysteine, sodium bisulphite or a combination of both was found to support a high meat binding product tested on pork which exhibited optimum cooking conditions at 40 °C for 60 minutes and 90 °C for 20 minutes (Chung et al., 2000).

Such a product does not employ the use of tumbling which can ultimately lead to tissue alterations in raw material as well as use of salt which will be avoided in this study due to hypertensive sensitivity in dogs (Coleman et al., 1969). Although the reported case study carried out by Chung et al. (2000) was based on mackerel (*Scomber Australasicus*), opportunities exist to investigate the potential of other fish-based binders using reducing agents. Employing such methods can reduce fish waste, as well as offering low-cost by-products that can be converted into viable food grade binders at low cost. Such a binder as that described above would also be highly suitable for this study due to its superior binding qualities and stable cooking parameters, which should ideally be suitable for pasteurisation processes in ready to eat (RTE) dog rolls.

2.2.5.1 Fish Protein Isolates / Fish Protein Powder

Fish protein isolates (FPI), can now be extracted from by-products or lower commercial value / underutilised species of fish using pH–shift technology where pH is adjusted to extract proteins near their iso-electric points (Kristinsson et al., 2005). These proteins are then used as wet or dry ingredients in developing value-added convenience foods such as restructured meats (Shaviklo et al., 2012). Dried fish protein or fish protein powder (FPP) can be created via this method for use as meat binders, emulsifiers or dispersing agents due to their high gel forming ability and strong protein interactions (Ramirez et al., 1999). The only downside for the FPI / FPP extracted using the pH-shift technology is that fish proteins subsequently freeze dried for storage undergo more oxidative changes than whole minced fish muscles. This was evident during the pH shift process and carrying through the freeze drying and storage process. Research into the area of reducing oxidation during the freeze drying process is required (Shaviklo et al., 2012).

2.2.6 Gelatine

Gelatine is derived from the primary protein component of animal connective tissue, collagen (Ramachandran, 1967). Due to its high solubility in water, gelatine is regarded as a hydrocolloid employed mainly in the food, pharmaceuticals and photography industry. However, gelatine differs from other polysaccharide-based hydrocolloids (such as pectin and carrageenan) as it is fully digestible and contains all essential amino acids with the exception of tryptophan (Poppe, 1992). Among its many uses, gelatine performs in the following roles for meat products as depicted in table 2-1 below.

Table 2-1: Uses of gelatine in food processing and physical characteristics displayed at optimised levels (Poppe, 1992).

Products	Gel strength (Bloom)	High viscosity	Average viscosity	Others	Rate of use
			Meat Industries		
Jellies	150-250	X	X	Transparency / colour	(3-15) %
Binders for meat emulsions	150-250	X	X		(0.5-3) %
Hams, canned meat products	150-250	X	X	Transparency	(1-2) %
Coatings	150-250		X	Transparency	(5-20) %
			Fish Products		
Binders	150-250		X		(0.5-3) %
Aspics	150-250		X	Transparency / colour	(3-15) %

Pertaining to meat emulsion-based products, gelatine is employed primarily to correct cooking irregularities brought about by release of water and fat. Adding gelatine creates a homogenous textured product, however its effectiveness can be influenced by the presence of other binding agents, amount of collagen in the emulsion or other ingredients (Poppe, 1992).

Gelatine forms and stabilises hydrogen bonds in water which allows it to form a three-dimensional structure in aqueous solutions. The resulting gel strength can be measured as referred to in Table 2-1 as gel or bloom strength. Commercial products process gelatine with a bloom strength between 50 and 300 bloom (grams). Bloom strength is an acceptable indicator of different gel strengths when comparing between products (Baziwane et al., 2003).

The inclusion of gelatine as an ingredient for use in pet foods will most likely utilise its emulsification and water binding properties more than its potential as a binding agent over other binders, given its low melting point below 35 ^{o}C (Glicksman, 1969). Sources of gelatine for commercial use have now moved away from the traditional bovine and porcine hides / by-products given the occurrence of mad cow disease and limited use by Muslims and Jews pertaining to religious beliefs. (Regenstein et al., 2007). Researchers are now currently undertaking studies to improve gelatine extraction from marine sources and improving products through use of citric acid to exterminate fishy odour which have remained an obstacle to uptake in recent years (Sae-leaw et al., 2014). Commercial gelatine products harvested from fish sources have high levels of the amino acids glycine and alanine but lack cysteine (Sae-leaw et al., 2016), which is required for synthesis of taurine in combination with methionine (Case et al., 2011).

2.2.7 Fat

Meat proteins act as emulsifying agents in emulsion meat systems containing sufficient fat (Amini et al., 2015). Fat has a textural influence on meat emulsions, playing a role in binding to water in the presence of proteins. Myosin, which is the main structural protein involved in fat emulsions and water holding capacity, influences emulsification through its non-polar tail which attaches to fat and its polar amino acid head which attaches to the water interface (Sorapukdee et al., 2013). Meat products without fats must incorporate fat replacers such as hydrocolloids that mimic the chemistry of fat to be able to bind with water and form an emulsified system.

2.3 Plant Derived Binders

Meat binders derived from plants include plant proteins, starches, gums and cellulose components. The term “binder” has been used by scientists to describe several different substances that are used to modify or improve water holding capacity, emulsification and adherence of meat pieces (Siegel et al., 1979). Non- meat proteins derived from plants also possess binding capabilities and are commonly used in the human and pet food industries. The following is a review of the different non-meat binders of plant origin.

2.3.1 Hydrocolloids

Hydrocolloids are made up of heterogeneous long chain polysaccharide or protein polymers with the ability to form viscous solutions and gels upon dispersion in water. Therefore, they are hydrophilic compounds owing to the large number of hydroxyl (–OH) groups within their polymer chains which are readily soluble in water (Saha et al., 2010). These substances produce dispersions in water which are intermediate between a true solution and a suspension taking on the behavioural characteristics of a colloid system. The term ‘hydrocolloid” arises from these two properties of being hydrophilic and colloids, thus coining the term hydrocolloid (Glicksman, 1983).

While gel formation is the main interest for this study, hydrocolloids involved in forming a gel network do so through the formation of polymer chains that form three dimensional structures that entrap water and immobilise the solution rendering it resistant to flow. This increases the viscoelastic property of the solution and imparts a physical gel with unique textural properties for each type of hydrocolloid used (Glicksman, 1983). Table 2-2 below lists the common hydrocolloids used as gels and thickeners in the food industry. The drivers for the current global demand for utilising hydrocolloids are their functional properties to reduce perceived unhealthy ingredients such as sodium (salt), fat and sugar (Hotchkiss et al., 2016).

The main use of hydrocolloids as thickening and gelling agents are due to their ability to modify the rheology of foods. They find uses as thickeners, emulsifiers, gels, stabilisers and regulators of crystal growth in ice and sugar products (Saha et al., 2010).

Table 2-2: List of some common hydrocolloids and their predominant functions (Saha et al., 2010).

Thickening hydrocolloids	**Gelling hydrocolloids**
Starch	Alginates
Xanthan gum	Pectin
Guar gum	Carrageenan
Locust bean gum	Gelatine
Gum Arabic	Gellan
Cellulose derivatives	Agar

A unique characteristic of hydrocolloids is their ability to improve freeze thaw stability as well as improving cooking yields, moisture retention and textural characteristics (Shand et al., 1990). Therefore, hydrocolloids with added binding properties have good potential for use in dog rolls.

2.3.2 Carrageenan

Carrageenan has been associated as an ingredient in human food systems dating back as far as 600 BC. This substance is a naturally occurring polysaccharide which fills the void in cellulosic plant structures (Figure 2-1). It has excellent water binding properties and interacts well with milk proteins and is a widely applied hydrocolloid used as a gelling, thickening and stabilising agent (Thomas, 1997). It was also one of the first hydrocolloids used for the purpose of binding water in high moisture pet foods and involved an application which was granted a patent (Pat: US4495208A) in 1984, that has now expired (Friedman et al., 1985).

Figure 2-1: Intercellular carrageenan using Laser Scanning Confocal Microscopy (Batista et al. 2017).

Figure 2-2: Gel formation ability of kappa and iota carrageenan via formation of helices (Dea, 1993).

Derived from red seaweeds, it is not nutritionally valuable in its role as a food additive but highly regarded for its functional properties as a hydrocolloid (Necas et al., 2013). In the food industry, carrageenan comprises two main types under separate additive numbers. Under European Union (EU) legislation, carrageenan is given the additive E407 for refined carrageenan and E407a as Processed Eucheuma Seaweed (PES). The difference between the two additives is that PES contains cellulose and can sometimes be referred to as “semi-refined carrageenan” or SRC (Hotchkiss et al., 2016).

Carrageenan comprises three main types, kappa, lambda and iota designated by the prevalent polysaccharide present as defined by the FAO (2001). It is widely used in milk systems due to its ionic content which is highly susceptible to gel formation in the presence of potassium (K^+) or calcium (Ca^{2+}), the latter being abundant in milk products. Only kappa and iota carrageenan can form gels. Kappa gels are dependent on the presence of both potassium and calcium whereas iota will only form a gel in the presence of calcium ions. Lambda carrageenan does not gel due to its inability to form helices, unlike the gel forming characteristic of its other two counterparts, and thus it finds many uses in other applications in food industry such as a viscosity agent. Refined kappa carrageenan forms stronger gels in the ratio of 1.2 % carrageenan with 0.3 % KCl, than SRC. Inferior gel strengths using SRC are due to the presence of low amounts of cellulose which interfere and disrupt the helix structure in gel formation (Hotchkiss et al., 2016).

While all types of carrageenan are soluble in hot water, only sodium salts of iota and kappa types are soluble in cold water. Carrageenan gels can be set at (40–70) °C and are relatively stable at room temperatures. Gels are reversible and reversing the gel formation can be achieved by heating the gel to (5–10) °C above the gelling temperature (Thomas, 1997). This means that the gel will remain stable after setting at typical ambient temperatures which is ideal for pet foods. However, the choice of gel structure and type for use would need to consider its firmness upon setting. This will favour iota carrageenan as it produces soft elastic gels compared to brittle gels achieved with kappa carrageenan (Table 2-3). The association of iota carrageenan with protein matrixes to stabilise emulsion-based food systems has application in meat canning and pet food industries (Imeson, 2009).

Currently following fourth in line from the leading hydrocolloids used in the food texture market following starch, gelatine and pectin, carrageenan is today widely sought after as a hydrocolloid to impart processing benefits such as improved slice ability, improved yields, increased shelf life and most importantly for the purpose of this study, improved freeze thaw stability (Hotchkiss et al., 2016).

Table 2-3: Functional properties of the three types of carrageenan (Hotchkiss et al., 2016).

Property	Kappa	Iota	Lambda
Protein Interaction	Strong	Moderate	Weak
Gel Strength Ability	Highest of all 3. Forms self-supporting brittle gels	Soft elastic to strong gels in presence of Ca^{2+}	Forms weak gels
Uses	Gelling agent	Gelling agent	Thickening agent
Freeze Thaw Stability	Poor	Good	Poor
Economical Dosage Rates	Very low (cost effective)	Low to moderate	Expensive

Iota carrageenan is widely used in frozen desserts due to its good freeze thaw stability, which prevents oozing after thawing. In respect of its good freeze thaw stability, it can be used together with kappa carrageenan due to kappa's strong protein interaction and moderately lower cost in comparison to lambda carrageenan. However, Iota carrageenan does not show synergistic relationships with other gums (Hotchkiss et al., 2016).

2.3.2.1 Use in Pet Food

Semi refined carrageenan containing kappa is used in retort canned pet foods as a stabiliser, processing aid and gelling agent together with other gums, phosphates, salts, sugars and minerals. High temperature retort processing allows carrageenan and other gums to dissolve and form gels upon cooling. Carrageenan acts as stabiliser when preventing seeping of blood from meat into the gel matrix during cooking. It also serves as binder in chewable products, single serve pouches for cats and gravy type products (Hotchkiss et al., 2016).

Controversies have unfolded over the use of carrageenan in human foods due to reports of associations with digestive disorders (David et al., 2018). However, no restrictions are placed on commercial carrageenan for use as a food additive, and there is increasing popularity for its use in new trend wet/moist pet foods. Furthermore, the USFDA has not placed an upper limit on the addition of carrageenan as a food additive to achieve functionality (Campbell et al., 2017). However, carrageenan is typically added in the range of (0.1–2) % in food products (Hotchkiss et al., 2016), and may be an ideal ingredient for dog roll products.

2.3.2.2 Iota Carrageenan, Kappa Carrageenan & Locust Bean Gum

As described by Hotchkiss et al. (2016) iota carrageenan displays good freeze thaw stability. However, iota carrageenan does not form synergistic interactions with other gums. Kappa carrageen however forms the strongest gel when used with locust bean gum. In this thesis, trials for freeze thaw stability utilised this relationship described by Hotchkiss et al. (2016) to incorporate Iota carrageenan into the formulation as a separate hydrocolloid. An important consideration when using iota carrageenan was the presence of calcium carbonate in the formulation which is a common additive in pet foods and importantly, iotas dependency for Ca^{2+} ions to form a gel with good freeze thaw stability.

Calcium ions have been found to be more effective in inducing gelling of iota carrageenan than monovalent ions such as sodium and potassium (Morris et al., 1980). Therefore, testing was carried out on iota carrageenan using calcium carbonate to test its effectiveness in reducing syneresis after freeze thaw treatment.

2.3.3 Cassia Gum

Cassia gum is approved for use in Europe as a stabilizer with functionality as thickener and gelling agent in human and pet foods (E499). It finds application as thickener, emulsifier, foam stabilizer, moisture retention agent and is included as a texture improver in cheese, meat, poultry products and frozen dairy desserts.

Cassia gum is extracted from the seeds of *Cassia tora*, *C. occidentallis* and *C. obtusifolia*, a group of ruderal plants that belong to the family of *Leguminosae*. Raw cassia gum contains several diverse aromatic compounds known as anthraquinones which find useful applications as laxatives and treatment of fungal skin disease, however, their use has been associated with causing nausea, vomiting, abdominal cramps as well as diarrhoea (Dave et al., 2012). In addition, presence of 0.1 % *C. occidentalis* seeds pose a toxicity risk as these have been associated with muscle toxicity. However, processing and refining to extract cassia gum results in reducing the anthraquinone presence to an acceptable level below 0.05 %.

Cassia gum is classified as a high molecular weight polysaccharide comprising a mannose: galactose ratio of 5:1. Although cassia gum resembles guar gum in terms of structure and chemical properties, it resembles locust bean gum (LBG) in its functionality. However, the refined cassia gum differs from both LBG and guar gum that it contains less galactose molecules next to the extended mannose backbone chains which form synergistic interactions with anionic polymer food gums such as carrageenan and xanthan.

This property results in a higher break strength of cassia and carrageenan combinations compared with locust bean gum and carrageenan at the same concentrations. Because of the high break strength, cassia gum and carrageenan synergy can reduce the total amount of hydrocolloids required potentially resulting in lower cost (Mahungu et al., 2008).

Another synergistic advantage of a combination of cassia gum and xanthan gum is that the resulting gum formed is freeze thaw stable at a ratio of 50:50 (Renn et al., 1990). Given these two synergistic properties, increased break strength and improved freeze thaw stability, cassia gum remains an ideal candidate to test for improving textural hardness whilst minimizing thaw drip loss after freeze thaw treatments while maintaining cohesiveness.

2.3.3.1 Permitted use

In being considered as a technical and functional additive for use in dog and cat foods, the panel on Additives and Products used in Animal Feeds (FEEDAP) commissioned by the European Commission advised that only purified semi-refined cassia gum which satisfy specifications of being a food additive (i.e.; contain 0.5 mg anthraquinones /kg) are considered safe for use in dog and cat foods with the maximum permitted content of 13,200 mg/kg (1.32 %) in a standardized complete feed with 12 % water content (Beynen, 2019).

Reported findings from the investigation also concluded the following:

1. It was highly cautioned that handling of cassia gum by workers is potentially harmful regarding the product being a skin and respiratory sensitizer with potential to irritate skin and eyes.
2. Could not conclude on the efficacy of cassia gum used as a gelling agent and thickener in feedstuffs for cats and dogs.

This considerations by FEEDAP confirms the listing of cassia gum as a flavouring agent (CAS number: 5373-11-5) for use in pet foods under MPI New Zealand GRAS status but not as a thickening and gelling agent. The same applies on the USFDA approved food additives where it is not stated as a “GRAS” additive.

2.3.4 Curdlan

While Hotchkiss (2016) describes the use of hydrocolloids to correct freeze thaw stability issues in food systems, Williams et al. (2009) reported the highest freeze thaw stability when using curdlan gum in combination with xanthan gum which reduced syneresis and improved heat stability and adhesiveness compared to other combinations involving hydrogels. Although this research applies to hydrogel stability, it shows promise if incorporated into dog roll formulations prior to heat processing which would allow the interaction of these gums with free moisture during the cooking process and promote reduced syneresis during freeze thaw cycles.

Given this property of curdlan, it has gained favour in pet foods and has been recorded as an additive incorporated into a patented pet food product (US20180343894A1) for improving texture in a wet pet food with a solid component (Lammers et al., 2018). However, curdlan gum is insoluble in water and only soluble in organic solvents due to its extensive hydrogen bonding holding granules together (Nishinari et al., 2009).

2.3.5 Xanthan gum

Xanthan gum is a polysaccharide secreted by *Xanthomonas campestris*, a bacterium used commercially to produce xanthan gum via a fermentation process. The particular polysaccharide is produced at the cell wall of the bacteria which are commonly present on the leaves of Brassica vegetables (Sworn, 2009).

It is common for industrial products to include more than one polysaccharide in formulations to achieve desired outcomes and performance in products. Combining some polysaccharides to achieve the desired effect is dependent on the properties of each polysaccharide involved. However, for others the combination of a gelling polymer with a non-gelling polymer results in a synergistic effect that is superior to the action of the individual polymers through the interaction of different polymer chains and junction zone formations that interact under given conditions. Such a state exists between xanthan gum, a non-gelling component and locust bean gum, a galactomannan possessing β-1-4 linkages in its polymer chains. Combining the two results in formation of junction zones between the two polymers giving rise to enhanced synergistic effects corresponding in a firm, thermo-reversible gel (Copetti et al., 1997).

Such properties work well in surimi, a concentrated fish muscle protein product, through the interaction with myofibrillar proteins when used in the ratio of 0.25:0.75 (xanthan: LBG). This ratio increases firmness, related to textural hardness. However, at higher levels from 0.75 – 1.00 the gel forming ability of xanthan was reduced in part by the presence of calcium ions up to a level of 0.4 % (Ramírez et al., 2002). Hydration of xanthan gum requires the addition of a dispersing agent such as sugar and oil or hydration under high shear mixing conditions. A combination of both yields good hydration of the gum preventing clumping or swollen lumps of partially hydrated gum. Dispersing agents at a ratio blend of 1:10 (xanthan: dispersant) is usually sufficient to impart proper hydration (Sworn, 2009).

A dispersing agent (vegetable oil) was applied to aid hydration of xanthan gum during trials. Synergy between xanthan gum and LBG was also investigated to test for stabilising textural hardness after freeze thaw treatment of dog rolls.

2.3.6 Alginates

Alginates are naturally occurring anionic polymers extracted from cell walls and intracellular spaces of brown seaweed (brown algae) and are considered significant hydrocolloids used in human and pet foods due to their low toxicity, biocompatibility, low cost and gelling characteristics in the presence of Ca^{2+} (Lee et al., 2012). Such unique properties have been supported by Lu et al. (2006), who reported further on the hydrophilicity and biodegradability which make alginate an ideal candidate for use in this study.

Commercial applications for alginates in food rely on interaction between sodium alginate and cations that lead to modification or generation of rheological characteristics of the product. Alginates are also freeze thaw stable due to their characteristic ability to maintain gel structure at any given temperature brought about by the formation of cross-linked calcium cations and alginate structure. This property is advantageous, has been widely applied to meat chunks for pet foods (Onsøyen, 1997), and has potential to improve freeze thaw stability in dog rolls. The gelling structure of alginate/calcium mechanisms is beneficial and has been adapted for utilisation in restructured meats. Optimum levels for restructured meats reported by Means and Schmidt (1986) were (0.8-1.2) % sodium alginate and (0.14-0.27) % calcium carbonate.

Clarke et al. (1988) also reported that restructured meat products formulated with 0.6 % alginate, 0.1 % calcium carbonate and 0.15 % lactate were found to possess acceptable palatability and cohesion characteristics. It was also found that at a binding level of 0.57 % resulted in high cooking yields and greater binding strength. Alginate will form thermo-irreversible gels in acid or with calcium ions at low temperature, which gives it its most important feature and ability to make heat stable gels (Onsøyen, 1997).

Of interest for the present study, is that alginates have been utilised to stabilise syneresis in ice cream, thereby improving freeze thaw stability (Helgerud et al., 2009). This is achieved by their unique ion exchange relationship with calcium ions where a gel network entraps water as well as influencing smaller ice crystal growth in frozen desserts. Helgerud et al. (2009) also reported that in food systems with free calcium ions present, a sequestrant is usually required to prevent premature gelling or by processing to temperatures above 70 ^{o}C and cooling.

Alginates also act as soluble dietary fibre through increasing viscosity in the gut (Wolf et al., 2002), thus being beneficial to gut health. Alginates are notably soluble in cold water, however, the addition of a dispersing agent such as oil is required to prevent clumping after which shear force can be applied to achieve maximum hydration.

Such characteristics of alginates is beneficial due to its freeze thaw stability, low quantity usage and non-toxic effects. Its added property of being able to act as a meat binder increased its potential as an ingredient for this study.

2.3.7 Agar

Agar is the oldest known hydrocolloid and is renowned for its strong gelling ability and properties as a stabiliser and thickening agent in foods. Extracted from red seaweed, the unique property of agar which sets it apart from carrageenan and alginates is its ability to form reversible gels without interacting with other ions to set gelation. Instead, it is dependent on the formation of hydrogen bonds, and has unique properties to form gels at exceptionally low concentrations. Another property of agar gel is its relatively low sulphate content which is the lowest (<4.5 %) compared to carrageenan in general (Armisen et al., 2009).

Generally, agar is insoluble in cold water but becomes soluble at higher temperatures. It forms a stable gel upon cooling to (34-43) ^{o}C and will remain in a gel state up to a temperature of 85 ^{o}C. Agar is recognised by the USDA with approved “GRAS” status and finds many applications in the food industry (Armisen et al., 1987), particularly in meat and fish preserves or aspics including pie filling (Modliszewski, 1990).

Agars solubility temperature would reduce its potential for use in this project as all gums employed were hydrated in cold water prior to batching.

2.3.8 Cellulose Derivatives

Cellulose, together with lignin and hemicelluloses (comprising other polysaccharides such as mannan) is the main constituent of cell walls in plants and is the world’s most abundant natural organic material (Nussinovitch, 1997). Cellulose itself is insoluble in water and remains undigested in the human body (Zecher et al., 1997). While cellulose itself remains limited in its use for creating edible food products, its derivatives find wide application in the food industry. Examples such as methylcellulose (MC), hydro propyl cellulose (HPC) and carboxy methylcellulose (CMC) are all produced by etherification of cellulose where hydroxyl groups are replaced with the various groups to produce the mentioned derivatives. Combining two or more reagents reacting with cellulose can produce mixed derivatives such as methyl hydropropylcellulose (MHPC).

- Methylcellulose (MC) - is derived from reacting alkali cellulose with methyl chloride (Zecher et al., 1997). Being water soluble, methylcellulose is classified as an industrial gum which can be used in food and non-food product applications (Grover, 1993). Methylcellulose was tested and found to be an effective meat binder for reconstructed meat products by Benard et al. (1989) and it was found to interact with meat proteins at the particle interface.
- Hydroxypropylcellulose (HPC) - is produced by reacting alkali (ether) cellulose with propylene oxide yielding a non-ionic, water soluble polymer which is used as a binder in tablets, cosmetics formulations and thickening agent (Zecher et al., 1997).
- Carboxymethylcellulose (CMC) - is produced by the reaction of ether cellulose with sodium chloroacetate (Zecher et al., 1997). CMC is considered a fibre option to replace the protein and fat actions on binding meat batters such as sausages. Such considerations for using cellulose derived products in meat products arises from the trend to reduce caloric content in products as well as reducing the CO_2 footprint of end products which ultimately are leading to the uprising of meat analogues and less usage of animal meat products (Alamanou et al., 1996). CMC in combination with gum arabic has been reported to improve frozen dough

performance in bread making by controlling ice crystallisation (Asghar et al., 2006). Such a characteristic may be advantageous in dog rolls.

- Methyl hydroxypropylcellulose (MHPC) - is produced by reacting alkali cellulose with two methyl chloride and propylene oxide (Zecher et al., 1997). Both MHPC and MC are uniquely soluble in cold water but insoluble in hot water. Gel formation is achieved by heating cold solutions to a thermal gel temperature of between 50 ^{o}C and 90 ^{o}C (Gibson et al., 1983).

Of all derivatives of cellulose, the USFDA has classed ethyl cellulose as a GRAS food additive permitted in feed and drinking water of animals (USFDA, 2019).

2.3.9 Guar Gum

Seed gums are composed of galactomannans, which are carbohydrates made up of chains of mannose units accommodating galactose side groups. Galactomannans possess the ability to act as thickeners in their interaction imparting stickiness and particle distribution in wet human and pet foods. The inclusion level of either mixed or single seed gums used in wet pet food may range from (0.01 – 0.5) %. Guar gum on its own was observed to reduce the net uptake of protein in dogs and cats at a concentration of 0.5 %, however at this level it does not impede gut health (Beynen, 2019).

Guar gum aids in preventing syneresis as well as contributing to textural appeal in processed cheese (Klis, 1966). It has been noted more recently that guar gum can be applied in concentrations as low as 0.0025 % - 0.01 % w/v to low fat cheese without changing the rheology and texture of the product. Apart from its effectiveness and viability, it is also one of the more economical gums available and it is widely used in ice cream manufacture to promote uniformity in ice crystal sizes (Mudgil et al., 2014). Such behaviour of guar gum in reducing syneresis at low concentrations would be ideal to test for improving freeze thaw stability in dog rolls.

Guar gum can also be used together with carrageenan in place of locust bean gum where it has been shown to hydrate better and is a preferred hydrocolloid to use in high temperature - short time (HTST) processes requiring thermal shock (Weinstein, 1958).

In processed meat products, guar gum is effective in controlling syneresis, binding water, emulsifying fat and generally contributing to the stability of sausages and comminuted meat products (Ercelebi et al., 2010). When used with xanthan gum, the blend was noted to be effective in retarding staling in gluten free breads, by controlling retrogradation of starch (Sumnu et al., 2010). Having considered all properties of guar gum as a potential candidate for improving freeze thaw stability of dog rolls, positive outcomes were expected when used in combination with xanthan gum.

2.3.10 Use of Cyclodextrin

Cyclodextrins are a group of oligosaccharides possessing a cyclic chemical structure comprising of either six (α-cyclodextrin), seven (β-cyclodextrin) or eight (γ-cyclodextrin) glucose units and extending to more complex forms linked by α– (1,4) bonds. They are also known as cycloamyloses, cyclomaltoses and schardinger dextrins (Eastburn et al., 1994). Cyclodextrins are a product of enzymatic degradation of starch by the enzyme cyclodextrin glucanotransferase (CGTase).

Of interest to this study, was the ability of cyclodextrins to form “inclusion complexes” with a variety of hydrophobic guest molecules due to their hydrophilic exterior and an apolar cavity providing a micro heterogeneous environment (Szejtli, 1989).

Cyclodextrins protect kappa carrageenan from shrinking, and thereby improve the freeze thaw stability of kappa carrageenan gels (Yuan et al., 2016). According to Del Valle (2004), of the three common cyclodextrins, β-cyclodextrin is the more easily accessible and economically viable form available.

2.3.11 Use of Sodium Tripolyphosphates (STPP)

Various other intrinsic factors may relate to freeze thaw stability of sausage-like products apart from the use of hydrocolloids including various salts, pH and tripolyphosphates. In such circumstances, it was reported by DeFreitas et al. (1997) that the addition of STPP eventuated in appreciable improvements in the water holding capacity of pork sausages after freeze thaw treatments. Increased pH also showed a significant reduction in moisture loss and increasing product texture when using kappa and iota carrageenan (DeFreitas et al., 1997). The use of STPP is common in dog foods as a sequestrant however, certain precautions must be taken as it may inflict skin problems in some breeds of dogs (Jackson, 2019).

It has been shown by DeFreitas et al. (1997), that STPP decreases thaw drip loss and increases hardness in pork sausages when used with carrageenan at concentrations of 0.5 % STPP to 0.5 % kappa carrageenan. Kappa carrageenan and iota carrageenan work best in the presence of STPP during freeze thaw cycles. Defreitas et al. (1997) also reported that the presence of phosphates improves gelation of ground meat products by either increasing pH or solubilising actomyosin. It was reported also that the presence of potassium chloride (KCl) in cooked pork sausages increased thaw drip and decreased hardness after freeze thaw treatment.

2.4 Carbohydrates

Carbohydrates can generally be classified as either structural or none–structural. Carbohydrates include digestible carbohydrates such as sugars and starches, and fibre, which remains indigestible to a dog's digestive enzymes. Starch is the main digestible carbohydrate in dog food while sugars have limited use. Starches are widely sourced from grains that are used extensively in pet food. However, more recently, there is a trend in dog food for it to be produced "grain free" as grains are considered to not be part of a dogs' natural diet. While grains are considered an unfavourable source of starch, these have in some cases been replaced with other sources of starchy material such as roots and tubers which seem to be readily accepted by consumers (Beynen, 2016). Completely omitting carbohydrates from dog diet is practiced although this has raised some issues. For example, it has been found that excessive phosphorous intake from mainly protein meals increases risk of developing kidney disease in aging dogs (Beynen, 2015).

On the other hand, there is a growing interest to include carbohydrates and particularly fibre in dog diets to improve stool quality and health. For example, Wichert et al. (2002), studied the effects of different celluloses on stool quality and found that stool quality was greatly improved with celluloses that had fibre lengths of 60-300µm.

2.4.1 Starches

Starches as a group are the cheapest and most widely sought-after hydrocolloid in the food and industrial sectors. In broad scope, starch is widely abundant in nature and many varieties exist in grains, roots or tubers and their functionality in foods may differ according to their botanical origin (Li et al., 2003). However, for the benefit of this study, only those that have found application for use with meat products was discussed. Starches in general are recognised as fillers, used to increase firmness of products (Verrez-Bagnis et al., 1993), as well as enhancing gel strength (Kim et al., 1987). However, when it comes to meat emulsions, the role of starch is to induce a stronger heat induced protein network (Carballo et al., 1996). Dexter et al. (1993), reported that the interaction of starch with batters were influenced by starch type, water to starch ratio and the presence of other components such as fat or even processing conditions. Inevitably the choice of which starch to use is dependent on its role to increase a firm protein network which would likely increase the binding strength of the product.

Many different starches are used in foods, but not all have the same functionality. For example, the swelling power of starch is an index for use in making noodles (Konik et al., 1992). While this characteristic reveals a critical factor for consideration, the full index of screening starch properties is still being investigated. To do this, one would need to investigate the rheological characteristics of starch/meat complexes as carried out by Li and Yeh (2003). Interestingly, from their investigation, the authors concluded that swelling power of starch was an ideal index for selecting the type to be used for starch/meat complexes. Investigation of corn, potato and pea starches for their suitability in meat patties, revealed that pea starches were ideal at a 5 % inclusion which yielded lower cooking drip loss (moisture retention) while acting as meat binder (Kilincceker, 2018). However, due to reported health concerns surrounding starch in dog foods it was not considered for use in this project.

2.4.2 Plantain Peel Flour

Plantain (cooking banana) is used in third world countries mainly as a staple food. Recent use of plantain peel flour as a binder in Frankfurter type sausages showed that it was successfully able to increase water holding capacity without effecting emulsion stability and pH at a substitution of 25 % wheat flour (Rosero-Chasoy et al., 2017). According to Arun et al. (2015) and Emaga et al. (2007), plantain peel was reported to contain the following nutrients and minerals provided in Table 2-4.

Table 2-4: Macronutrient and selected vitamin and mineral content of plantain flour and plantain peels.

Plantain Peel Flour (Composition)	Plantain Peel (vitamins)	Plantain Peel (minerals)
Protein (5.89 %)	Ascorbic acid (0.08 mg/100 mg)	Potassium (35.61 mg/100 mg)
Fat (5.12 %	Riboflavin (0.065 mg/100 mg)	Calcium (28.63 mg/100 mg)
Ash (7.83 %)	Niacin (0.12 mg/100 mg)	Sodium (14.49 mg/100 mg)
Carbohydrate (11.03)	Folic acid (33.12 mg/100 mg)	Iron (6.96 mg/100 mg)

Plantain peel has a protein content of (8-11) %, with the essential amino acids leucine, valine, phenylalanine and threonine existing in significant quantities (Emaga et al., 2007). These essential amino acids are part of the ten essential amino acids required by dogs (Agar, 2001). Plantain peel was also reported by Agar (2001) to contain high levels of fibre (40 - 50 %).

Further to its potential as a binder, plantain peel flour also possesses high natural antioxidant properties due to its phenol content (Agama-Acevedo et al., 2016), and has anti-ulcerogenic properties as shown in an aspirin-induced stomach ulcerations model in rats (Best et al., 1984).

In respect to its use traditionally as a stomach soothing agent, research has shown the potential of plantain as an ingredient in formulating dog food due to its unique antioxidant properties, and high calcium and potassium levels which are available to bind with carrageenan and alginates for stabilisation of freeze thaw operations in dog roll manufacturing and storage.

Hypothetically, plantain or plantain peel may possibly be an indirect food source for dogs, given their behaviour in scavenging intestinal remains of their prey (e.g., pigs, birds), who directly consumer plantain through natural foraging prior to becoming prey to wild dogs. Such investigations would prove highly feasible for such a commodity to be incorporated into dog rolls given its many positive characteristics of antioxidant activity, binding ability and being an important carbohydrate (fibre) source. An evaluation of the viability of natural plantain as an ingredient in dog food should be carried out.

2.5 Natural Antioxidants

The most common form of chemical spoilage in meat products occurs due to lipid oxidation brought about by factors such as meat composition, light and presence of oxygen (Kanner, 1994). Oxidation leads to the formation of certain chemicals (e.g. peroxides), which adversely affect sensory (colour, texture, flavour) and nutritional qualities of meat products which can be reduced by the activity and presence of antioxidants (Karakaya et al., 2011).

According to observations by Astridge (2000), dog rolls are limited in their shelf life at refrigerated temperatures due to the presence of hydrogen peroxide produced from the proliferation of lactic acid bacteria after approximately 9 weeks, a time at which presumably the antioxidant effect of additives (e.g., garlic or ginseng) have worn off or excess peroxides have been produced over time.

Either way, the use of antioxidants can be used to reduce or inhibit lipid peroxidation as described by Shah et al. (2014) through:

- Acting as oxygen scavengers and delocalizing free radicals.
- Decomposing peroxides.
- Reduce binding chain metal ion concentrations (precursors).

2.5.1 Natural Plant Extracts

Some of the most recent work has been directed in identifying plant extracts for potential antioxidant activity and viability for use in meat products. Compared to synthetic antioxidants on the market (e.g. BHA, BHT, TBHQ), grape seed, rosemary, cinnamon, green tea, pomegranate, pine bark and nettle have demonstrated a similar if not superior antioxidant activity (Shah et al., 2014). Other research has also reported sources of extracts from fruits, vegetables, spices, and herbs which have been identified to reduce lipid oxidation (Table 2-5).

Table 2-5: Common natural plant extracts, properties and possible applications for use in meat products.

Plant Extract	Product Tested	Property	Application	Dose	Storage Time
Green tea extract, Pepper extract (Wójciak et al., 2011)	Cured, cooked ground pork meat	Supports colour stability by inducing nitroso myoglobin formation	Cured meats (pork)	0.5 %	30 days
Grape seed extract (Mc Carthy et al., 2001; Rojas et al., 2007)	Cooked refrigerated beef and pork	Antioxidant property, effective in reducing colour (green) in refrigerated patties	Cooked refrigerated beef and pork patties	0.2 %	Refrigerated storage
Rosemary extract (Rojas et al., 2008)	Precooked chilled pork patties (21.1 % fat)	High lipid antioxidant activity	More effective compared to green tea, coffee, grape skin	200 ppm.	Retail conditions (aerobic). 10 days @ 4 °C
Tea catechins (Wójciak et al., 2011)	Raw pork patties	Antioxidant activity	More effective compared to aloe vera, fenugreek, ginseng,	-	Stored up to 9 days
Tea catechins, rosemary and sage (Wójciak et al., 2011)	Patties from previously frozen pork	Reducing lipid oxidation	Meat patties	0.25 % TC, 0.1 % R, 0.05 % S	6 days at refrigeration
Ginger rhizome (Hu et al., 2002; Mansour et al., 2000)	Ground beef patties	High antioxidant activity	Beef patties in cold storage	Activity decreases when cooked	Raw refrigerated storage
Pidgeon pea hull extract (Kanatt et al., 2011)	General	Retards Bacillus cereus. High antioxidant activity	Food general	-	-
Mint leaves (Kanatt et al., 2007)	Radiated processed lamb meat	Effective in radiated processed meats	Meats to undergo radiation processing	0.1 %	4 weeks chilled storage
Irradiated chitosan (Kanatt et al., 2004)	Radiated processed lamb meat	High reducing power over autoclaved chitosan in controlling lipid oxidation	Radiated meat products)	1 % solution	Storage at(0-3) °C

2.5.1.1 Lotus Root & Leaf Extract

Lotus exists as a dietary staple in Eastern Asia. The root is used as a common vegetable as well as a herb to stop bleeding. It has been used by ancient Chinese to package meat and improve taste. The leaf and root extracts are highly effective against lipid oxidation in meat products (Hu et al., 2002).

It is important to note that microbial inhibition by lotus plant extracts leads to an extended lag phase, increased generation time, decreased maximum growth or various combinations of these effects on microbial growth (Bahk et al., 1990; Duffy et al., 1994). Similar findings were also reported for ginseng and garlic in dog rolls.

While the recent studies of using plant extracts are increasing, it is important to choose an extract that can tolerate and be effective after processing (e.g., radiation treatment, pasteurisation) of dog rolls and be able to remain effective over prolonged storage conditions including compatibility with freeze thaw cycles.

2.5.1.2 *Moringa Oleifera Leaf Extract (MOL)*

When extracted from the leaves of mature moringa plants, moringa oleifera leaf extract (MOL) demonstrated superior antioxidant effects on cooked goat meat patties under refrigerated storage. This was due to the high phenolic and flavonoid component of the leaves. When used at a rate of 0.001 %, MOLs effectiveness in protecting goat patties against oxidative rancidity was sustained longer than synthetic antioxidants such as BHT (Das et al., 2012).

2.6 Processing

Dog rolls are heat processed to achieve a “pasteurised” state rather than being regarded as commercially sterile given the heat treatment and time of exposure. Pasteurisation is carried out to inactivate or destroy viable pathogens but may not be effective in delocalising spoilage organisms which may proliferate under incorrect storage or defective packaging conditions. Standard processing parameters are 85 ^{o}C for 30 minutes with the fresh minced meat passed though the pasteurisation process in a batch jacketed steam vessel after which it is filled hot into casings, crimped and cooled in a water bath before refrigerated storage (Astridge, 2000). Improving the freeze thaw stability of dog rolls should maintain acceptable texture, slice-ability, and reduced moisture loss (thaw drip loss) after freeze thaw cycles.

2.6.1 Freeze Thaw Stability

Dog rolls exhibit textural quality degradation after freezing which includes the release of bound moisture (and thus increasing water activity). Such characteristics limit the storage conditions of dog rolls to refrigerated storage. Currently, refrigerated storage is limited to a short shelf life of 12 weeks for current products on market (Astridge, 2000). This limitation prevents exporting of the product from New Zealand to overseas markets.

Freeze thaw stability refers to a products ability to maintain its physiochemical integrity upon exposure to freeze thaw cycles. Dog rolls are heat processed (pasteurised) products and an emphasis of this project was based on freeze thaw stability and the improvement of current physiochemical properties to minimise thaw drip loss as well as minimising the textural changes observed after such temperature abuse.

3 Baseline Evaluation of Commercial Factory Dog Rolls

3.1 Introduction

To evaluate binding strength and freeze thaw stability in the proposed dog roll prototypes, certain preliminary evaluations must be carried out on the current factory products which will be used to compare the performance of pilot-scale replicates against developed prototypes. The research was directed at evaluating factory made “chicken and pumpkin” and “beef and beetroot” samples, however, only the “chicken and pumpkin” dog rolls were used for pilot scale replication (Chapter 4) to evaluate developed prototypes using selected functional ingredients.

The physio-chemical properties of vegetables included as ingredients in dog rolls may have a potential to influence textural properties. For example, Betalain is responsible for the intense red colour in beetroot as well as providing added benefits in antioxidant and anti-inflammatory activity (Georgiev et al., 2010). Such properties may be useful in aiding post process antioxidant activity for future research.

To investigate appropriate methods of analysis, a comparison of results was carried out for the following tests and analysis:

1. Freeze thaw drip loss over one and two freeze thaw treatments.
2. Instrumental texture analysis for:
    a. Hardness using the double compression test.
    b. Cohesiveness based on double compression testing.
    c. Shear force using the Warner Bratzler Shear Force test.

3.1.1 Aim and Hypothesis Statement

The aim of evaluating the existing factory produced dog rolls was to establish baseline data to:

a) Develop methods for instrumental textural measurements and thaw drip loss before and after freeze thaw cycles.
b) Replicate acceptable control samples produced at pilot scale using same equipment and methods for developing successive prototypes.

It is hypothesised that freeze thaw treatments will increase thaw drip loss and change instrumental textural measurements such as hardness, cohesiveness and Warner-Bratzler shear force.

3.2 Materials and Methods

3.2.1 Commercial Dog Rolls

Two current market products (Perfect Grain Free Chicken with pumpkin and Perfect Grain Free Beef with beetroot dog roll; manufactured by Medallion Pet Foods (Waipukurau, New Zealand) were evaluated under treatment conditions. However, for the purpose of conducting trials for improved freeze thaw stability, just the “chicken and pumpkin” dog rolls were used on the basis that successful performance of the chicken-based dog rolls could then be tested on “beef and beetroot” dog rolls using recommended functional ingredients to conclude the study.

Figure 3-1. Perfect Grain Free Chicken with pumpkin & Beef with beetroot dog roll.

3.2.2 Thaw Drip Loss Measurement

Both product samples, wrapped in commercial packaging as shown in Figure 3-1 were placed in a freezer at -18 °C for 24 hours. The samples were then thawed over 48 hours by placing in a chiller at 5 °C. In order to make preliminary observations of thawing behaviour, samples were removed from the chiller after 24 hours (Stage 1) and cut in half (perpendicular to the length of the rolls) through the casing which was retained. Each half was placed into a sealable freezer bag and returned to the chiller at 5 °C for the final 24 hours of thawing (Stage 2). A two-stage thawing operation was necessary; the first partial thawing of the product after freezing to enable cutting open the product to expose the product surface for drip loss to migrate out of the product via the cut surface. The second phase allowed a full thaw, after which the drip loss and texture profile analysis were performed. Thaw drip loss was deduced by weight loss calculations as below.

% drip loss = $\left((weight\ before\ thawing - weight\ after\ thawing) \div weightbefore\ thawing\right) \times 100$

3.2.3 Freeze Thaw Cycles Protocol

The method to measure freeze thaw stability in dog rolls was adapted from DeFreitas et al, (1997) and adjusted to suit experimental conditions and materials available for this study as depicted in appendix A.

The following treatment conditions were applied to the samples prior to analysis:

- No freeze thaw treatment (**0 FT**) – Only refrigerated samples.
- Single freeze thaw cycle (**1 FT**) - Sample dog rolls were subjected to blast freezing conditions at -18 °C for 24 h, after which a two-stage thawing was carried out over 48 h at 5 °C. Thaw drip loss was measured after phase 2 thawing.
- Double freeze thaw cycle (**2 FT**) - Sample dog rolls were subjected initially to blast freezing conditions at -18 °C for 24 h, after which two stage thawing was carried out over 48 h at 5 °C. The freeze thaw process was repeated, i.e., the samples were submitted to a second treatment of -18 °C for 24 h, after which a two-stage thawing was carried out over 48 h at 5 °C. Drip loss was carried out following the two freeze-thaw cycle.

Measurement of the drip loss was carried out using single replicate samples as would be consistent throughout the project trials due to sample and time availability to reproduce more than one sample for analysis. Therefore, thaw drip loss behaviour was observed for a general response by the product where the amount lost after treatment can be compared generically to test samples and the outcome measured as "improved", by a decline or vice versa.

3.2.4 Instrumental Texture Analysis

Texture profile analysis (TPA) was conducted using the TA.XTPlus Texture Analyser (Stable Micro Systems, Godalming, Surrey, United Kingdom). Instrumental analysis was carried out to deduce textural changes upon freeze thaw treatments (1 FT, 2 FT) affecting textural hardness, cohesiveness, and shear force to fulfil aims as stated in Chapter 3.1.1.

Upon using the texture analyser, a force over distance curve is generated for the data displayed. Using this curve, the force over distance relationship evaluates outcomes for TPA hardness (Force 1) and cohesiveness.

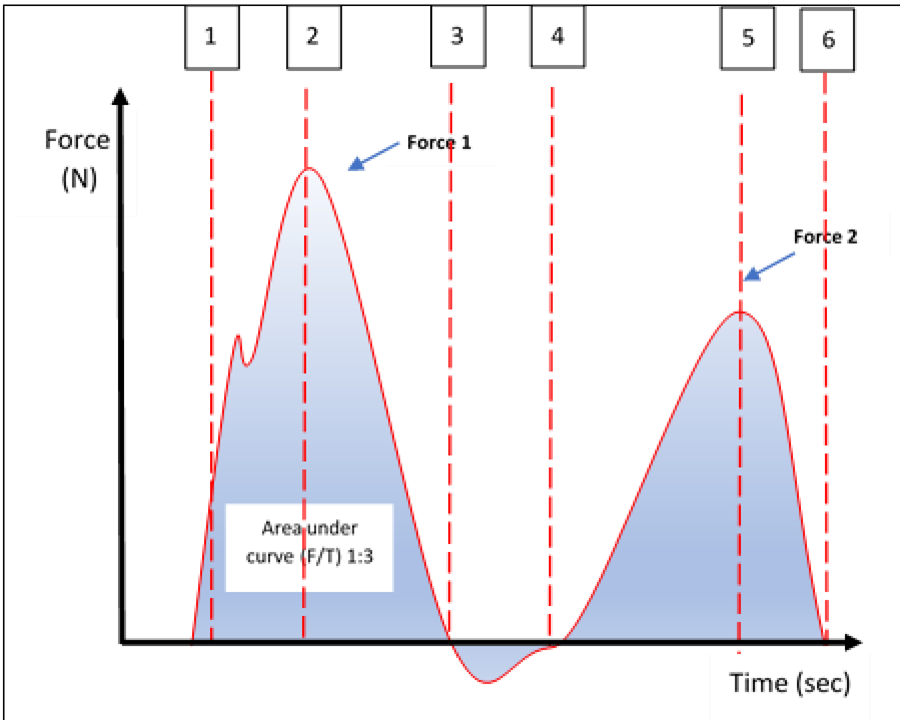


Figure 3-2: Graphical representation based on TX.XTplus texture analysis data. Force 1 indicates textural hardness.

Determining successful textural analysis profiles for hardness, cohesiveness and shear force would support their potential to be used for later trials. Hardness and cohesiveness values were analysed from the double compression test using the TA. XTPlus texture analyser based on specific data generated according to Table 3.1.

Table 3-1: TA. XTPlus texture analyser data labels used for determining Hardness and calculating cohesiveness.

Hardness	Cohesiveness
N	Ratio
Force 2	Area F-T (4:6)/Area F-T (1:3)

The settings for the TA.XTPlus texture analyser are given in Appendix A2.

Cubed product samples for instrumental textural analysis were prepared using a double-edged knife (Fig 3-3)

Figure 3-3: Test samples for TPA analysis prepared using a double-edged knife to obtain cubes approximately 2 cm^3.

3.2.5 Warner Bratzler Shear Force (WBS)

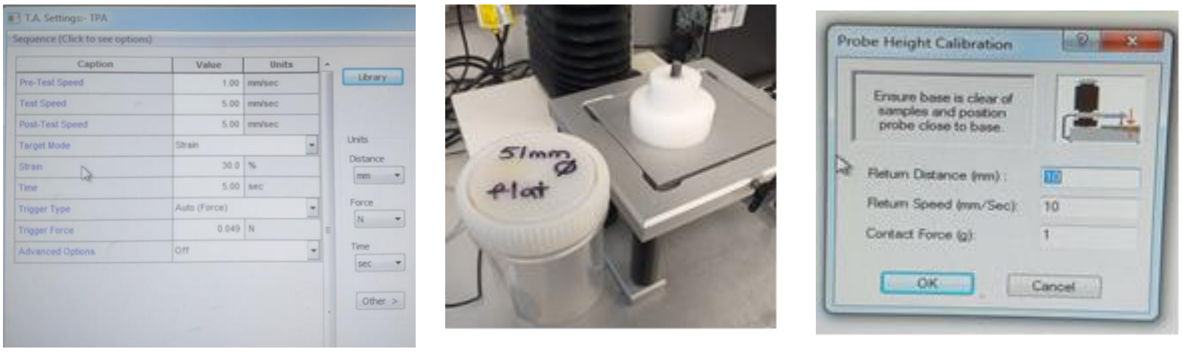
Mechanical shear force assessment using the Warner-Bratzler shear force test (WBS) is the common approach to measuring beef tenderness and has been shown to correlate with trained sensory panel evaluation (Caine et al., 2003). It is hypothesised that the binding strength of product after freeze thaw treatments will decrease and result in lower WBS compared with non-freeze thawed samples.

The shear force measurements were carried out using the Warner-Bratzler inverted *v* shear blade mounted on a TA. XTPlus texture analyser using a 5 kg load cell. This was used to measure cross sectional cutting force as an indication of binding strength. All samples were equilibrated to room temperature for at least two hours prior to testing. Ten blocks were prepared for instrumental texture analysis for each treatment. The warner Bratzler shear force analysis was carried out using a test speed of 2 mm/sec, a pre-test speed of 1 mm/sec and a post-test speed of 2 mm/sec. The crosshead distance was 45 mm with a trigger force of 0.049 N.

3.2.6 TPA Hardness

Textural hardness was evaluated using 10 samples submitted to the double compression test via the TA. XTPlus texture analyser with a 5 kg load cell. The double compression test based on texture analysis replicates the mastication process carried out by the mouth and returns mechanical properties of physiological changes occurring in the tissue of the food (Herrero et al., 2008). Hardness is defined as the maximum peak force for the first compression and relates to the product's resistance to deformation under standardised conditions (Peleg, 1976). Using these observations, I tested the hypothesis that freeze thaw treatment will reduce textural hardness.

Double compression testing of samples was carried out using a circular 51 mm diameter compression gauge to apply a bite force of 0.049 N for 5 s (Fig 3-4). The test speed and post-test speed were set at 5 mm/s and a strain of 30 % was applied. The instrument was calibrated with a load cell of 4.8 kg prior to analysis. A full reference of the standard operating procedure for the TPA analysis is provided in appendix A2.



T.A. Settings:- TPA

Sequence (Click to see options)

Caption	Value	Units
Pre-Test Speed	1.00	mm/sec
Test Speed	5.00	mm/sec
Post-Test Speed	5.00	mm/sec
Target Mode	Strain	
Strain	30.0	%
Time	5.00	sec
Trigger Type	Auto (Force)	
Trigger Force	0.049	N
Advanced Options	Off	

Units
Distance: mm
Force: N
Time: sec

Probe Height Calibration

Ensure base is clear of samples and position probe close to base.

Return Distance (mm): 10
Return Speed (mm/Sec): 10
Contact Force (g): 1

Figure 3-4: Double compression test setting, compression gauge and calibration for TA. XTPlus texture analyser.

3.2.7 TPA Cohesiveness

Cohesiveness is defined as “the degree to which sample deforms before shearing” (Chen et al., 1991). Cohesiveness when relating to mechanical action by instrument was described originally by Szczcniak et al. (1963) as the “strength of internal bonds making up the body of the product.”

Cohesiveness is therefore measured as the work done in the second compression divided by work done during the first compression or “bite”. This work is measured as force over time (Szczesniak, 1963), and is determined from TPA double compression results from the TA. XTPlus texture analyser data log:

*Cohesiveness = (Area F-T 4:6) / (Area F-T 1:3) (*see Fig 3-2)

Therefore, cohesiveness in this sense is a dimensionless ratio and the measure of cohesiveness can be the best indication of binding strength (internal bonds that make up the body of the product) including how well the product withstands a second deformation relative to its resistance under the first compression (Bourne et al., 1978).

In accordance with Bourne et al. (1978), a small change in cohesiveness will mean less difference in binding strength affected after each treatment. This will represent a successful indicator of acceptable cohesiveness.

3.3 Results and Discussion

Both commercial products displayed evidence of increased thaw drip loss following repeated freeze thaw treatments as shown in Figure 3-5. Visual evidence also displays evident syneresis after freeze thaw treatment (Fig 3-6). After 1 freeze thaw treatment (1 FT) both products showed a thaw drip loss of about 0.7 % compared to the unfrozen treatment samples (0 FT) which had near 0 % drip loss. The second freeze thaw cycle resulted in more purge resulting in a further increase in thaw drip loss of up to 1.6 % for the beef and beetroot roll and approximately 2.4 % for the chicken and pumpkin roll. This demonstrates that the extent of drip loss for both products were increased with repeated freeze thaw treatments. This behaviour validates the hypothesis put forward and supports the use of thaw drip loss as a useful indicator to measure improved product stability after exposure to freezing and subsequent thawing operations.

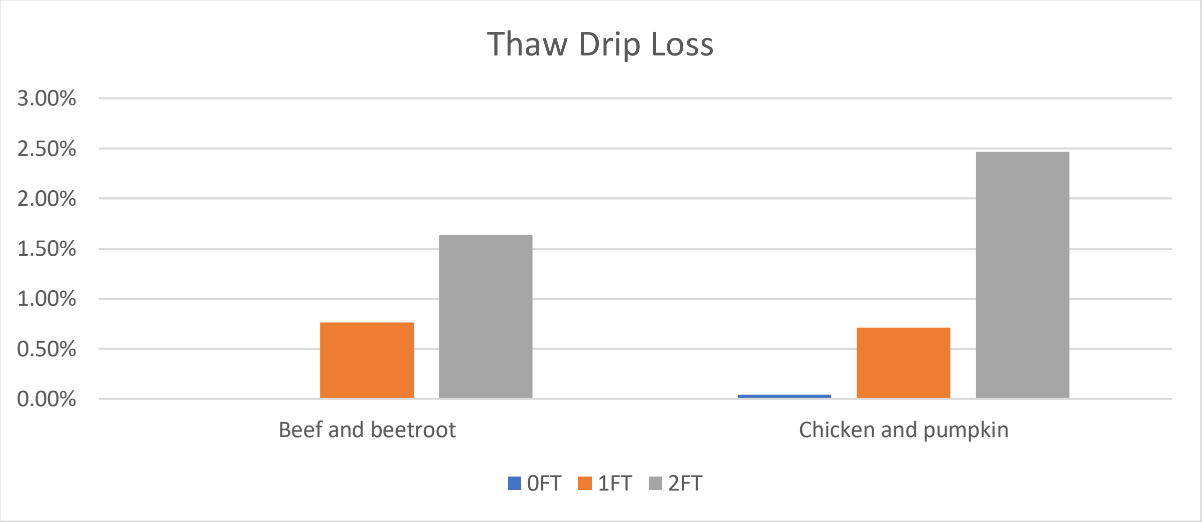


Figure 3-5: Thaw drip loss for commercial chicken with pumpkin and beef with beetroot dog rolls after 0 (O FT), 1 (1 FT) and 2 (2 FT) freeze-thaw treatments.

Figure 3-6: Factory dog roll samples exhibiting thaw drip loss after the freeze thaw treatments.

The Warner Bratzler shear force measurements are given in Figure 3-8 and show higher values for the beef and beetroot rolls than for the chicken and pumpkin rolls. However, there were problems as the non-uniformity of both products affected the results. Vegetable and pieces of intact whole meat tissues in the samples (Fig 3-7) caused higher hardness measurements when the blade sliced through these, but low values were observed when the blade did not pass through these components. This occurrence is supported by the high mean standard error for each treatment ranging from 2–9.75. Although the beef and beetroot dog rolls showed a higher shear force value over its chicken and pumpkin counterpart, the validity of the analysis remains inconclusive due to interference by vegetables and whole muscle tissue in products. Therefore, the Warner Bratzler shear force proved an unsuitable method of analysis for these products.

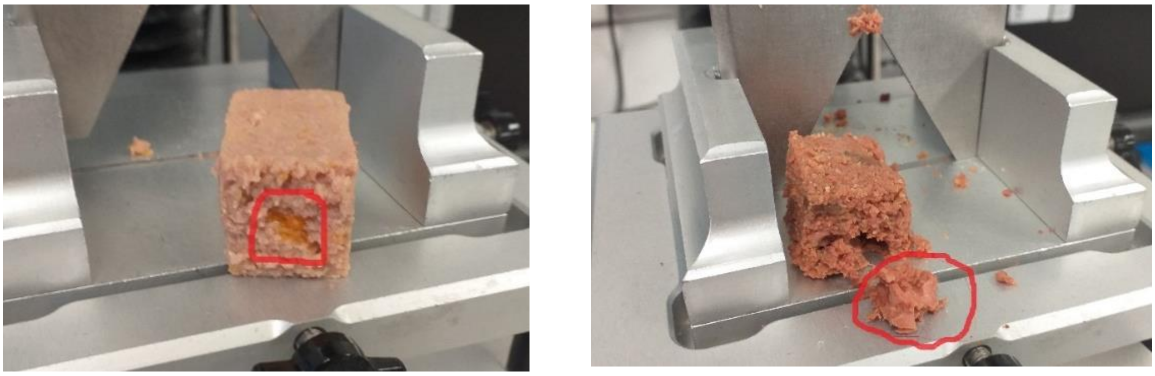


Figure 3-8: Samples causing overload for the Warner Bratzler measurements with chicken and pumpkin (left) and beef and beetroot (right) dog rolls.

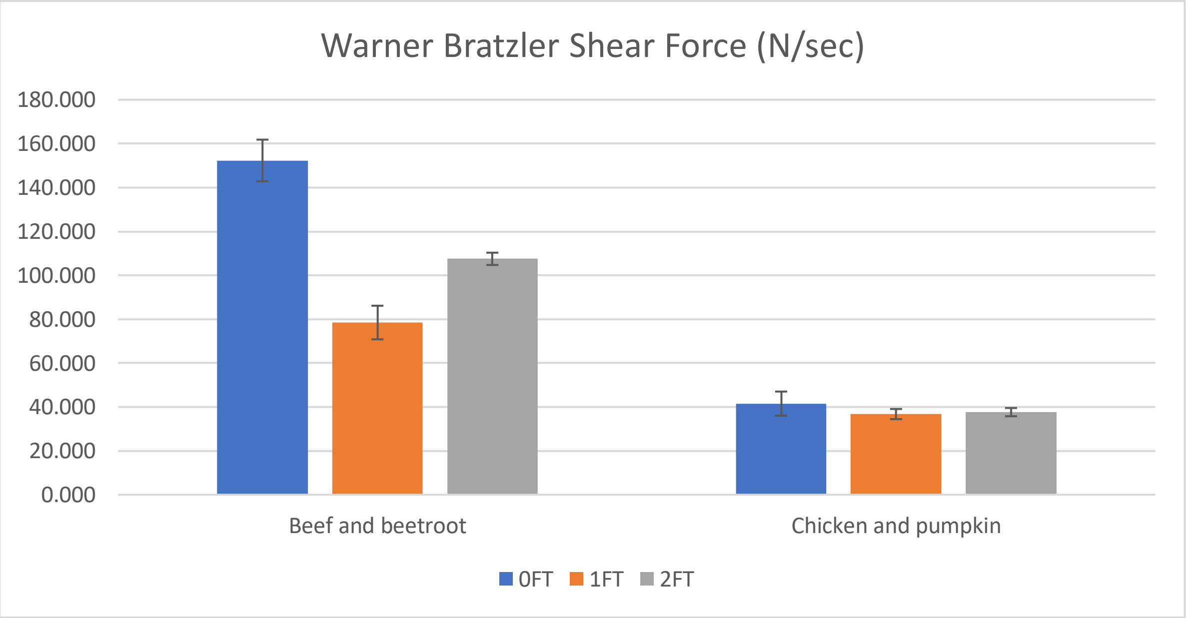


Figure 3-7: Warner Bratzler shear force comparisons for Factory made chicken and pumpkin and beef and beetroot dog rolls.

Texture profile analysis (TPA) for hardness is given in Figure 3-9. The results show initially that the beef and beetroot roll displayed a higher hardness, but both products showed a successive reduction in hardness after freeze thaw treatments. This TPA behaviour after freeze thaw cycles is consistent with the observed loss in texture that is anecdotally observed and prevents the use of freezing as a method of preservation for these commercial products. Also, the variation between replicate TPA testing is small and gives confidence moving forward that this analysis as appropriate for this study.

The TPA for cohesiveness is given in Figure 3-10 and all samples showed a cohesiveness of less than 1, indicating that the area under the second compression curve is lower than the first compression curve. This was expected as the first compression causes permanent damage to the structure of the sample that is then less able to resist the second compression. However, the cohesiveness was found to increase with freeze thaw cycles which counters the observed “loss” of texture with freeze thaw cycles. The increasing cohesiveness was due to the lower peak of the first compression curve of the TPA cycle after freeze thaw treatments resulting in higher ratios of the area under the second versus first compression curve and apparent increase in cohesiveness with increased freeze thawing. This indicated that the measurement of cohesiveness was not a useful method to assess performance under freeze thaw cycles so was discarded from further analysis.

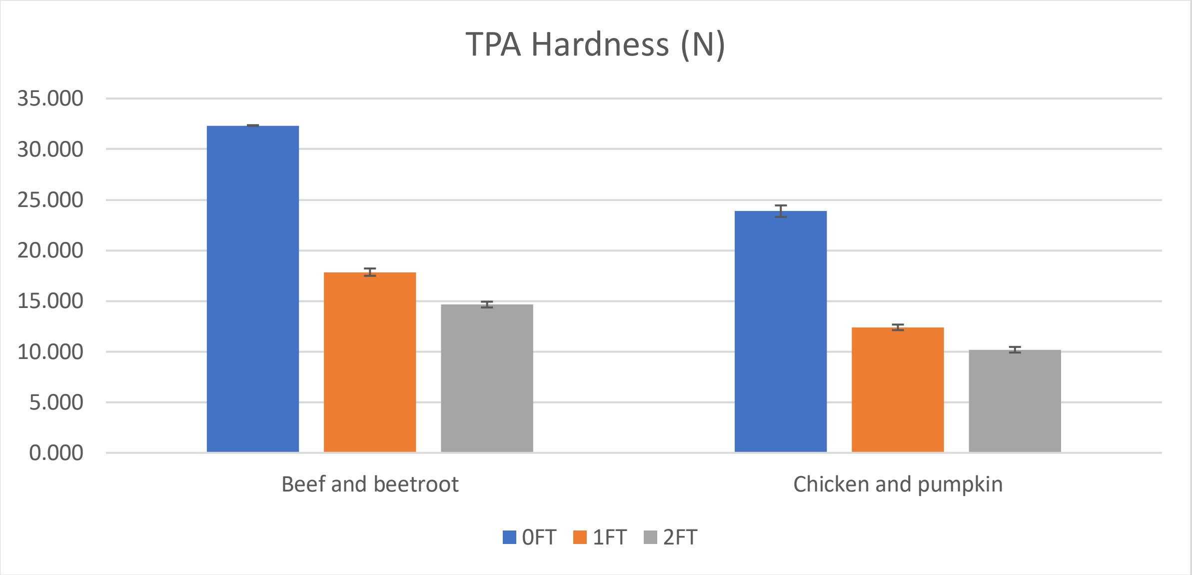


Figure 3-9: TPA hardness showing response from both factory samples after 0, 1 and 2 freeze-thaw treatments. Mean standard error is low (see appendix B1).

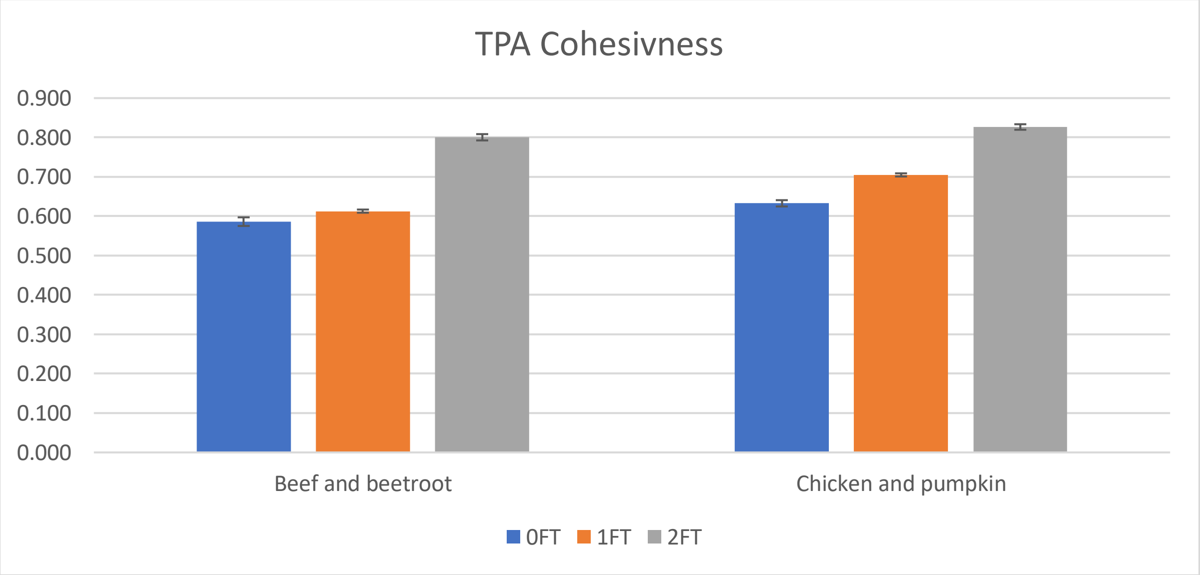


Figure 3-10: Cohesiveness showing similar variation upon 0, 1 and 2 freeze-thaw treatments. Mean standard error is less than 0.1 (appendix B1).

3.4 Conclusion and Recommendations

The thaw drip loss and TPA hardness are good instrumental measures to be applied for evaluation of freeze thaw stability performance of the dog rolls in later prototypes. TPA hardness showed good repeatability and is consistent with the observed 'loss of texture' observed following freeze thaw treatments.

It was concluded that the Warner Bratzler shear force method was not an accurate method to assess the dog rolls as the presence of the vegetables chunks and whole meat pieces influenced the results of the shear force texture profile analysis.

TPA cohesiveness was also not a suitable measurement for analysing the effect of freeze and thaw cycles on dog rolls.

Following the conclusion of baseline measurements, it is recommended that:

- continuation of the evaluation methodology was to include thaw drip loss and texture profiling for hardness while abandoning the TPA cohesiveness and Warner Bratzler shear force measurements.

4 Product Replication on Pilot Scale

4.1 Introduction

Following baseline testing of factory-made products, a pilot scale production process was then carried out to replicate the performance of the factory product using pilot scale equipment after which its performance was tested applying the analysis methods used in Chapter 3. This step was necessary to compare any differences due to reproducing the dog rolls on a smaller scale compared to a large-scale commercial production. The successful outcome of this pilot scale replication process was to establish sample viability to be used as the "controls" for comparison of all future product prototypes to deduce the best outcome for low thaw drip loss and acceptable instrumental textural hardness performance. At this stage, only the chicken and pumpkin dog rolls were replicated before evaluation against potential test prototypes using selected functional ingredients to improve freeze thaw treatment outcomes.

4.1.1 Aim

- To validate the use of equipment selected for pilot scale production of dog rolls on site.
- Obtain analytical data comparable to previous baseline measurements to form a control baseline data for reference with prototype development.
- Observe any difference for a pilot scale replication process that may affect accuracy of results in comparison to factory products.

4.2 Materials and Methods

Processing of dog rolls requires a pasteurisation process at 85 °C. Factory processing methods involve placing the comminuted chicken meats, offal and additives into a processing vessel that provides indirect heating while undergoing agitation until it reached 85 °C. The temperature inside the vessel is maintained at 85 °C for a holding time of 15 minutes under agitation. After 15 minutes, a binding agent is added together with pumpkin then held at 85 °C under agitation for a further 10 minutes prior to pumping and filling into casings that are then sealed with metal clips and cooled by spraying water over the surface of the packaging.

A process flow chart for the Massey process to simulate the industrial process is provided in Figure 4-1. The heat processing equipment used was the *Blentech CC10* (Fig. 4-2) that contains a chamber with two rotating screws laid horizontally which provides mixing and agitation. Heating to the chamber is provided by steam which can be directly injected into the chamber or can be used to indirectly heat the chamber through the enclosing metal jacket. Indirect heating was used for all experimental work.

Filling of products was completed immediately after the final stage of heat processing while the meat mixture was still hot. Filled products weighed approximately 0.8 kg in synthetic casings that were smaller than the commercial product and were sealed using a Tipper tai clipping tool from Press Tai® (Fig. 4-3) then cooled in an ice water bath rather than a water spray for practical reasons.

Replication of dog roll base formulations and ingredients were provided by Medallion Pet Foods to mimic a miniature batch assembly of current products (Table 4-1). Chicken meat consisted of a blend of mechanically deboned meats and a range of offal that were pre-blended by Medallion according to their recipe frozen and stored at -18°C and then shipped to Massey University. Medallion use frozen meat in their batch thereby mimicking the process was carried out identical to the factory operation.

The blend is not provided here due to commercial sensitivity. Immediately on arrival to Massey University the frozen chicken meat was placed into storage at -18 °C at Massey University and enough meat for a batch was thawed prior to processing. Pumpkin was provided diced and stored at -18 °C then thawed prior to use. Kappa carrageenan, locust bean gum powders, a vitamin premix, preservatives, and vegetable oil were also provided by Medallion in sealed packages and stored at ambient room temperature prior to use.

Table 4-1: Replicate product formulation for chicken and pumpkin dog rolls.

Pilot Replicate Formulation	
Ingredients	**% Composition (w/w)**
Chicken meat	*75.09 %*
Water	*16.22 %*
Pumpkin	*7.00 %*
Preservatives	*0.53 %*
Kappa Carrageenan	*0.57 %*
Essential vitamins / nutrients	*0.43 %*
LBG	*0.14 %*
Total Batch Composition	**100 %**
Control 1	

4.2.1 Standard Processing Methodology

Processing of chicken and pumpkin dog rolls was replicated based on factory methods. Ingredients were weighed to constitute a 3.5 kg batch weight. The process involved initially placing the chicken meat, dissolved salts and preservatives using approximately 40 % of the water and vegetable oil into the Blentech steam cooker then starting the mixing and heat processing via indirect steam injection to reach a chamber temperature of 85 °C. A further 15 minutes holding time was added to the cook time whilst maintaining the pasteurisation temperature of 85 °C, after which the rest of the ingredients were added including the hydrocolloid emulsion prepared using the remaining water and pumpkin. Ten minutes of extra holding time was included to the total cook time after which the product was ejected from the chamber and filled hot into casings, clipped, and immediately placed into an ice-filled water bath.

Total processing time included 15 minutes come up time to reach 85 °C, another 15 minutes holding time at 85 °C and then after the addition of the hydrocolloid emulsion and pumpkin a further 10 minutes holding at 85 °C after adding.

- Total processing time = 40 minutes
- Total holding time @85 °C = 25 minutes

Full standard operating procedure using the Blentech including cleaning and sanitation of the Blentech were carried out according to SOP indicated in appendix C.

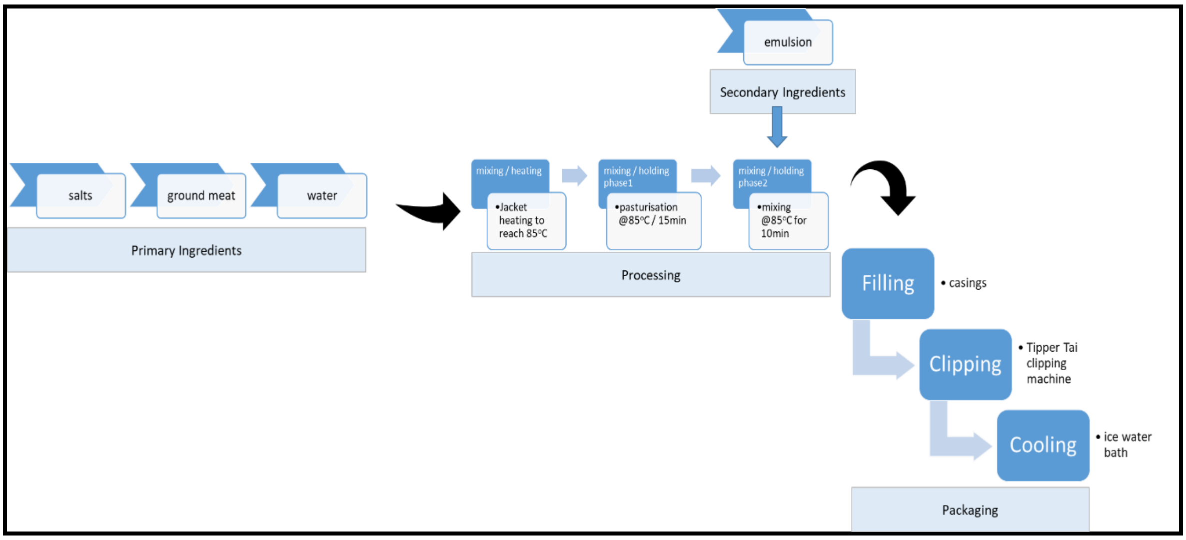


Figure 4-1: Process flow diagram for pilot plant dog roll prototypes at Massey University, Palmerston North, New Zealand.

4.2.2 Processing Equipment

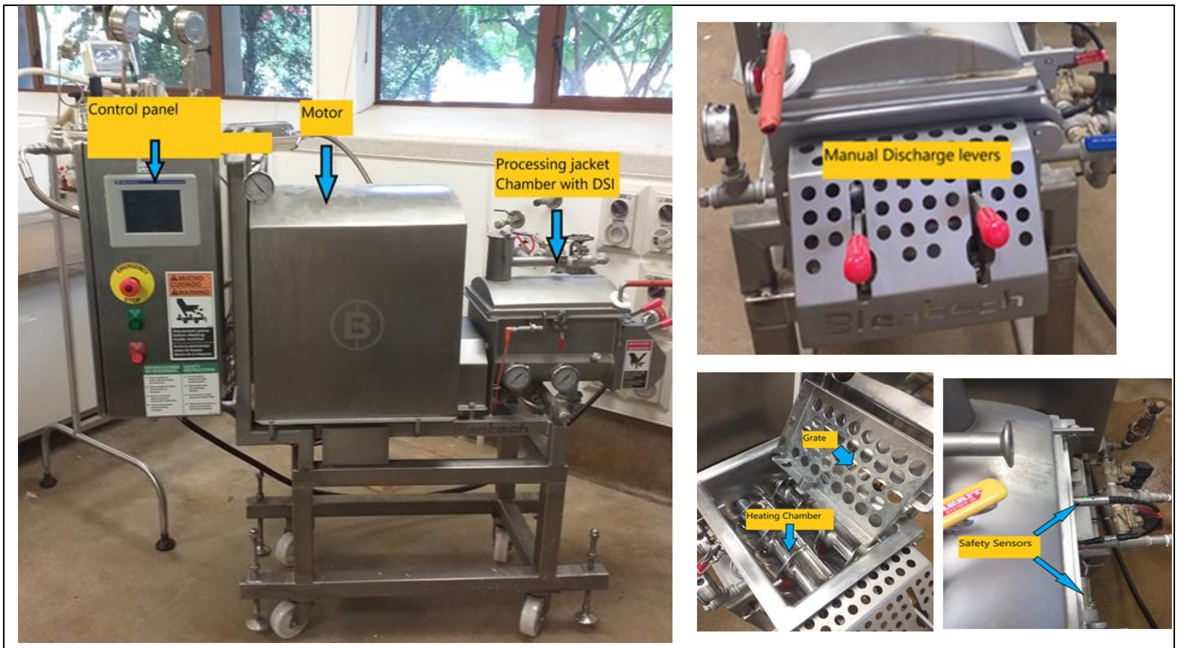


Figure 4-2: The Blentech CC10 Steam injected pasteuriser.

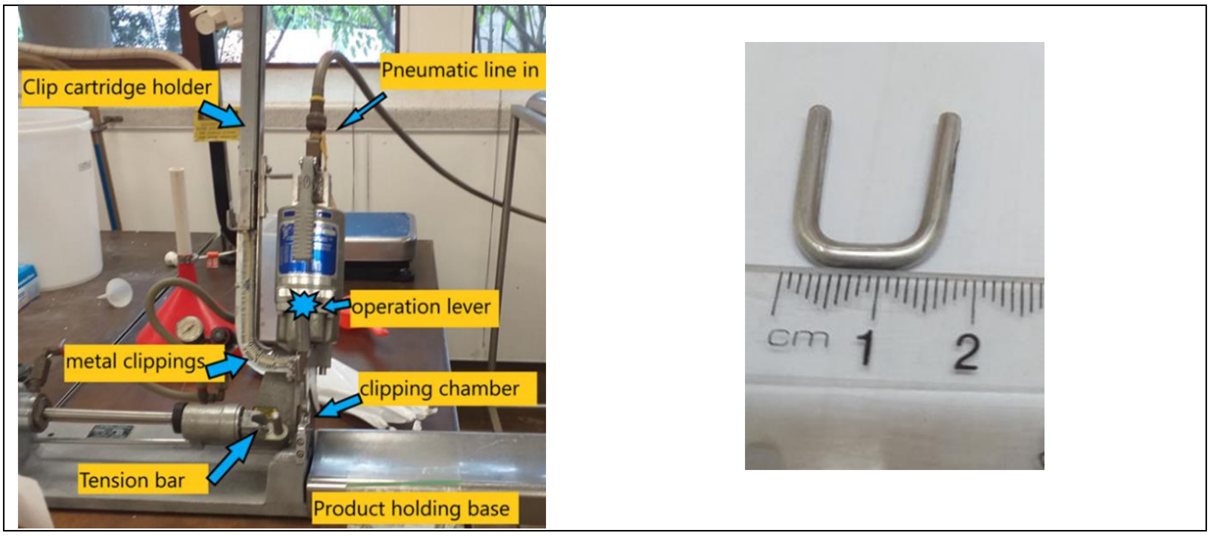


Figure 4-3: Tipper Tai manual clipping tool from Press Tie®. Metal clips are used for sealing casings after product is "hot" filled.

4.3 Results and Discussion

4.3.1 Thaw Drip Loss

Comparative results between the factory product and pilot scale replicated samples exhibited a similar trend with an increase in thaw drip loss after successive freeze thaw treatments (Fig 4-4). The initial thaw drip loss prior to freezing was slightly higher for the pilot replicate than the factory product. There was also greater thaw drip loss after 1 FT cycle for the pilot replicate. Although a larger increase was observed for factory products after a second treatment (2 FT), this behaviour may have played out due to the difference in product sample sizes since the factory products weight approximately 2 kg whilst the replicated samples produced on a pilot scale weighed about 0.8 kg. The variation in sample sizes may have affected the freezing process independent of both samples. As the freezing and thawing periods were carried out over a 24 h periods in different pack sizes, it is expected that complete thawing will have occurred in both samples. The difference in thaw drip observed may have reflected the different surface area to volume ratio and different distance to the centre of the rolls due to the differences in roll dimensions.

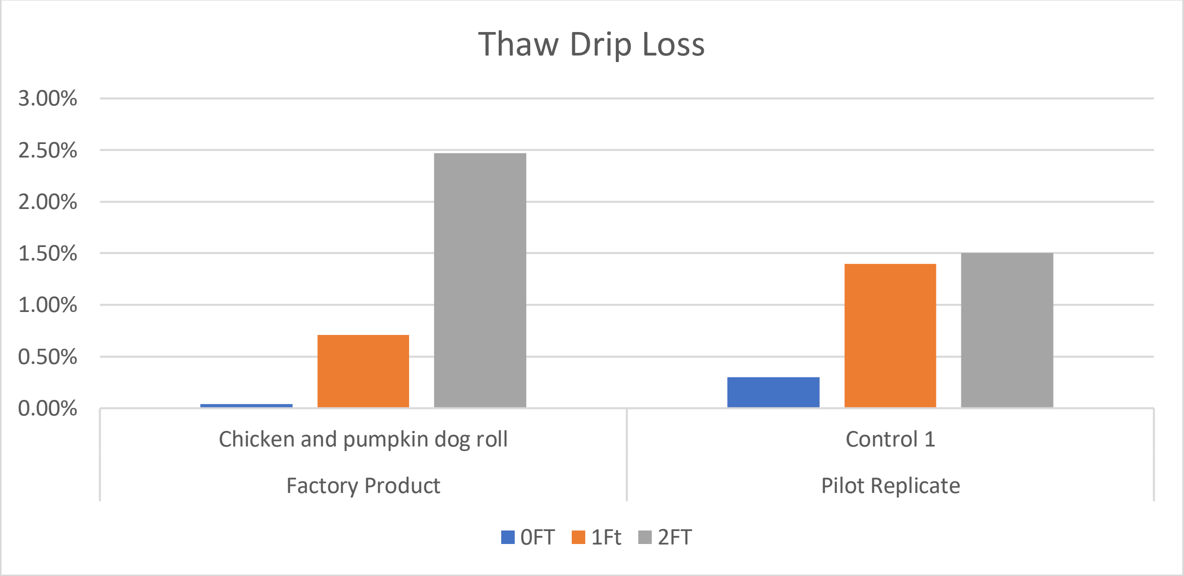


Figure 4-4: Thaw drip loss comparisons between factory made and pilot replicate chicken and pumpkin dog rolls.

4.3.2 Instrumental Texture Profile Analysis – TPA Hardness

TPA hardness for samples (Fig 4-5) showed similar behaviour with a decrease in hardness with successive freeze thaw treatments. Similarly, the difference in change of textural hardness remained smaller between 1 FT and 2 FT treatments. The hardness prior to freeze thaw cycles was higher for the factory samples and the higher hardness was seen after both freeze thaw cycles. Again, these differences may have been brought about by the fact that the pilot plant samples were produced using a smaller batch size (3.5 kg) than at factory level where batch weights are equal to or exceed 1000 kg. Also, the factory product and pilot scale product were made with different batches of raw materials. While the absolute value of hardness is different between the factory product and pilot replicate, the pilot plant replicate samples behaved similarly to factory made products upon subjecting to freeze thaw treatments.

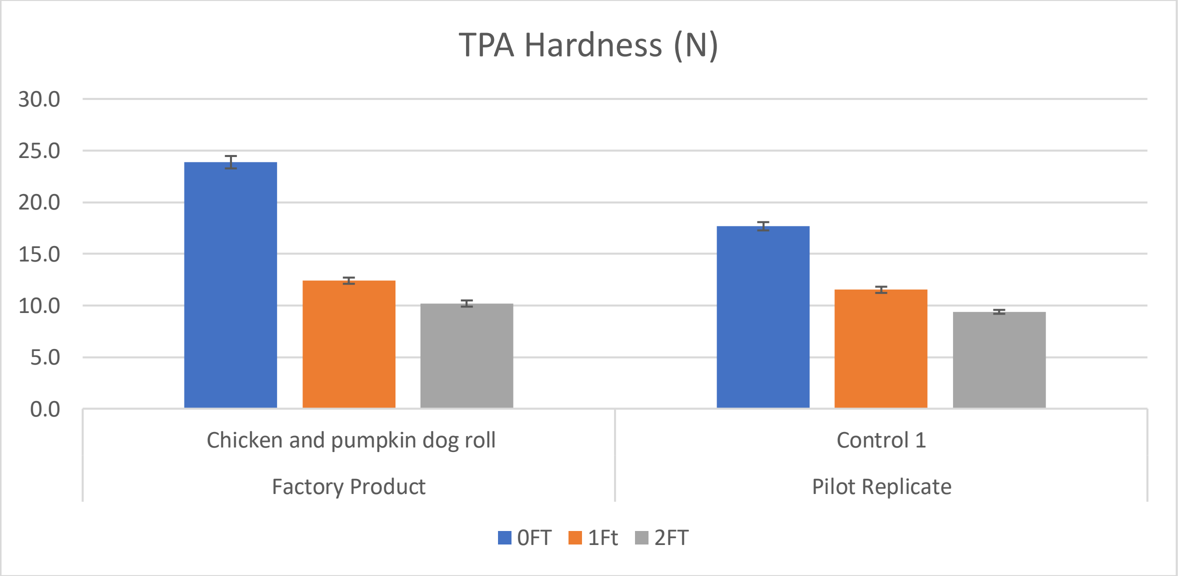


Figure 4-5: TPA hardness displaying similar response between factory and pilot scale replicates.

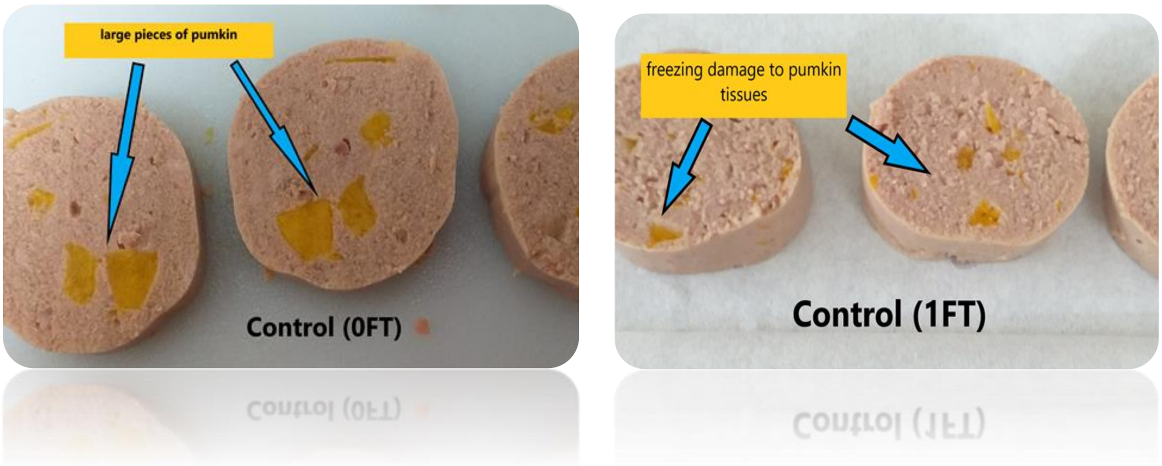


Figure 4-6: Pilot plant samples showing effect of freezing on the pumpkin in product.

Figure 4-6 compared the pilot plant samples before and after 1 freeze thaw treatment. There was a shrinkage in the overall structure, likely due to the moisture loss. Interestingly there were changes in both the meat structure and the vegetables which was likely due to ice crystal formation within the cell walls of the pumpkin and structures within the meat tissue leading to decreased textural hardness in products after freezing.

While hardness and thaw drip loss values were different between the dog rolls from the factory product and the pilot replicate, they were alike and displayed a similar behaviour when subjected to freeze thaw cycles. Thaw drip loss was found to increase with successive freeze thaw cycles while TPA hardness decreased with successive freeze thaw cycles for both factory and pilot plant replicates.

The difference between the factory and the pilot scale replicate could be due to the differences in raw materials in particular, the meat component which were from different batches, the effect of process scale or the difference in equipment.

4.4 Conclusion and Recommendations

Both the factory product and pilot scale replicate show similar behaviour with increased thaw drip loss and reduced TPA hardness with successive freeze thaw cycles. However, there were differences in values for TPA hardness and thaw drip loss.

It was concluded that the pilot scale process would be used to compare formulations with different binders to the current formulation using the same batch of meat, with samples compared using thaw drip loss and TPA hardness before and after freeze thaw cycles.

5 Product Development and Testing – Chicken and Pumpkin Dog Roll

5.1 Introduction

This chapter investigated possible functional ingredients that meet a selection criterion outlined in Table 5-3 in an effort to improve freeze thaw stability in dog roll formulations.

The following product prototypes were developed for the chicken and pumpkin dog rolls and trialled for their response to a 2-stage freeze – thaw treatment at -18 °C/5 °C. Following recommendations from Chapter 4.4, the performance of prototypes produced at pilot scale were assessed by differences between 1 FT and 2 FT in all tests prior to scaling methodology back to implementation of only a single freeze thaw treatment to observe performance under treatment.

It was considered that a two-stage freeze thaw treatment (2 FT) comparison was necessary to deduce the best outcome comparisons for initial trials to the control pilot scale prototype to achieve a better overall evaluation in performance of selection of potential candidates to improve freeze thaw stability. Such assessments will consider retaining of acceptable TPA hardness and thaw drip loss as well as overall performance (visual and physical acceptability) to be assessed after a single (1 FT) and double (2 FT) freeze thaw treatment.

5.1.1 Aim

Develop and test chicken and pumpkin dog roll prototypes using selected functional ingredients to:

a) Improve freeze thaw stability and increase performance based on current factory product (Pilot scale replicates).

b) Optimise levels of functional ingredients for use to obtain maximum effect for chicken and pumpkin dog rolls.

5.2 Considerations for Product Development

While individual hydrocolloid gums are applied extensively in meat products as emulsifying, thickening or gelling agents, a combination of two or more may be important for investigating their known synergistic effects with other hydrocolloids as the basis for improving freeze thaw stability in dog rolls.

From literature review of possible candidates, Table 5-1 lists the main choice of additives which hold potential to improve the freeze thaw stability of current dog roll products.

Table 5-1: Proposed functional ingredients and combinations for improving freeze thaw stability in dog rolls.

Formulation	Ingredient	Beneficial Properties of Interest
1	Curdlan, Xanthan gum	Hydrogels involving this combinations stabilised syneresis up to 5 FTT cycles(P. Williams, 2011).
2	Guar gum	Guar gum displays good FTT stability in frozen products with ability to reduce ice crystal formation (Mudgil et al., 2014).
3	Xanthan, LBG	Performs well in surimi emulsion gels at 0.25/0.75 with xanthan gum: LBG (Ramırez et al., 2002).
4	Iota carrageenan, LBG	A combination of iota carrageenan with LBG, improves freeze thaw stability in minced ham (Sepúlveda Cossio et al., 2013).
5	Sodium carboxy methylcellulose	Improves freeze thaw stability when used with gum Arabic by controlling ice crystallisation in bread dough (Asghar et al., 2006).
6	Sodium tripolyphosphate (STPP)	Reduces thaw drip loss in pork sausages when used in combination with carrageenan (DeFreitas et al., 1997).
7	Gelatine	Used as meat binder due to good water binding properties and stabiliser for ice creams (Poppe, 1992).
8	Cyclodextrin	Improves freeze thaw stability of carrageenan gels(Yuan et al., 2016).
9	Sodium alginate	Improves freeze thaw stability of hydrogels in the presence of calcium ions (Onsøyen, 1997).

5.2.1 Commercial Considerations for Ingredient Selection

As this project was centred on investigating the freeze thaw stability of current commercial dog roll products, the business viability was an important aspect of consideration alongside the functional properties of the ingredients. While the economic viability of choosing the right ingredients was important, consumer expectations and general perception of safe and acceptable ingredients also formed key considerations for the successful outcome. Key product attributes were discussed with Medallion Pet Foods and highlighted in Table 5-2 below.

5.2.2 Consumer and Product Considerations

Table 5-2: Key considerations for developing the Medallion brand of freeze thaw stable dog rolls (personal communication, Alastair Haliburton, Medallion Pet Foods - March 2020).

Product Attributes	• Freeze thaw stable exhibiting minimal thaw drip. • Minimum change of texture upon freeze thaw treatments. • Texture – dense clean break on flexing. • Smooth and shiny product appearance. • Ready to eat (RTE).
Product Formulation	• Needs to remain a meat dominant product. • Gluten free. • Starch free. • Ingredients are from a natural source. • Ingredients that are easily understood by consumers.

These considerations form the commercial aspect involved with developing a commercially acceptable product that meets customer expectations as well as satisfying business goals.

5.2.3 Product Development Strategy

The ultimate project goal was to identify an additive, or combined additives that would improve the stability of current dog roll products. This goal would be achieved through a combination of reducing thaw drip loss whilst exhibiting acceptable minimal textural changes during freezing and subsequent thawing operations.

Certain likely candidates of binders, gelling agents and other functional ingredients have been identified through literature review originating from both animal and plants including identified salts with potential to interact on a molecular level with ingredients and produce a product with the desired outcome.

In considering types of functional ingredients for use, the potential for that ingredient or additive to suit the processing method of dog roll production was also considered. As such, selected functional ingredients with a potential to improve freeze thaw stability and reduce syneresis were selected from a list of hydrocolloids. Initially, these hydrocolloids were selected based on being available locally as common pet food ingredients and their reported potential to improving freeze thaw stability in other meat-based products of similar physiochemical properties (e.g., meat-based luncheon rolls). From this, a selection criterion was drawn and presented in Table 5-3.

Table 5-3: Selection criterion for potential additives to improve freeze thaw stability of dog rolls.

INPUT	Quality Control	✓ Non starch based. ✓ Non grain based (Gluten free). ✓ Non legume based.
	Suitability as Binder (properties)	✓ Ability to form gels through binding of free. water or interaction with protein. ✓ Good freeze thaw stability. ✓ Ability to set or tolerate temperatures of up to 80 °C without losing functionality. ✓ Can be used at low dosage <1.0 %.
	Commercial Viability	✓ Economical (cost effective). ✓ Accessible in New Zealand.
	Regulation (Animal health)	✓ Approved for use in dog food (AAFCO). ✓ Permitted for use in New Zealand (MPI).
OUTPUT	Product Characteristics (measurable outcomes)	➢ Maintain minimum changes in TPA hardness and cohesiveness after freeze / thaw treatment. ➢ Reduce thaw drip. ➢ Provides visual acceptability.

Using the above criteria, all candidates were assessed on their ability to fulfil the objective of identifying the ultimate functional ingredient(s) to improve freeze thaw stability and satisfy customer acceptability from a business point of view.

Key attributes in the criteria were:

a) Being subjected to the “GRAS register” for oral nutritional compounds under the Ministry of Primary Industries, New Zealand (mpi.govt.nz, 2020) and

b) included as an ingredient listed in 21 CFR part 582 referenced by AAFCO for substances generally recognized as safe under the electronic code of federal regulations (USFDA, 2020) which form the governing legislative ordinance with pet food ingredients.

Using the following selection criteria in Table 5-4 below, the use of gelatine and cyclodextrin, originally identified on a scientific basis for trials were discarded from the project as they did not satisfy acceptability criteria based on both the commercial and scientific basis. Instead, functional ingredients comprising guar gum, iota carrageenan, alginate, xanthan gum and sodium tripolyphosphate (STPP) including calcium carbonate, required for gelling by iota carrageenan and sodium alginate were to be the focus of trials as they satisfied the selection criteria on Table 5-4.

The selection criteria outlined were derived based on scientific and commercial considerations as well as legislative regulations in the selection of ingredients. The scientific basis for selection remains the basis for exploration of their effectiveness.

Table 5-4: Selection criterion used for guidance in selecting functional ingredients for testing product stability after freeze thaw treatments.

Criteria	Basis	iota CGN	guar gum	xanthan gum	Alginate	STPP	CMC	cyclodextrin	cassia gum	Curdlan gum	gelatin
Quality Control	Non - gluten base	☑	☑	☑	☑	☑	☑	☒	☑	☑	☑
	Non - grain based	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑
	Non - synthetic	☑	☑	☑	☑	☑	☒	☑	☑	☑	☑
Suitability as Binder	Ability to form gels through interaction with proteins, ions or other gums	☑	☑	☑	☑	☒	☑	☒	☑	☑	☑
	Improve freeze thaw stability in processed meat products	☑	☑	☒	☑	☑	☒	☑	☑	☑	☑
	Ability to remain tolerant to temperatures up to 85 °C without losing functionality	☑	☑	☑	☑	☑	☑	☑	☑	☑	☒
	Can be used at low dosage <1 %	☑	☑	☑	☑	☑	☑	☑	☑	☑	☒
Commercial considerations	Economical and cost effective										
	Easy to access in New Zealand	☑	☑	☑	☑	☑	☒	☒	☒	☒	☑
	Meets expectations for clean label requirements	☑	☑	☑	☑	☒	☒	☒	☑	☑	☑
Approved for use in Pet Foods	Approved CFR GRAS status under USFDA/ AAFCO	☑	☑	☑	☑	☑	☑	☒	☒	☒	☑
	Permitted for use by MPI New Zealand	☑	☑	☑	☑	☑	☑	☒	☒	☒	☑

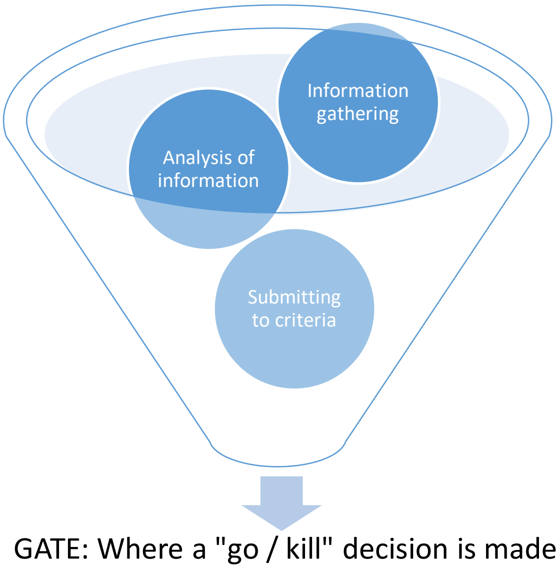


Figure 5-1: Template of model concept for approving additives for trials.

In summary, the template adopted a product development concept where each criterion was treated as a stage in the decision-making process, analogous to a stage gate model utilised for screening of potential ideas (Fig 5-1). Using the selection criteria, depicted in Table 5-4, favourable functional ingredients were screened and selected for their viability to proceed onto the prototype development stage of the project.

5.3 Testing of Selected Functional Ingredients

As stated in the aims, this project chapter focussed on identifying functional ingredients and then demonstrating analytical evidence to potentially improve freeze thaw stability and reduce thaw drip loss in dog rolls.

5.3.1 Iota Carrageenan – Trial A

According to Hotchkiss et al. (2016), iota carrageenan exhibits good freeze thaw stability. However, by itself it remains inert to synergistic interactions with other gums. The use of iota carrageenan to formulate a freeze thaw stable dog roll prototype would utilise its known effectiveness in stabilising freeze thaw stability through reducing thaw drip loss as mentioned by the above author. The only other addition apart from iota carrageenan will be to test the addition of calcium carbonate ($CaCO_3$) in the formulation which supports iota carrageenan's dependency for Ca^{2+} ions to form a gel with good freeze thaw stability. The addition of calcium carbonate was to determine if sufficient levels existed as part of the base formulation of the product (0.3 %) or if more was required (personal communication, Alastair Haliburton, 2020).

This test was to mimic optimum levels described by (Sepúlveda Cossio et al., 2013) when used in the ratio of 25:56.25:18.75 (LBG:KC:IC). The test formulation (Table 5-6) includes the current total proportions (w/w) of normal base ingredients for the current chicken and pumpkin product combined with test ingredients to a pilot scale batch weight of 3.5 kg. No extra water was added to hydrate the hydrocolloids for any of the test prototypes. For the purpose of illustrating formulations, percentage (%) composition only was presented in the product formulation tables.

Table 5-5: Optimisation of iota carrageenan with kappa carrageenan and locust bean gum (source; (Sepúlveda Cossio et al., 2013).

Trial Ingredient	Composition	Test Formulation Weight (g) / 3.5 kg Batch Weight
Locust bean gum	25.00 %	6.01
Kappa carrageenan	56.25 %	13.52
Iota carrageenan	18.75 %	4.51
Total	**100 %**	**24.04**

Furthermore, current formulations were in line with optimum levels recommended for kappa carrageenan ratios to locust bean gum ratio (4:1) as reported by Femandes *et al*. (1991). Since Medallion Pet Foods have confirmed a calcium level at 0.3 % from lab analysis in current product (Personal communications, Alastair Haliburton, 2020), added calcium was assessed for its performance with iota carrageenan at both increased and reduced levels.

Trial A-1 included the addition of $CaCO_3$ to the emulsion whereas trial A-2 was carried out omitting $CaCO_3$ from the product. This second trial excluded $CaCO_3$ in this formula, while keeping all values the same to observe if there was enough calcium in the meat to react with iota carrageenan in forming a softer gel.

Alterations:

- 5.7 g flaxseed oil (50 %) was used as a dispersant for the hydrocolloids prior to hydration whilst the rest were mixed with meat prior to processing.
- Using an electrical blender helped the dispersal of the hydrocolloid gums into water forming a uniform emulsion.

Table 5-6: Trial formulations using iota carrageenan with and without $CaCO_3$.

A1 - Iota carrageenan (+ $CaCO_3$)			**A2 - Iota carrageenan (no $CaCO_3$)**	
Ingredients	**Composition**		**Ingredients**	**Composition**
Total Base Ingredients	**82.97 %**		**Total Base Ingredients**	**83.09 %**
Water	16.20 %		Water	16.23 %
Kappa carrageenan	0.39 %		Kappa carrageenan	0.39 %
LBG	0.17 %		LBG	0.17 %
Calcium carbonate	0.14 %		Calcium carbonate	0.00 %
Iota carrageenan	0.13 %		Iota carrageenan	0.13 %
Total Test Ingredients	**17.03 %**		**Total Test Ingredients**	**16.91 %**
Total Batch Composition	**100.00 %**		**Total Batch Composition**	**100.00 %**
Substituting 25 % kappa carrageenan with iota carrageenan in factory formulation.			*Substituting 0.13 % kappa carrageenan with iota carrageenan without $CaCO_3$.*	

The above formulation (Table 5-6) was used to test iotas compatibility in the presence of added extra $CaCO_3$ in reducing thaw drip loss whilst retaining acceptable TPA - hardness after a two-stage freeze thaw treatment.

5.3.2 Guar Gum - Trial B

The next trial tested guar gums' effectiveness in improving freeze thaw stability through controlling ice crystal formation and subsequent thaw drip loss due to minimizing cellular damage caused by freezing operations. LBG was substituted for guar gum (w/w) in this trial.

A repeat trial was carried out due to initially submitting the former test (B1) to a prolonged storage time before analysis by which 2 FT analysis for thaw drip and instrumental texture analysis was conducted more than a week after production. This was not consistent in time after treatment for the testing compared to all other trials where testing occurred within a week. However, this experiment revealed differences in response to freeze thaw treatment influenced by extending storage times up to seven (7) days after freeze thaw treatment.

Table 5-7: Trial formulation using guar gum to replace LBG in the original factory formulation.

B1 - Guar gum			**B2 - Guar gum**	
Ingredients	**Composition**		**Ingredients**	**Composition**
Total Base Ingredients	**83.06 %**		**Total Base Ingredients**	**83.06 %**
Water	16.22 %		Water	16.22 %
Kappa carrageenan	0.57 %		Kappa carrageenan	0.57 %
Guar gum	0.14 %		Guar gum	0.14 %
Total Test Ingredients	**16.94 %**		**Total Test Ingredients**	**16.94 %**
Total Batch Composition	**100.00 %**		**Total Batch Composition**	**100.00 %**
*Replacing LBG with guar gum in factory formulation. Analysed **after** 1 week.*			*Repeat formulation B1, analysed **within** 1 week.*	

5.3.3 Guar Gum + Iota Carrageenan – Trial C

This trial was carried out to assess the synergy between guar gum and kappa carrageenan including iota carrageenan while excluding locust bean gum - which was substituted (w/w) with guar gum. This formulation was similar to section 5.3.1, where optimised levels for LBG: KC: IC were observed to work well for minced ham (Sepúlveda Cossio et al., 2013) but replaced LBG with guar gum.

The formulation used the optimised levels used for ham which were identical to Trial A but replaced LBG with guar gum. Iota carrageenan was also added to this batch along with $CaCO_3$ (C1)

Trial (C2) was carried out using the same test ingredients as in Trial C1 but excluded $CaCO_3$. This was designed to test if sufficient $CaCO_3$ existed in the product to react with iota carrageenan to improve thaw drip loss after freeze thaw treatment. The iota carrageenan component was also doubled to assess whether enough levels existed to influence change upon freeze thaw treatments.

A proportion of polyunsaturated vegetable oil (0.32 %) of the total used as a dispersant to aid in the formation of a consistent hydrated emulsion suspension. This proportion of vegetable oil remained consistent throughout all tests.

Table 5-8: Trial formulations combining guar gum and iota carrageenan.

C1 - Guar gum + iota carrageenan (+$CaCO_3$)	
Ingredients	**Composition**
Total Base Ingredients	**82.97 %**
Water	16.20 %
Kappa carrageenan	0.39 %
Guar gum	0.17 %
Calcium carbonate	0.14 %
Iota carrageenan	0.13 %
Total Test Ingredients	**17.03 %**
Total Batch Composition	**100.00 %**
Substituting LBG with guar gum including iota carrageenan + $CaCO_3$.	

C2 - Guar gum + iota carrageenan	
Ingredients	**Composition**
Total Base Ingredients	**82.98 %**
Water	16.20 %
Kappa carrageenan	0.39 %
Guar gum	0.17 %
Calcium carbonate	0.00 %
Iota carrageenan	0.26 %
Total Test Ingredients	**17.02 %**
Total Batch Composition	**100.00 %**
Substituting LBG with guar gum including 2x iota carrageenan without $CaCO_3$.	

5.3.4 Synergistic Effect of Xanthan Gum with LBG – Trial D

As reported by (Ramırez et al., 2002), a combination of xanthan and LBG at a ratio of 0.25:0.75 increased textural hardness, stiffness and cohesiveness in surimi gels. This relationship was tested in the next trial in the presence of iota carrageenan (Trial D-1) and sodium alginate (Trial D-2) while including calcium carbonate in both formulations to deduce which combined synergy has the better effect after a two-stage freeze thaw treatment.

Table 5-9: Testing xanthan gum synergy with LBG in the presence of iota carrageenan (D1) and sodium alginate (D2).

D1 - Xanthan gum + iota carrageenan (+$CaCO_3$)	
Ingredients	**Composition**
Total Base Ingredients	**82.97 %**
Water	16.20 %
Kappa carrageenan	0.39 %
Calcium carbonate	0.14 %
Iota carrageenan	0.13 %
LBG	0.13 %
Xanthan gum	0.04 %
Total Test Ingredients	**17.03 %**
Total Batch Composition	**100.00 %**
Using ratio of xanthan: LBG (0.25:0.75; 1.5g:4.5g).	

D2 - Xanthan gum + sodium alginate (+$CaCO_3$)	
Ingredients	**Composition**
Total Base Ingredients	**83.03 %**
Water	16.21 %
Kappa carrageenan	0.39 %
Sodium alginate	0.17 %
LBG	0.13 %
Xanthan gum	0.04 %
Calcium carbonate	0.03 %
Total Test Ingredients	**16.97 %**
Total Batch Composition	**100.00 %**
Using ratio xanthan: LBG (0.25:0.75; 1.5:4.5). Sodium alginate added with reduced $CaCO_3$.	

5.3.5 Results and Discussion

All products (Fig 5-2) displayed an acceptable level of similarity in physical appearance to the pilot scale replicate (Control 1) dog rolls, except for irregularities (e.g., porous cavities and apparent gel pieces) on the surface of all test products. This indicated insufficient hydration of hydrocolloid during emulsion preparation.

Manual hydration using a spatula to apply mixing was attributed to the poor emulsion formation which included conglomerates of un-hydrated gums which later appeared as whole gels including the effect of porous manifestations in appearance of the product. The aid of an electric blender and the use of vegetable oil as a dispersing agent improved this characteristic (Figure 5-3).

Figure 5-2: Porous cavities and presence of gel pieces in product as a result of un-emulsified hydrocolloids.

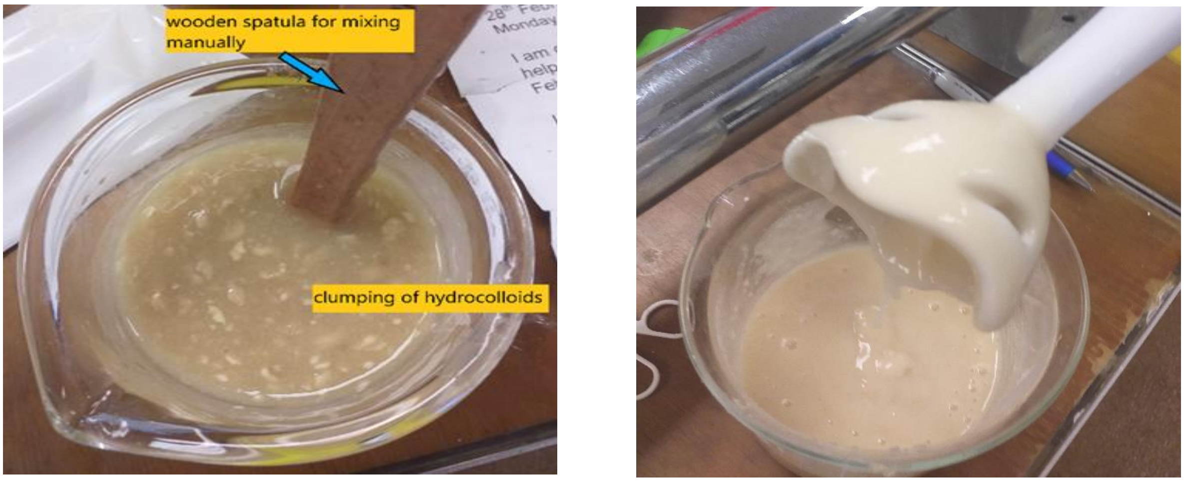


Figure 5-3: The use of vegetable oil and an electric blender (shear force) improved emulsion formation.

Mixing of hydrocolloid gums aided by a dispersing agent and shear force created a better consistency in emulsion formation. However, sodium alginate, iota carrageenan and guar gum presented thick and viscous emulsions.

These emulsions were found to be smooth but extremely thick and viscous and the Blentech had to be stopped and the emulsion added manually onto the meat before continuing mixing (Fig 5-4). These pre-processing attributes did not hinder the products outcome during and after processing.

Figure 5-4: Addition of thick emulsion to Blentech by opening grate.

Trial A:

Iota carrageenan in combination with calcium carbonate (A1) reduced the thaw drip loss after one freeze thaw cycle compared to the control (Fig 5-6). However, this effect was not observed when iota carrageenan was used without $CaCO_3$ (A2). It was therefore concluded that the addition of $CaCO_3$ was required by iota carrageenan to reduce thaw drip loss caused by freeze thawing. This can be seen by an obvious increase in thaw drip loss when $CaCO_3$ is not included in the formulation as depicted by prototype A2 (Fig 5-6). This observation required the further investigation of iota carrageenan at increased concentration in the formulation whilst including added $CaCO_3$. TPA hardness as shown in Figure 5-5 was similar for A1 and A2 which showed that $CaCO_3$ was not critical for TPA hardness. However, the TPA hardness was less than the control prior to freeze thawing but showed moderate decline over 2 FT compared to other candidates (Fig 5-5). This result justified iota carrageenan's continued use for further investigations given its proven potential to reduce thaw drip loss over freeze thaw treatments.

Trial B:

The use of guar gum instead of locust bean gum (B1 & B2) resulted in a thaw drip loss lower than the control for both fresh and freeze thaw cycles and was similar to that of trial D-2 which utilised the combined synergistic effect of xanthan gum and LBG in the presence of sodium alginate and calcium ions. Although a repeat test was carried out (B-2), there was a difference observed between the two tests where the former (B-1) exhibited a lower thaw drip loss. It was noted that B1 was thawed and chilled for seven (7) days before measuring the thaw drip loss, while the thaw drip loss for B2 was analysed within one day of thawing. A possible explanation is that freeze thawing operations causes water to accumulate resulting in thaw drip loss however, further chilled storage enables some of the free water to bind to the different components. This remains speculation and would require further testing. For other experiments, the thaw drip loss was measured the day after thawing to eliminate the possible effect of storage at chilled temperatures.

TPA hardness was lower than the control for both B1 and B2 in which there was a decline after 1 FT but displayed the smallest change between 1 FT and 2 FT (Table 5-10) indicating favourable performance and supports the need for further evaluation.

Trial C:

The combination of iota carrageenan and replacing the LBG with guar gum resulted in reduced thaw drip loss compared to the control provided $CaCO_3$ was added (Trial C1). However, there was in an increase thaw drip loss after 1 FT, even at double the concentration of iota carrageenan without the addition of $CaCO_3$ (Trial C2), which then was reduced after 2FT treatment (Table 5-10). Although exhibiting acceptable thaw drip loss, both tests for trial-C displayed the lowest hardness values across all prototypes (Fig.5-5) which does not satisfy key considerations to meet acceptable hardness requirements. A useful outcome gained from this test shows that iota carrageenan improves thaw drip loss in the presence of added $CaCO_3$. Optimising levels of $CaCO_3$ were to be considered going forward with further testing of iota carrageenan and guar gum / kappa carrageenan. Combining guar gum and iota carrageenan formed thick viscous emulsions that could not flow freely and exhibited increased demand for water to form a free-flowing emulsion. This limits the amount of iota carrageenan and guar gum that can be added and needs to be considered for further evaluation. This is mainly due to the amount of water for hydrating and forming emulsion. The total water component in all tests remained constant and comprised 16.2 % of total batch ingredients and was used for the mixing of salts as well as emulsions. Of the water added, 60 % was required for hydrating the emulsion while the rest was used for dissolving salts.

Trial D:

Trial D was carried out to investigate the effect of adding xanthan gum to increase hardness while maintaining LBG in the presence of iota carrageenan, sodium alginate and added $CaCO_3$. However, when iota was replaced with alginate in the presence of added $CaCO_3$, a reduction in thaw drip loss was observed (Table 5-10), although there was a slight decrease in hardness. Therefore, the use of sodium alginate combined with $CaCO_3$ remained a candidate for further evaluation.

General responses for TPA hardness indicated less differences between 1 FT and 2 FT treatments across all samples. Although thaw drip loss behaviour across all samples showed higher levels of variation, minimum change was displayed for the better performing products (B1, B2, C1 & D2) between 1 FT and 2 FT treatments. These results favoured the elimination of a two-stage freeze thaw cycle for further product development and testing using best performing candidates.

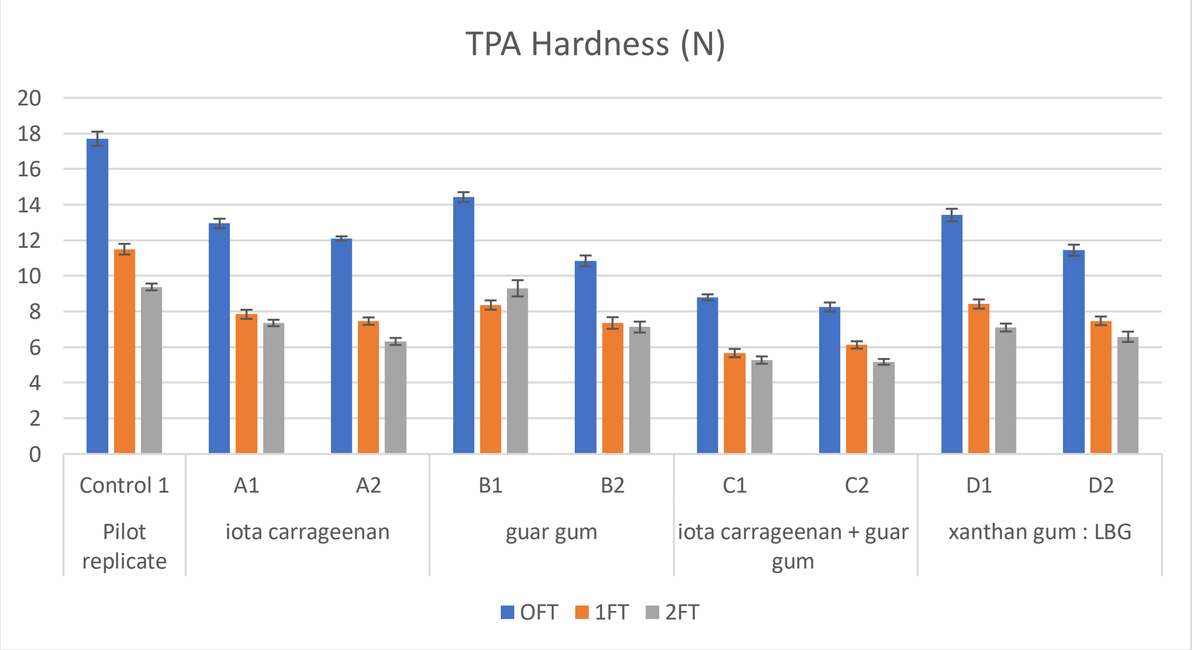


Figure 5-5: Comparison of TPA Hardness across tested functional ingredients measured as Force (N).

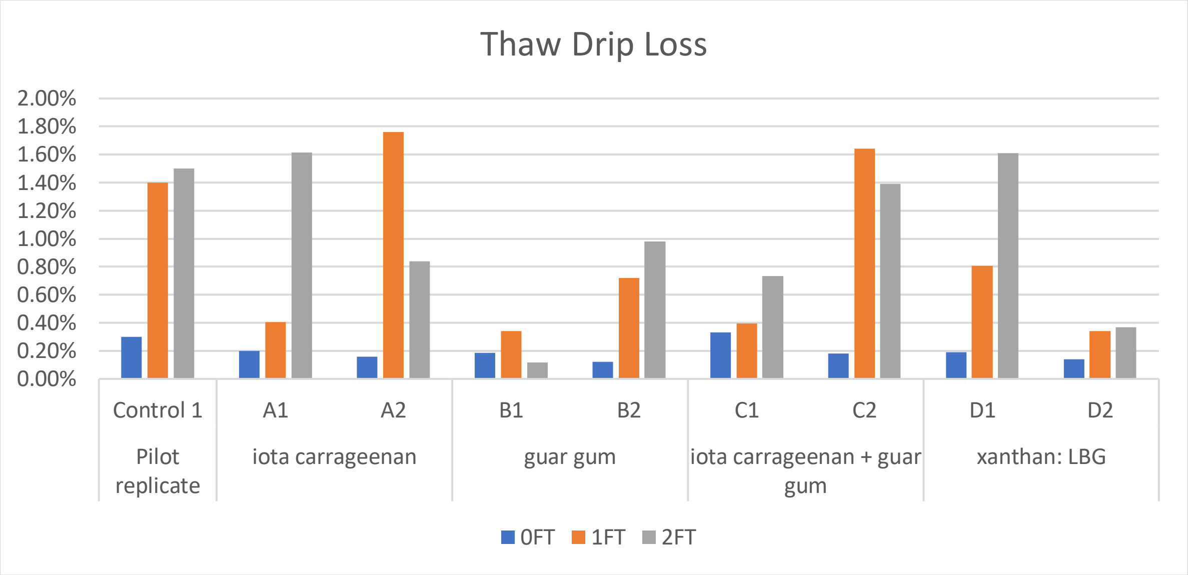


Figure 5-6: Thaw drip loss comparisons over 2 freeze thaw (2 FT) treatments for chicken and pumpkin dog roll prototypes compared against pilot scale product replicate (control 1).

Table 5-10: Result summary of test combinations and differences observed during 1 freeze thaw (1 FT) and 2 freeze thaw (2 FT) treatments during initial testing of selected functional ingredients.

Label	Variables								% Difference in change						Difference (Absolute Value)		
	kappa CGN	LBG	Iota CGN		Guar gum	Xanthan gum	Alginate		TPA Hardness			TPA Cohesiveness			Thaw drip		
			(+) $CaCO_3$	(-) $CaCO_3$			(+) $CaCO_3$	(-) $CaCO_3$		0 -1FT	1-2FT		0 -1FT	1-2FT		0 -1FT	1-2FT
Control	☑	☑							Δ (FT)	-35%	-12%	Δ (FT)	31%	10%	Δ (FT)	1.1%	0.1%
A1	☑	☑	☑							-39%	-4%		33%	6%		0.2%	1.2%
A2	☑	☑		☑						-38%	-9%		33%	3%		1.6%	-0.9%
B1	☑				☑					-42%	7%		31%	18%		0.2%	-0.1%
B2	☑				☑					-32%	-2%		47%	13%		0.6%	0.3%
C1	☑		☑		☑					-36%	-4%		33%	4%		0.1%	0.3%
C2	☑			☑	☑					-26%	-12%		60%	-1%		1.5%	-0.3%
D1	☑	☑	☑			☑				-37%	-10%		25%	11%		0.6%	0.8%
D2	☑	☑				☑	☑			-35%	-8%		29%	2%		0.2%	0.0%

Key:	☑	**standard dog roll ingredient used**
	☑	**test ingredient applied**
	Δ (FT)	**difference between treatments**

5.3.6 Conclusion and Recommendations

The addition of guar gum, iota carrageenan and xanthan gum synergy with LBG combined with sodium alginate showed good freeze thaw stability by reducing thaw drip loss. The TPA hardness was less than the control for all the experiment formulations and did decline with freeze thaw treatments to a similar extent as the control sample. The addition of $CaCO_3$ must be included to any product containing iota carrageenan and was also added to the sodium alginate trial. The combination of xanthan and LBG was effective in stabilising textural hardness after freeze thaw treatment and remained a promising approach to attempted processing optimisation for the future trials.

In addition, for all future testing, the analyses were carried out in the same uniform timeframe with thaw drip loss and TPA hardness measured within a day after thawing. Further investigations from the previous trials have considered:

- Using increased quantities of iota carrageenan, guar gum, xanthan and alginate levels.
- Testing the use of sodium tripolyphosphate (STPP) on improving carrageenan's performance.
- Implementing only one freeze thaw treatment (1 FT) for sample evaluation.

5.4 Optimization for Product Performance

This phase of prototype development investigated the optimization of the concentrations of the already tested binding ingredients and included the use of sodium tripolyphosphate (STPP). The trial with STPP was recommended after consultation with Medallion Pet Foods. It was screened out previously as consumers would perceive it to be a chemical additive. However, its potential to improve the hardness in dog rolls at low concentrations when combined with other binders warranted investigation.

Product development was conducted to deduce the effectiveness of the following candidates in improving thaw drip loss and textural hardness. Figure 5-7 Illustrates the roadmap used for development and selection of the best performing candidates to continue optimisation stages of product development.

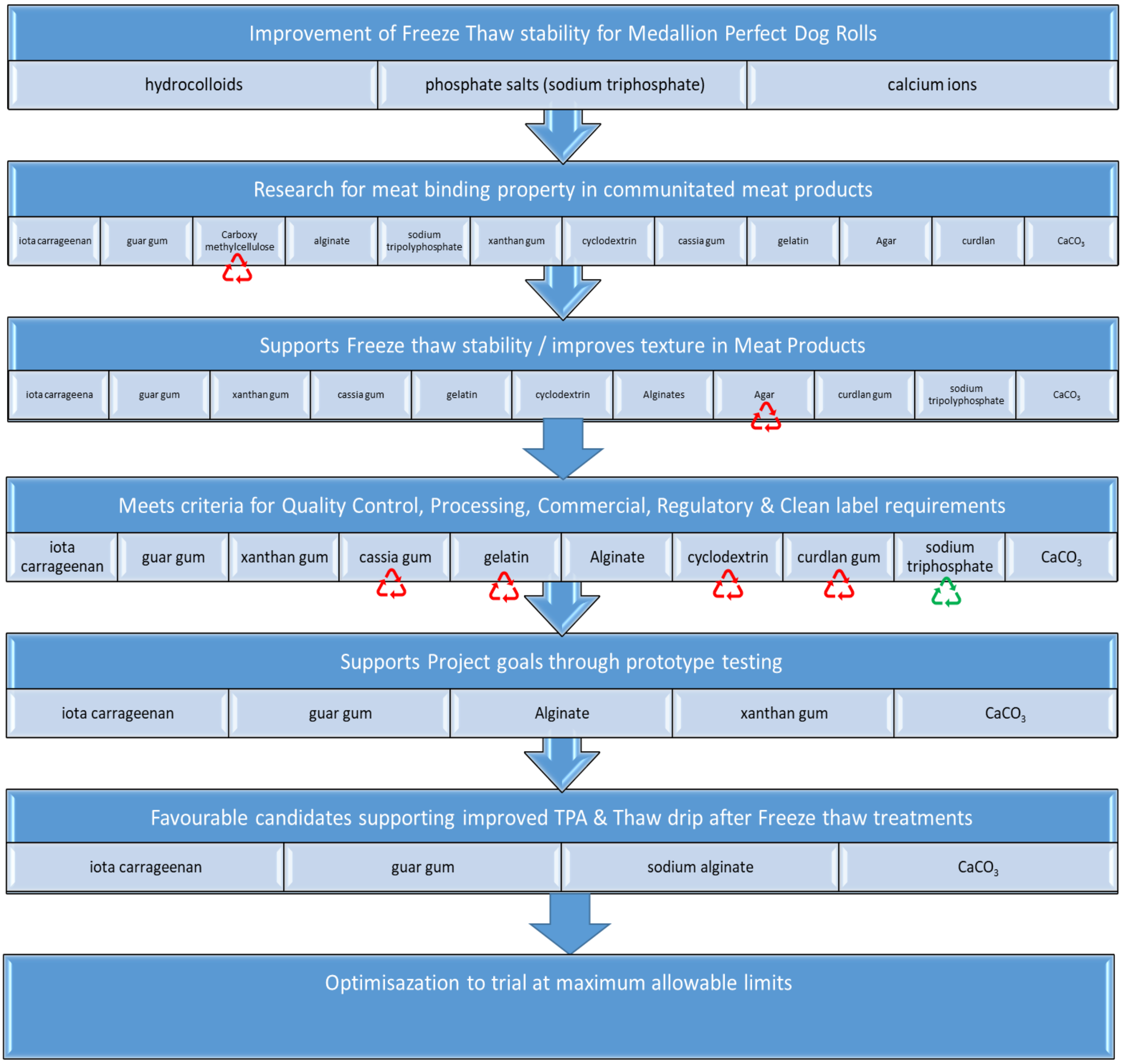


Figure 5-7: Project road map of functional ingredient selection up to the point of optimisation.

Key:	♻	**Has potential but discarded**
	♻	**Has potential and reserved**

5.4.1 Iota Carrageenan / $CaCO_3$ -Trial A3/A4

Trial A3 was conducted to observe the effect of using double the amount of iota carrageenan originally used in the current formula of the factory dog rolls while using optimised levels of kappa carrageenan and LBG ratios (4:1) as reported by Femandes *et al*. (1991). Thus, the use of kappa carrageenan was reduced from 0.57 % to 0.50 % and LBG from 0.17 % in the normal formulation to 0.13 %. If this formula returned favourable results, then a further optimization trial could be carried out using normal or reduced KCl levels in the formulation as described by DeFreitas et al. (1997) where the presence of KCl reduced textural hardness and increased thaw drip in the presence of STPP.

Trial A4 was formulated to compare the effect of using iota carrageenan instead of sodium alginate in improving thaw drip loss as well as maintaining textural hardness. This formulation substituted iota carrageenan for sodium alginate into the same formula used for D4. Comparisons were made to observe iota carrageenan's performance against sodium alginate using the observed improved performance in the previous trials utilising the synergy between xanthan: LBG to improve textural hardness after freeze thaw treatment.

In both trials the use of $CaCO_3$ was reduced to 0.03 % as previous levels used in the former trial were observed to interfere with salt hydration given the amount of water available for mixing. Such a small amount would complement the pre-existing amount present in the product reported by Medallion Pet Foods to be 0.3 %.

Table 5-11: Formulations for Trials A3 and A4.

Trial A3	
Ingredients	**Composition**
Total Base Ingredients	**82.92 %**
Water	16.19 %
Kappa Carrageenan	0.50 %
LBG	0.13 %
Calcium carbonate	0.03 %
Iota carrageenan	0.23 %
Total Test Ingredients	**17.08 %**
Total Batch Composition	**100.00 %**
Ratio kappa carrageenan: LBG (4:1).	

Trial A4	
Ingredients	**Composition**
Total Base Ingredients	**82.88 %**
Water	16.19 %
Kappa carrageenan	0.38 %
Xanthan: LBG (0.25:0.75; 2.5g:7.5g)	0.29 %
Calcium carbonate	0.03 %
Iota carrageenan	0.23 %
Total Test Ingredients	**17.12 %**
Total Batch Composition	**100.00 %**
Substituted iota for sodium alginate.	

5.4.2 Increased Guar Gum + (STPP) - Trial B3 & B4

Guar gum appeared to reduce thaw drip loss in previous experiments. The aim of trial B3 and B4 are based on further tests using higher concentrations of guar gum and tested improvement of textural hardness when using STPP in the presence of kappa carrageenan. The upper limit of guar gum addition and STPP are controlled by relative solubility in forming emulsion and dissolving salts given the proportion of water available for blending. STPP was added to salts and dissolved separately from the emulsion.

Table 5-12: Investigating the effect of using guar gum at increased levels with and without STPP.

Trial B3 - Increased guar gum + STPP	
Ingredients	**Composition**
Total Base Ingredients	**82.53 %**
Water	16.12 %
Kappa carrageenan	0.50 %
STPP	0.43 %
Guar gum	0.43 %
Total Test Ingredients	**17.47 %**
Total Batch Composition	**100.00 %**

Trial B4 - Increased guar gum (no STPP)	
Ingredients	**Composition**
Total Base Ingredients	82.89 %
Water	16.19 %
Kappa carrageenan	0.50 %
STPP	0.00 %
Guar gum	0.43 %
Total Test Ingredients	**17.11 %**
Total Batch Composition	**100.00 %**

5.4.3 Testing Synergy of Xanthan Gum and Guar Gum/ LBG. - Trial D3 & D4

This test observed the use of xanthan gum in combination with LBG and xanthan gum in combination with guar gum. Sodium alginate was added to reduce thaw drip loss as observed in previous trials, therefore was present in both formulations as was kappa carrageenan.

Table 5-13: Comparing the effect of synergy between xanthan gum: guar gum (D3) and xanthan : LBG (D4) at proposed optimised levels for xanthan: LBG.

Trial D3 - Xanthan gum: guar gum + sodium alginate	
Ingredients	**Composition**
Total Base Ingredients	82.88 %
Water	16.18 %
Xanthan: guar gum (0.25:0.75; 2.5g:7.5g)	0.29 %
Calcium carbonate	0.04 %
Sodium alginate	0.23 %
Kappa carrageenan	0.38 %
Total Test Ingredients	**17.12 %**
Total Batch Composition	**100.00 %**

Trial D4 - Xanthan gum: LBG + sodium alginate	
Ingredients	**Composition**
Total Base Ingredients	82.88 %
Water	16.18 %
Xanthan: LBG (0.25:0.75; 2.5g:7.5g)	0.29 %
Calcium carbonate	0.04 %
Sodium alginate	0.23 %
Kappa carrageenan	0.38 %
Total Test Ingredients	**17.12 %**
Total Batch Composition	**100.00 %**

5.4.4 Sodium Tripolyphosphate (STPP) - Trial E & F

The use of STPP to improve freeze thaw stability in combination with kappa carrageenan was tested in combination with sodium alginate in the original product to improve both textural hardness and improved thaw drip loss. The outcome of this test determined STTPs suitability for use with kappa carrageenan. The level of STPP was increased to 0.5 % in trial F which mimicked the original product as per the optimised levels in pork sausages reported by Defreitas et al. (1997). This was compared against a better performing formula using sodium alginate and calcium carbonate which contained a lower level of STPP depicted in trial E. Trial E also employed lower levels of kappa carrageenan to achieve better hydration with added sodium alginate.

Table 5-14: Comparing the effect of STPP using upper and lower limits.

TRIAL E-STPP + sodium alginate (+$CaCO_3$)	
Ingredients	**Composition**
Total Base Ingredients	82.91 %
Water	16.19 %
Kappa carrageenan	0.39 %
STPP	0.29 %
Sodium alginate	0.23 %
Calcium carbonate	0.04 %
Total Test Ingredients	17.09 %
Total Batch Composition	100.00 %
With reduced kappa carrageenan + $CaCO_3$.	

TRIAL F -Adding STPP to original product	
Ingredients	**Composition**
Total Base Ingredients	**82.7 %**
Water	16.15 %
STPP	0.50 %
Kappa carrageenan	0.50 %
LBG	0.14 %
Total Test Ingredients	**17.3 %**
Total Batch Composition	**100.00 %**
Adding STPP to the factory formulation with reduced kappa carrageenan.	

5.4.5 Results and Discussion

Thaw drip loss for all formulations before and after one freeze thaw cycle is given in Figure 5-9. All experimental formulations showed less thaw drip loss then the control both, before and after the freeze thaw treatment. The thaw drip loss for all formulations increased after freezing and thawing, but for many treatments this increase was exceedingly small.

TPA hardness results are shown in Figure 5-8, and all experimental formulations showed a lower TPA hardness than the control whether before or after freeze thaw treatment. All the experimental formulations behaved similarly by revealing a decline in TPA hardness after freeze thaw treatment.

Increasing the levels of guar gum and sodium alginate resulted in further improvement in thaw drip loss after freeze thaw treatment compared to previous trials using lesser amounts. Xanthan gum used in combination with LBG at a ratio of 0.25:0.75 was more effective in maintaining hardness after one freeze thaw treatment than guar gum, with the effect greatest in the presence of sodium alginate (D4) and iota carrageenan (A4). Further evaluation of results showed that using the combination of xanthan: LBG in the presence of sodium alginate resulted in better textural hardness (Fig 5-8), whilst maintaining low thaw drip loss after treatment (Fig 5-9). Iota carrageenan’s performance was improved in combination with xanthan: LBG (A4) achieving low thaw drip loss differences and comparable TPA hardness to the better performing formulation (D4) as summarised in Table 5-15. This observation supports a better outcome due to the synergistic effect of xanthan gum and LBG combined with sodium alginate.

Sodium tripolyphosphate (STTP) was observed to perform poorly in controlling thaw drip loss when combined with guar gum (Trial B3) and sodium alginate (Trial E) depicted by summary differences in Table 5-5. STPP added to the control formulation that contained kappa carrageenan and LBG (Trial F) also showed a high thaw drip loss. However, STPP when used with guar gum and sodium alginate did minimise thaw drip loss, even though it showed a poor ability to reduce thaw drip on its own without other test ingredients present. Therefore, the improvement shown by other formulations using STPP was due to the influence of the other test ingredients used such as guar gum and sodium alginate. Furthermore, STPP showed poor response to improving textural hardness overall in the product when used with guar gum and sodium alginate (Fig 5-8).

It was concluded that the best formulations for further development were A4 and D4 as these formulations displayed minimal thaw drip loss and relatively high TPA hardness, although this was less than the control. Both these formulations included kappa carrageenan combined with xanthan gum and LBG in the ratio of 0.25:0.75, where A4 included the addition of iota carrageenan and $CaCO_3$ and D4, the addition of sodium alginate and $CaCO_3$.

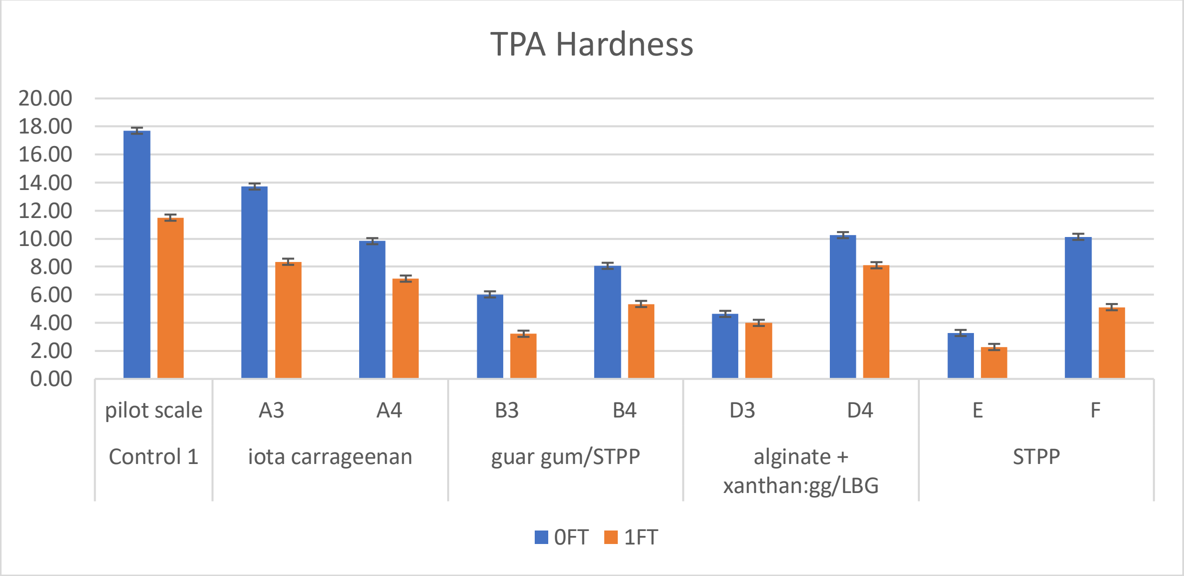


Figure 5-8: Comparison of TPA hardness using elevated levels of ingredients listed either used alone or in combination with the other test ingredients.

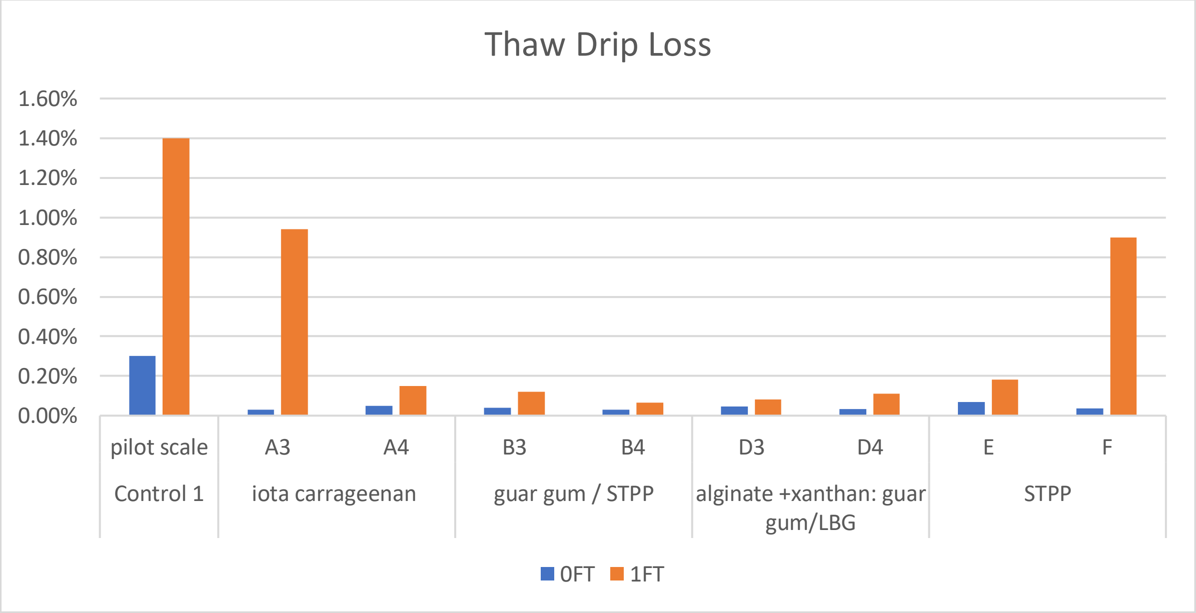


Figure 5-9: Comparison of thaw drip loss using elevated levels of ingredients listed either used alone or in combination with the other test ingredients.

Table 5-15: Summary of test differences (%) in TPA hardness and thaw drip loss after 1 freeze thaw treatment.

Chicken & pumpkin product trials	Variables									% Difference in Treatments				Differene (Absolute value)	
	kappa CGN	LBG	Iota CGN		Guar gum	Xanthan gum	Alginate		STPP	TPA Hardness		TPA Cohesiveness		Thaw drip	
			(+) $CaCo_3$	(-) $CaCo_3$			(+) $CaCO_3$	(-) $CaCO_3$		Δ (FT)	0 -1FT	Δ (FT)	0 -1FT	Δ (FT)	0 -1FT
Control 1	☑	☑									-35%		31%		1.1%
A1	☑	☑	☑								-39%		33%		0.2%
A2	☑	☑		☑							-38%		33%		1.6%
B1	☑				☑						-42%		31%		0.2%
B2	☑				☑						-32%		47%		0.6%
C1	☑		☑		☑						-36%		33%		0.1%
C2	☑			☑	☑						-26%		60%		1.5%
D1	☑	☑	☑			☑					-37%		25%		0.6%
D2	☑	☑				☑	☑				-35%		29%		0.2%
A3	☑	☑	☑								-39%		27%		0.9%
A4	☑	☑	☑			☑					-27%		8%		0.1%
B3	☑				☑				☑		-47%		18%		0.1%
B4	☑				☑						-34%		31%		0.1%
D3	☑				☑	☑	☑				-14%		24%		0.1%
D4	☑	☑				☑	☑				-21%		26%		0.1%
E	☑						☑		☑		-30%		40%		0.1%
F	☑	☑							☑		-49%		40%		0.9%

Key:		
	☑	standard dog roll ingredient used
	☑	test ingredient applied
	Δ (FT)	difference between treatments

5.4.6 Conclusion and Recommendation

The use of guar gum was discarded at this point as it did not fulfil requirements for acceptable hardness although thaw drip loss was improved. Further optimisation trials were to be conducted using both iota carrageenan and sodium alginate combined with xanthan: LBG as these formulations showed similar levels of improved performance so far.

While STPP results were not promising, a further test in combination with iota carrageenan was performed as it was reported by DeFreitas et al, (1997) that STPP combined with kappa and iota carrageenan promoted good freeze thaw stability in pork sausages.

5.5 Effect on Change of Raw Meat Composition

Meat composition variation is an industrial reality given changing raw material specifications and sourcing of appropriate ingredients dictated by price, availability and changing costs. Given such conditions, the supply of raw minced meat, originally comprising a two part mix for both chicken and beef dog rolls was changed to a single finely minced meat emulsion for the remainder of the work.

As such, a re-evaluation of the pilot replication of factory products (control 1) for both dog roll flavours (chicken and pumpkin and beef and beetroot) was required and these were reproduced at pilot scale for the continuation of evaluating prototype development and testing. The other raw materials in the control formulations for both products remained unaffected.

5.5.1 Results and Discussion

Both products were reassessed for their response to instrumental textural changes in comparison to their counterpart factory products manufactured using identical ingredients. The chicken and pumpkin dog rolls samples continued with the optimisation process and the beef and beetroot control samples were compared with appropriate prototypes in Chapter 5-7.

Comparing chicken dog roll replicates (Fig 5-11), a difference in TPA hardness can be seen upon using different raw meat composition. This distinct variation of textural hardness between the former meat type used for earlier trials (control 1) and current meat sourced by medallion Pet Foods for factory operations (control 2) is shown in Table 5-16. The control 2 replicate was used to provide the base factory sample at pilot scale for comparison against improved prototypes for the remainder of the work.

Thaw drip loss (Fig 5-12) when compared to the former product remained within 0.01 % (Table 5-16) by differences in % loss. This almost identical response between the two products using different pre-formulated raw meat ingredients indicated that changing the raw meat ingredient did not affect thaw drip loss.

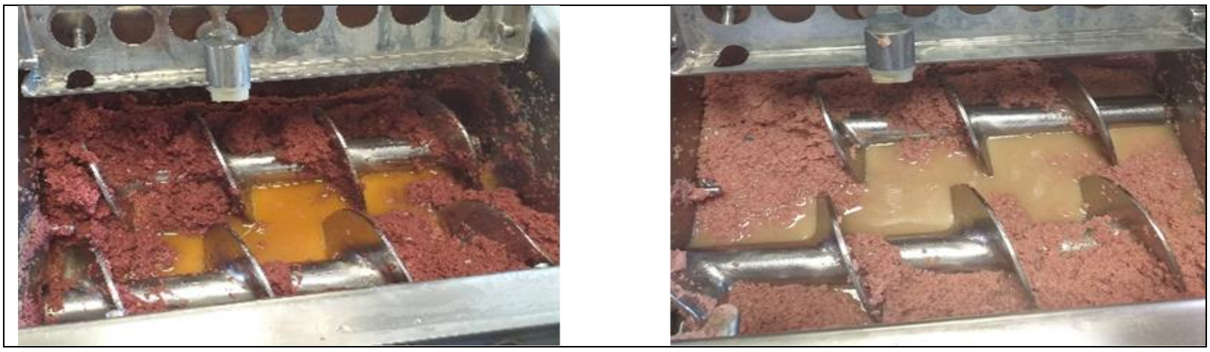


Figure 5-10: New Beef (left) and Chicken meat (right) after processing @ 85 °C for 15 minutes.

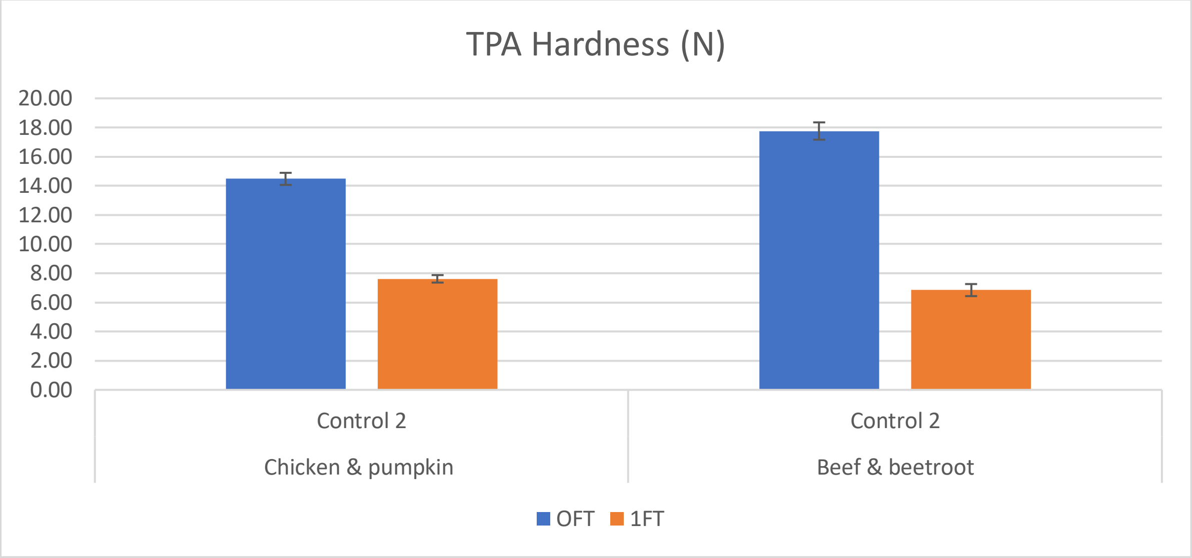


Figure 5-11: TPA hardness for pilot plant replicate (control 2) samples prepared using a different raw meat material supply as previously used for the initial work.

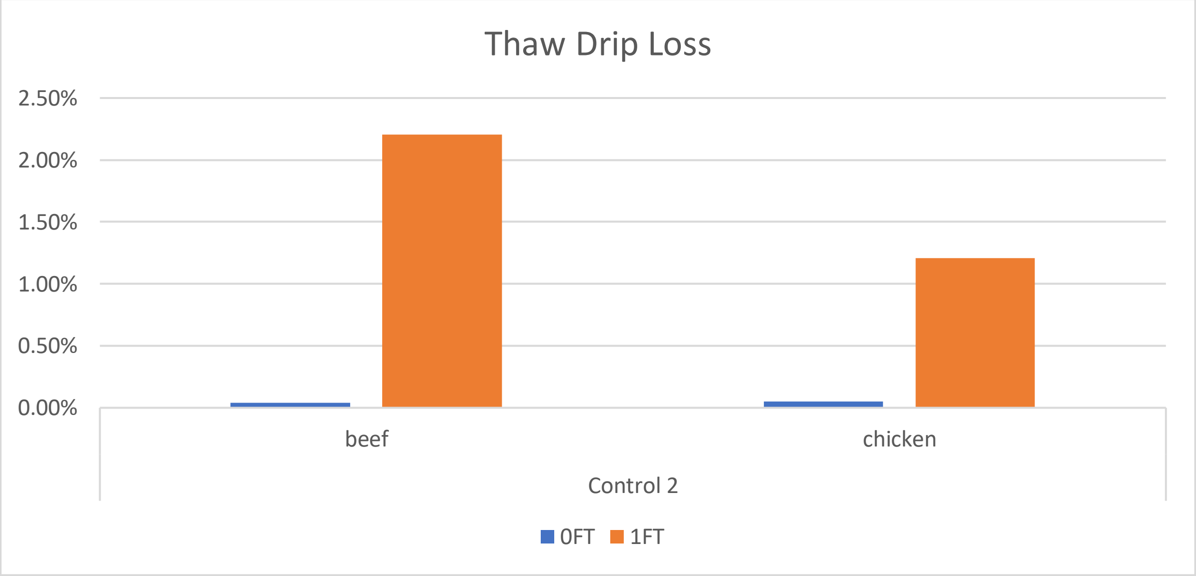


Figure 5-12: Thaw drip loss for pilot plant replicate (control 2) samples prepared using a different raw meat material supply as previously used for the initial work.

Table 5-16: Summary of differences in change in thaw drip loss and instrumental textural hardness after one freeze thaw treatment of the two control products.

Chicken & pumpkin product trials	Variables									% Difference		Difference (Absolute value)	
			Iota CGN				Alginate			TPA Hardness		Thaw drip	
	kappa CGN	LBG	(+) CaCo$_3$	(-) CaCo$_3$	Guar gum	Xanthan gum	(+) CaCO$_3$	(-) CaCO$_3$	STPP	Δ (FT)	0 -1FT	Δ (FT)	0 -1FT
Control 1	☑	☑									-35%		1.1%
Control 2	☑	☑									-47%		1.2%

Key:		
	☑	standard dog roll ingredient used
	☑	test ingredient applied
	Δ (FT)	difference between treatments

5.5.2 Conclusion and Recommendation

Results of the subsequent trials were comparable to the former trials for response to thaw drip loss after treatment, however the difference between the TPA hardness assessment of the two control samples suggested that this was not possible for the instrumental textural analysis within the next optimization section.

5.6 Final Optimization for Processing Performance

The final optimization phase was purposefully carried out using the best performing test ingredients to both achieve satisfactory performance given commercial and economic considerations (Chapter 5.2) as well as suit processing for dog rolls and reduce issues with undissolved ingredients (gums and salts) as reported in Chapter 5.3. Optimization limits for added functional ingredients were dependent on the amount of water designated for dissolving salts and hydrocolloids which remained constant at 16.2 % throughout the product development stages.

The following formulations followed recommendations from best performing prototypes drawn from Chapter 5.3. These include xanthan gum in combination with sodium alginate and iota carrageenan. Iota carrageenan was further evaluated with STPP to observe whether hardness was improved to match the better response observed with sodium alginate. Further evaluation of iota carrageenan was considered on the basis that it satisfied commercial acceptability (easy to access, cost, regulations) as outlined in Chapter 5.2.

5.6.1 Sodium Alginate - Trial D5 and D6

The use of sodium alginate was further manipulated into a final three (3) formulations (D5, D6 & D7) to compare the performance of iota carrageenan with synergistic effects of xanthan: LBG. All three formulations were based on the best outcome observed so far displayed by formulation D4 (Alginate, Xanthan: LBG) discussed in Chapter 5.4. Both trials D5 and D6 reflected the better performing formulation D4 assessed in Chapter 5.4.

Trial D5 was conducted to utilise higher levels of kappa carrageenan and investigated intermediate levels of xanthan: LBG while sodium alginate composition was reduced from 0.23 % to 0.17 % as previous formulations display an increased requirement for water to form emulsion (Chapter 5.3.5).

Trial D6 was conducted to investigate the optimised levels for xanthan: LBG (at a ratio of (0.25:0.75) according to Ramirez et al. (2002), in combination with Kappa carrageenan which also has a synergistic effect with LBG (Femandes et al., 1991). Observing such difference would enable the optimisation of LBG usage in the formulation.

For the purpose of illustrating the altered quantities of test ingredients, the formulations given were expanded to reflect batch quantities as well as percentage composition for easier reference.

Therefore, at a composition of 6 g LBG the following proportions were calculated.

1. Kappa carrageenan: LBG at a ratio of 4:1, using 17.5 g kappa will require 4.4 g LBG, therefore 6 – 4.4 = 1.6 g LBG available for xanthan gum synergy.

   Therefore:
2. When using 6 g LBG, only 1.6 g will be available to react with xanthan gum. Given 1.6 g available LBG to interact with xanthan gum, the appropriate amount of xanthan gum was deduced from the ratio of xanthan: LBG (0.25:0.75) which amounts to 0.5 g. Therefore, the amount of xanthan gum required to react with the available 1.6 g of LBG equals 0.5 g (Trial D6).

Table 5-17: Comparing effect of reducing xanthan in formulation according to amount of LBG available after optimising kappa carrageenan.

Trial D5			Trial D6		
Ingredients	**QTY (g)**	**Composition**	**Ingredients**	**QTY (g)**	**Composition**
Total Base Ingredients	**2906.90**	82.88 %	**Total Base Ingredients**	**2906.90**	82.92 %
Water	567.70	16.19 %	Water	567.70	16.19 %
Kappa carrageenan	**17.50**	0.50 %	**Kappa carrageenan**	**17.50**	0.50 %
Xanthan: LBG (0.25:0.75; 2g:6g)	8.00	0.23 %	Xanthan: LBG (0.25:0.75; 0.5g:6g)	6.40	0.18 %
Sodium alginate	6.00	0.17 %	Sodium alginate	6.00	0.17 %
Calcium carbonate	**1.20**	0.03 %	**Calcium carbonate**	**1.20**	0.03 %
Total Test Ingredients	**600.40**	**17.12 %**	**Total Test Ingredients**	**598.80**	**17.08 %**
Total Batch composition	**3507.30**	**100.00 %**	**Total Batch composition**	**3505.70**	**100.00 %**
Ratio xanthan gum: LBG (0.4g:1.2g) /kappa carrageenan: LBG (4:1 = 17.5:4.4).			*Reducing xanthan in proportion of LBG available (xanthan gum: LBG, 0.25:0.75).*		

Trial D5 was compared to trial D4 in the previous serious of tests for overall performance at adjusted levels for kappa carrageenan. The results from trial D6 would be compared against D5 and if there was no difference than the combined synergies would be recommended for further trials with the beef and beetroot dog rolls.

5.6.2 Effect of Omitting Kappa Carrageenan - Trial D7

This test was conducted to observe the performance of sodium alginate used with xanthan: LBG without kappa carrageenan. The formulation was based on the best outcome observed previously (D4) and enabled a direct comparison to measure kappa carrageenan's importance in influencing improved freeze thaw stability as a key base ingredient. Kappa carrageenan was also excluded to reduce total hydrocolloids available in forming the emulsion and improve the hydration of sodium alginate as well as measuring the dependency of sodium alginate, xanthan and LBG to perform independently without kappa carrageenan. This test was designed to test the optimisation of ingredients as well as achieving economical gains in cost saving of ingredient usage.

Table 5-18: Best performing prototype for product optimization investigated without kappa carrageenan.

Trial D7		
Ingredients	**QTY (g)**	**Composition**
Total Base Ingredients	**2907.10**	83.20 %
Water	567.70	16.25 %
Xanthan: LBG (0.25:0.75; 2.5g:7.5g)	10.00	0.29 %
Sodium alginate	**8.00**	0.23 %
Calcium carbonate	1.20	0.03 %
Total Test Ingredients	**586.90**	**16.80 %**
Total Batch Composition	**3494.00**	**100.00 %**
Formulation D4 without kappa carrageenan.		

5.6.3 Iota Carrageenan with STPP – Trial A5

This test was conducted to evaluate STPPs performance with iota carrageenan in the presence of xanthan and LBG in improving thaw drip loss and maintaining acceptable textural hardness after treatment. The amount of STPP was reduced in consideration of available water for maintaining solubility with other salts.

Table 5-19: Testing STPP with iota carrageenan.

Trial A5		
Ingredients	**QTY (g)**	**Composition**
Total Base Ingredients	**2906.90**	99.99 %
Water	567.70	19.53 %
Kappa carrageenan	13.50	0.46 %
Xanthan: LBG (0.25:0.75; 2.5g:7.5g)	10.00	0.34 %
Iota carrageenan	8.00	0.28 %
Sodium tripolyphosphate (STPP)	8.00	0.28 %
Calcium carbonate	1.20	0.04 %
Total Test Ingredients	**608.40**	**20.93 %**
Total Batch Composition	**3515.30**	**120.92 %**
Iota carrageenan + STPP.		

5.6.4 Results and Discussion

Changing raw meat composition (supplied as comminuted mixed chicken meat) affected the variability in textural hardness, however, thaw drip loss was minimally altered by this change.

Therefore, the new raw meat was used to formulate prototypes A5, D5, D6 and D7, before comparison of the thaw drip loss and textural hardness was evaluated against the performance of the control 2 pilot scale product replicate.

TPA hardness and thaw drip loss results are given in Figure 5-13 and 5-14 respectively. All formulations showed a reduction in initial TPA hardness and thaw drip loss before freeze-thaw treatment compared to control 2. Overall results for D5 and D6 were favourable, showing sodium alginate together with the use of xanthan gum and LBG provided the best solution. Compared to formulation D4, both D5 and D6 have an increase amount of kappa carrageenan and a reduction in xanthan gum and LBG which had led to an increase in hardness compared to D7 which was expected as kappa carrageenan is responsible for gelling and providing TPA hardness. Comparing D5 and D6, there was a slightly higher initial hardness and lower drip loss upon thawing (Table 5-20) for formulation D5 which had the higher total concentration of gums.

Including STPP with iota carrageenan resulted in a slight increase in thaw drip loss and textural hardness. Considering past observations for STPP (Chapter 5.4), iota carrageenan's performance was not favourable in fulfilling the desired outcomes in combination with sodium alginate and guar gum. Therefore, displaying a poor performance in the presence of iota carrageenan now rules out STPPs suitability as a potential functional ingredient for improving freeze thaw stability in pasteurized dog rolls.

The exclusion of kappa carrageenan when used with xanthan gum: LBG (Trial D7) and sodium alginate results in an increased thaw drip loss. This formulation's poor performance in the absence of kappa carrageenan indicated kappa carrageenan's importance in contributing to improved thaw drip loss when combined - with sodium alginate, LBG and xanthan gum. This observation also supported the observations of Femandes et al. (1991) that removing kappa carrageenan results in weakening of the gel structure resulting in increased syneresis and proved the synergistic effect of kappa carrageenan and LBG in maintaining freeze thaw stability.

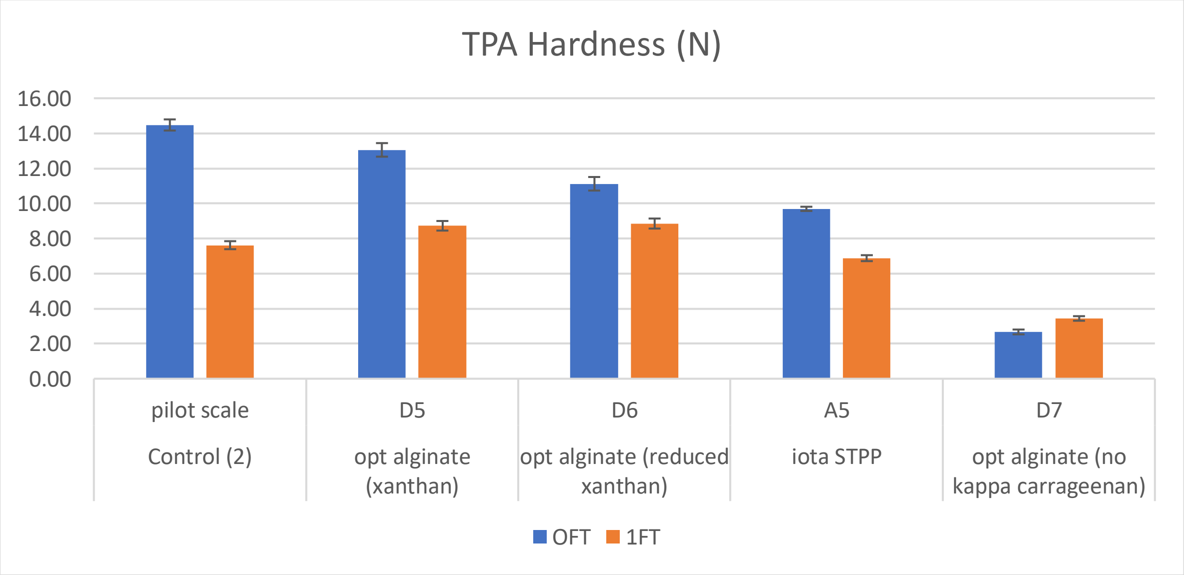


Figure 5-13: TPA hardness responses of the final formulations compared to Control 2.

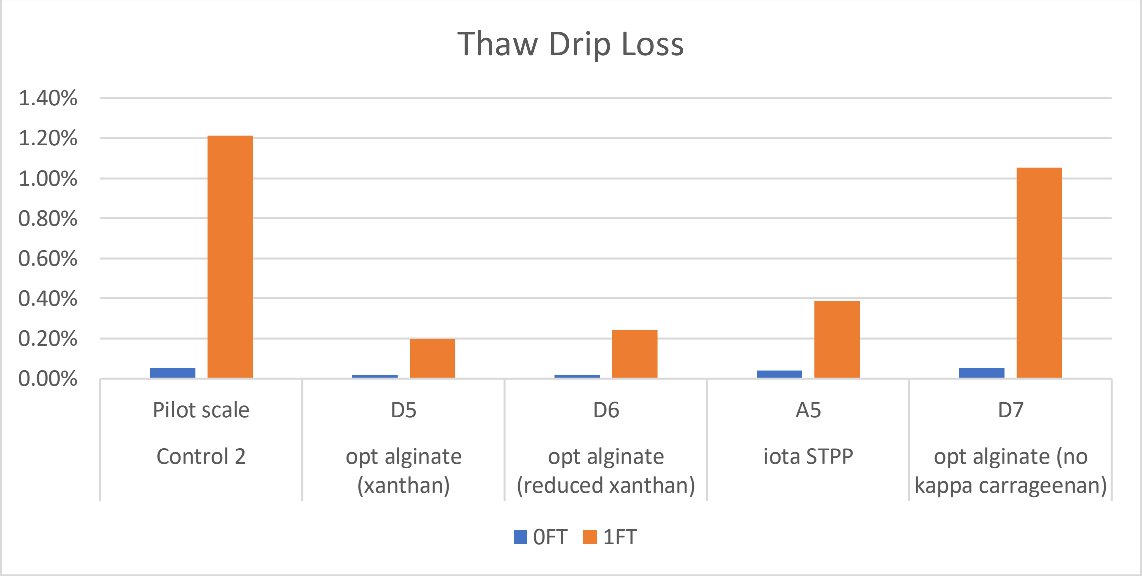


Figure 5-14: Comparison of thaw drip loss of the final formulations with Control 2.

Table 5-20: A complete summary of results for differences (%) in thaw drip loss and TPA hardness.

Chicken & pumpkin product trials	Variables									% Difference in Treatments				Difference (Absolute value)	
	kappa CGN	LBG	Iota CGN		Guar gum	Xanthan gum	Alginate		STPP	TPA Hardness		TPA Cohesiveness		Thaw drip	
			(+) $CaCo_3$	(-) $CaCo_3$			(+) $CaCO_3$	(-) $CaCO_3$			0 -1FT		0 -1FT		0 -1FT
Control 1	☑	☑								Δ (FT)	-35%	Δ (FT)	31%	Δ (FT)	1.1%
A1	☑	☑	☑								-39%		33%		0.2%
A2	☑	☑		☑							-38%		33%		1.6%
B1	☑				☑						-42%		31%		0.2%
B2	☑				☑						-32%		47%		0.6%
C1	☑		☑		☑						-36%		33%		0.1%
C2	☑			☑	☑						-26%		60%		1.5%
D1	☑	☑	☑			☑					-37%		25%		0.6%
D2	☑	☑				☑	☑				-35%		29%		0.2%
A3	☑	☑	☑								-39%		27%		0.9%
A4	☑	☑	☑			☑					-27%		8%		0.1%
B3	☑				☑				☑		-47%		18%		0.1%
B4	☑				☑						-34%		31%		0.1%
D3	☑				☑	☑	☑				-14%		24%		0.1%
D4	☑	☑				☑	☑				-21%		26%		0.1%
E	☑						☑		☑		-30%		40%		0.1%
F	☑	☑							☑		-49%		40%		0.9%
Control 2	☑	☑									-47%		22%		1.2%
A5	☑	☑	☑			☑			☑		-29%		18%		0.4%
D5	☑	☑				☑	☑				-33%		15%		0.2%
D6	☑	☑				☑	☑				-20%		14%		0.2%
D7		☑				☑	☑				29%		0%		1.0%

Key:	☑	standard dog roll ingredient used
	☑	test ingredient applied
	Δ (FT)	difference between treatments

5.6.5 Conclusion and Recommendation

The use of sodium alginate combined with xanthan gum and LBG at further optimised levels for processing performance (D5 & D6) based on trial D4 from previous trials displayed an acceptable performance however, trial D4 (Chapter 5.4.5) produced the better outcome in maintaining drip loss and TPA hardness. The use of iota carrageenan also displayed comparable performance, however, trial A4 which did not use STPP also displayed better outcomes in reducing thaw drip loss and TPA hardness.

6 Beef and Beetroot Prototypes with Best Outcomes for Chicken and Pumpkin Dog Roll

In the final series of trialled prototypes, beef and beetroot dog rolls were created based on the three formulations that exhibited the best performance for chicken and pumpkin dog rolls. These were tested to observe whether a similar gain in improvement of freeze thaw performance would be displayed. The following trial formulations were based on sodium alginate and iota carrageenan in combination with xanthan gum, LBG and maintaining kappa carrageenan levels at an optimised level of 0.39 %. These levels achieved positive performance in chicken and pumpkin dog roll prototypes and if the same was observed then further optimisation for beef and beetroot samples would be executed. Comparisons of performance of beet and beetroot prototypes were made against Control 2 for beef (Chapter 5.5)

The only alteration in the following test formulations was replacing comminuted chicken and diced pumpkin with comminuted beef and shredded beetroot. The individual components of ground beef are not known and supplied as a single comminuted raw ingredient. The best performing formulations observed in Chapter 5 reflecting trial A4 and D4 for chicken and pumpkin dog rolls were used to replicate beef and beetroot dog rolls. These were conducted to reflect optimised usage of ingredients satisfying both processing and product performance.

Table 6-1: Trial formulations using beef and beetroot based on best performing prototypes developed for chicken and pumpkin.

Trial D4 (using beef and beetroot)	
Ingredients	**Composition**
Total Base Ingredients	**99.06 %**
Kappa carrageenan	0.39 %
Xanthan: LBG (0.25:0.75; 2.5g:7.5g)	0.29 %
Calcium carbonate	0.04 %
Sodium alginate	0.23 %
Total Test Ingredients	**0.94 %**
Total Batch Composition	**100.00 %**
With sodium alginate.	

Trial A4 (using beef and beetroot)	
Ingredients	**Composition**
Total Base Ingredients	**99.07 %**
Kappa carrageenan	0.39 %
Xanthan: LBG (0.25:0.75; 2.5g:7.5g)	0.29 %
Calcium carbonate	0.03 %
Iota carrageenan	0.23 %
Total Test Ingredients	**0.93 %**
Total Batch Composition	**100.00 %**
With iota carrageenan.	

6.1 Results and Discussion

To evaluate and contrast outcomes, a comparison of results was made against pilot scale control formulation and experimental formulations for both the chicken and pumpkin dog rolls portraying identical formulations as those of beef and beetroot. Results for TPA hardness and thaw drip loss are given in Figure 6-1 and 6-2 respectively.

TPA hardness exhibited by beef and beetroot prototypes declined significantly with freeze thawing for both control and formulations A4 and D4. Visually the textural hardness was greatly reduced, and a large measurement of difference was recorded after one freeze thaw treatment compared to the performance of chicken and pumpkin dog roll prototypes (Fig 6-1). Iota carrageenan was found to perform poorly in the product as a whole with inferior textural hardness gained by the interaction of beef and beetroot with optimised test ingredients under the same conditions of treatment (Table 6-2).

Visual observations of beef and beetroot after treatment showed evident syneresis of liquid from sectioned test samples (Fig 6-3) compared to the chicken products (Fig 6-4) which remained visually satisfactory with no evident liquid expelled from the sectioned products. Comparison of beef and beetroot samples in Table 6-2 revealed greater than 2 % difference in thaw drip loss after treatment compared to chicken and pumpkin products which maintained thaw drip loss below 0.4 %.

The large differences associated with beef and beetroot prototypes may have been influenced by the potassium content of beetroot which was reported by DeFreitas et al. (1997) to reduce functionality of kappa and iota carrageenan. This related to discussion in Chapter 5.6 where omitting kapa carrageenan escalated thaw drip loss in chicken and pumpkin prototypes.

Judging by its performance, beef and beetroot dog rolls formulated with the same functional ingredients that are optimal in the chicken-based products did not perform as well and appear to be affected by the composition of beef or beetroot present in the product as these were the only difference between the two products. Therefore, formula’s optimised for chicken and pumpkin dog roll products are not optimal for beef and beetroot dog roll products under these processing conditions.

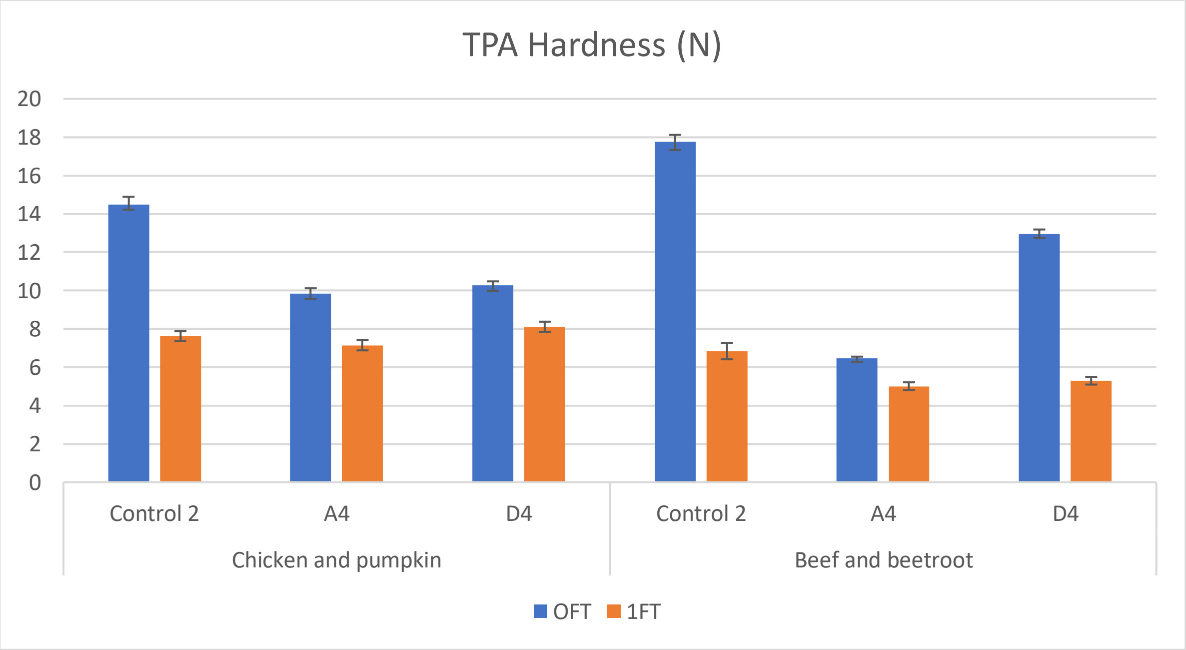


Figure 6-1: TPA hardness comparisons for the chicken and pumpkin and beef and beetroot dog rolls.

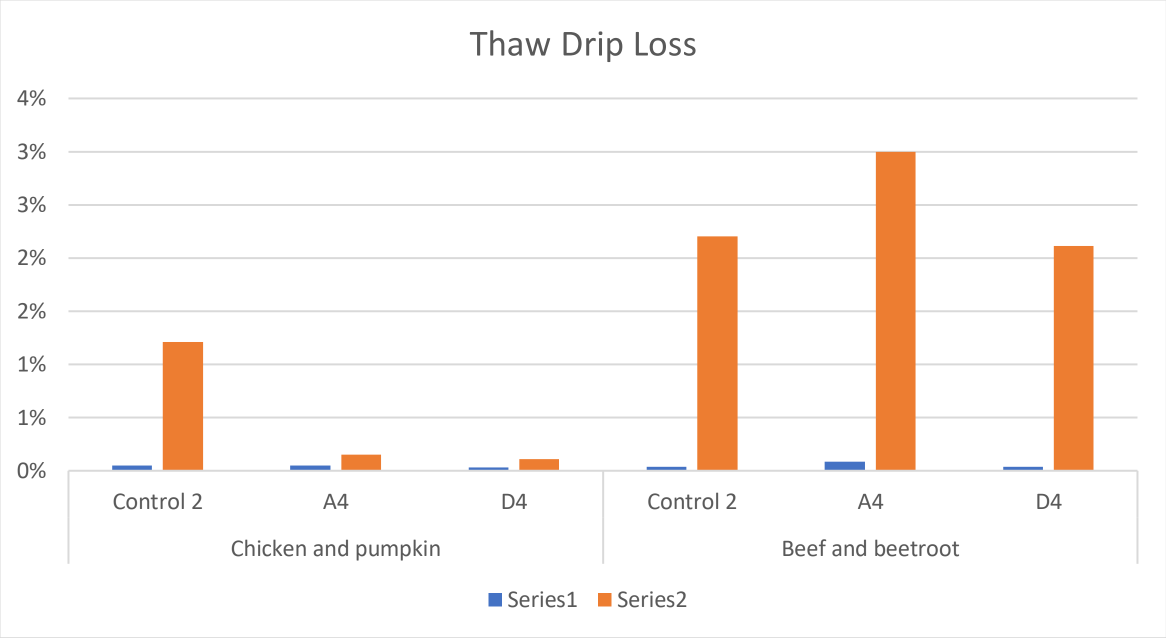


Figure 6-2: Thaw drip loss from chicken and pumpkin based products compared to beef and beetroot-based test prototypes using identical formulations.

Figure 6-3: Thaw drip loss for Beef and beetroot samples.

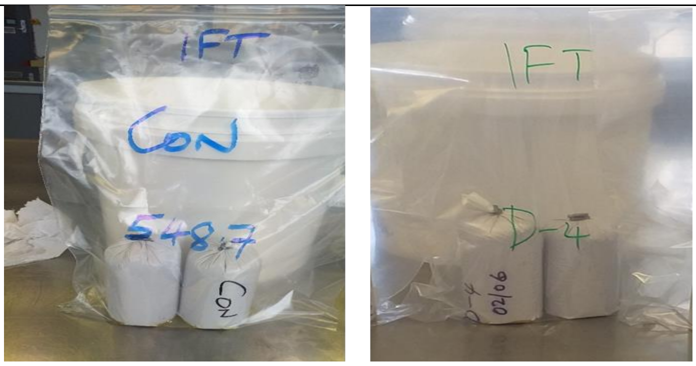


Figure 6-4: Comparison for thaw drip using chicken-based dog rolls showing evident improvement after one freeze thaw treatment compared to control 2 (left).

Table 6-2: A final comparison of the performance of chicken and pumpkin and beef and beetroot dog rolls after 1 freeze thaw cycle.

Product Base	Experiment	Variables									TPA		Thaw drip		% Difference		Differences (Absolute value)	
		kappa CGN	LBG	Iota CGN		Guar gum	Xanthan gum	Alginate		STPP	Hardness (N)				TPA Hardness		Thaw drip loss	
	Label			(+) $CaCo_3$	(-) $CaCo_3$			(+) $CaCO_3$	(-) $CaCO_3$		0FT	1FT	0FT	1FT		0 -1FT		0 -1FT
Chicken	Control 1	☑	☑								17.7	11.5	0.3%	1.4%	Δ (FT)	-35%	Δ (FT)	1.1%
	A4	☑	☑	☑			☑				9.8	7.2	0.1%	0.2%		-27%		0.1%
	D4	☑	☑				☑	☑			10.3	8.1	0.0%	0.1%		-21%		0.1%
	Control 2	☑	☑								14.5	7.6	0.1%	1.2%		-47%		1.2%
	A5	☑	☑	☑			☑			☑	9.7	6.9	0.0%	0.4%		-29%		0.4%
	D5	☑	☑				☑	☑			13.1	8.7	0.0%	0.2%		-33%		0.2%
	D6	☑	☑				☑	☑			11.1	8.9	0.0%	0.2%		-20%		0.2%
	D7		☑				☑	☑			2.7	3.4	0.1%	1.1%		29%		1.0%
Beef	Control 2	☑	☑								17.8	6.9	0.0%	2.2%		-61%		2.2%
	A4	☑	☑	☑			☑				6.5	5.0	0.1%	3.0%		-23%		2.9%
	D4	☑	☑				☑	☑			12.9	5.3	0.0%	2.1%		-59%		2.1%

Key:		
	☑	Standard dog roll ingredient used
	☑	Test ingredient applied
	Δ (FT)	Difference between treatments

6.2 Conclusion and Recommendations

Beef and beetroot prototypes did not respond in the same way as chicken and pumpkin dog rolls and displayed poor freeze thaw stability when formulated with sodium alginate and iota carrageenan in combination with xanthan and LBG. These combinations of gels had satisfied the performance requirements for the chicken and pumpkin dog roll prototypes. Future considerations for improving beef-based products should attempt revising this chapter using either:

1. a beef and pumpkin mix or
2. incorporating an alternative vegetable possessing lesser amounts of potassium compared to beetroot within its micronutrient content or
3. reducing the KCl content in the product batch and
4. reviewing the mineral content of the raw beef meat used.

7 Cost Comparison for Chicken and Pumpkin Dog Roll

Having identified a successful outcome for chicken and pumpkin dog roll using sodium alginate and iota carrageenan together with xanthan gum and LBG, a relative cost comparison was made against the control formula to deduce the differences in cost. Approximate prices for the ingredients used were provided by Medallion Pet Foods and included in Appendix E. (Suppliers for ingredients are not mentioned due to commercial sensitivity).

Table 7-1 below highlights the approximate cost for current products with respect to kappa carrageenan and LBG. Table 7-2 and 7-3 reflect the relative cost/kg for the two best performing formulas using sodium alginate and iota carrageenan which worked for chicken and pumpkin dog rolls compared against the normal product cost. Based on current ingredient prices, LBG remained the ingredient with the highest cost next to kappa carrageenan. Compared against the normal product, both formulations for freeze thaw stable chicken and pumpkin dog rolls remained within NZD$ 0.10 more in product cost. Iota carrageen was the cheaper alternative to sodium alginate and influenced a margin of only NZD$ 0.03 in cost of total test ingredients compared to the normal product based on current costs.

The marginal difference in cost between the normal product and freeze thaw stable products considers the reduction in kappa carrageenan from 0.57 % to 0.39 % as well as the cost of iota carrageenan itself which is comparatively the cheaper ingredient over sodium alginate.

Comparison for relative costings is based on the total test ingredients per batch. Cost per kilogram of test ingredients is calculated as:

$$Cost\ per\ kilo\ (Test\ Ingredients) = \frac{Total\ Test\ Ingredients}{Total\ Qty\ (kg)(Batch\ Ingredients)}$$

Table 7-1: Cost of functional ingredients in normal chicken and pumpkin dog roll.

Current Product Costing				
Ingredients	**QTY (kg)**	**Composition**	**Price/kg (NZD)**	**Batch Cost**
Total Base Ingredients	3466.60	99.28 %	**x**	**x**
Kappa carrageenan	19.95	0.57 %	$ 23.85	$ 475.81
LBG	5.04	0.14 %	$ 51.00	$ 257.04
Total Test Ingredients	24.99	0.72 %	x	**$ 732.85**
Total Batch Ingredients	**3491.59**	**100.00 %**	**x**	**x**
Cost per kg (Test Ingredients)				**$ 0.21**

Key:

X = unaccounted cost dependant on other raw materials.

Table 7-2: Relative cost using sodium alginate-based formula.

Formula D4 - Using Sodium Alginate				
Ingredients	**QTY (kg)**	**Composition**	**Price/kg (NZD)**	**Batch Cost**
Total Base Ingredients	3466.61	99.06 %	x	X
Kappa carrageenan	13.50	0.39 %	$ 23.85	$ 321.98
Xanthan gum	2.50	0.07 %	$ 6.00	$ 15.00
LBG	7.50	0.21 %	$ 51.00	$ 382.50
Calcium carbonate	1.40	0.04 %	$ 2.50	$ 3.50
Sodium alginate	8.00	0.23 %	$ 42.00	$ 336.00
Total Test Ingredients	32.90	0.94 %	x	**$ 1,058.98**
Total Batch Ingredients	**3499.51**	100.00 %	x	X
Cost per kg (Test Ingredients)				**$ 0.30**

Table 7-3: Relative cost using iota carrageenan-based formula.

Formula A4 - Using Iota Carrageenan				
Ingredients	**QTY (kg)**	**Composition**	**Price/kg (NZD)**	**Batch Cost**
Total Base Ingredients	3466.60	99.07 %	x	X
Kappa carrageenan	13.50	0.39 %	$ 23.85	$ 321.98
Xanthan gum	2.50	0.07 %	$ 6.00	$ 15.00
LBG	7.50	0.21 %	$ 51.00	$ 382.50
Calcium carbonate	1.20	0.03 %	$ 2.50	$ 3.00
Iota carrageenan	8.00	0.23 %	$ 15.00	$ 120.00
Total Test Ingredients	32.70	0.93 %	x	**$ 842.48**
Total Batch Ingredients	**3499.30**	100.00 %	x	X
Cost per kg (Test Ingredients)				**$ 0.24**

In conclusion, if considering more trials for the improvement of freeze thaw stability in dog rolls, iota carrageenan remained the better alternative between the two successful formulations due to its lower purchase cost over sodium alginate. The lower cost compared to the normal product was possible with a reduction in the use of kappa carrageenan in the formulation. The current evaluation of costs was based on 2020 prices of hydrocolloid gums procured within New Zealand and used only to contrast differences in product value between the current commercial product and an improved freeze thaw stable product.

8 Conclusion

This project aimed to reduce both the thaw drip loss and loss of textural hardness that resulted from the freezing and thawing of dog rolls. Gums were identified as suitable ingredients. For the chicken and pumpkin dog rolls the project identified the optimal combination of kappa carrageenan, locust bean gum, xanthan gum and sodium alginate with calcium carbonate that minimised thaw drip loss to acceptable levels. There was, however, still a decline in TPA hardness but the combination of ingredients minimised this and further consideration is required to determine whether this is acceptable. Further optimisation of these ingredients may be possible and is recommended.

However, the same optimised ingredient composition successfully found to work for the chicken and pumpkin dog rolls resulted in poor performance (high thaw drip loss and large differences in TPA hardness) after freeze thaw treatment in beef and beetroot dog rolls. This may be due to the differences in composition between chicken and beef and the beetroot and pumpkin.

The major limitation for incorporating higher levels of functional ingredients was the amount of water available in the product for dissolving salts and hydrating the respective functional gums to form an emulsion suspension that would be added after the initial cooking phase during processing. Economic considerations based on lowering ingredient costs may consider the use of iota carrageenan in combination with xanthan gum, LBG and calcium carbonate with a reduction in kappa carrageenan as depicted in Table 7-3 which would result in a value-added product within marginal cost limits.

The ingredients selected are considered modest in terms of clean labelling and customer expectations as they are gums similar to those already used and accepted in similar products which are used commercially and approved for use in dog foods under governing legislations within New Zealand and abroad.

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10 Appendices

10.1 Appendix A1 – Freeze Thaw Procedure

The freeze thaw cycles shall be carried out over 24 hours each for the freezing and thawing totalling 48 hours over the cycle prior to measuring thaw drip and texture analysis. The critical parameters will be the thawing cycle which should be carried out exactly at 24 hours prior to carrying out the two analysis.

Steps:

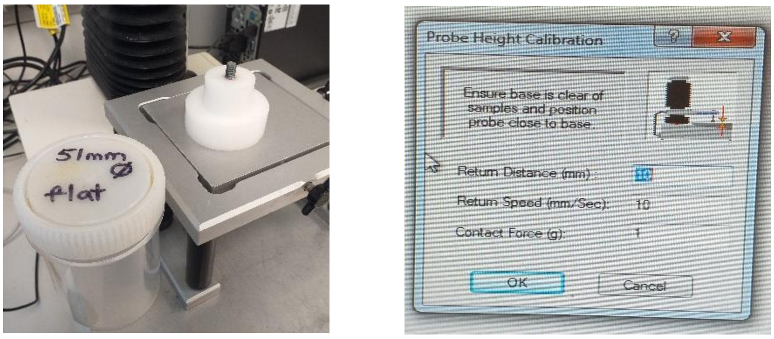
1. Samples of whole dog rolls shall be **weighed**, labelled and placed in the -18^{0}C freezer for 24 hours.
2. After freezing time, samples shall be removed and placed in the chiller at 5^{0}C for no less than 24 hours.
3. After thawing, samples for 1 cycle freeze thaw stability shall be removed for thaw drip and textural profiling analysis while samples for 2 cycle freeze thaw stability shall be placed back into the -18^{0}C freezer and kept indefinitely until thawing is carried out under same conditions as in step 2.

10.2 Appendix A2 – TA.XTPlus (Stable Microsystems) Settings

Texture profile analysis was carried out using the TA.XTPlus ® texture analyser (Stable Microsystems, Godalming, Surrey, UK).

Steps in setting up TPA on the TA.XTPLus.

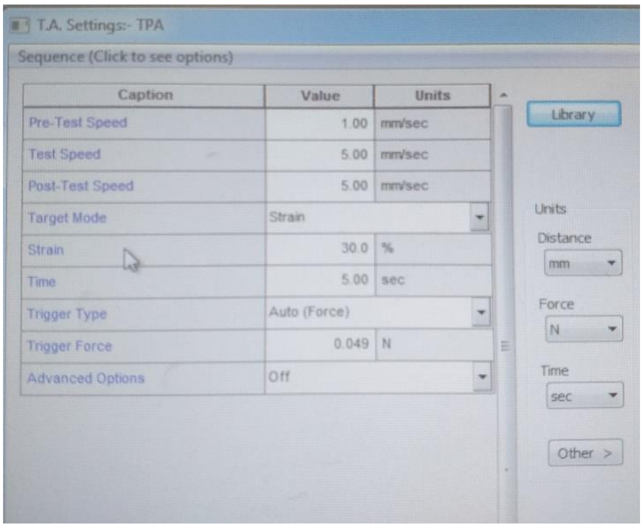
1. Switch on the TA.XTPlus by the switch located on the back of the instrument.
2. On the computer, open the program.
3. Open project and select file. This was initially set up by the laboratory supervisor for the double compression test.
4. Select run on the drop-down menu and calibrate force using the 4781g calibration weight.
5. Place calibration weight on weight plate and press ok if weight is matched on screen.
6. Use 51mm flat compression gauge (Fig 10-1) to the mounting of instrument and ensure base is clear.
7. Select calibrate height on run menu and set variables.
8. Select strain level and test speed settings as indicated.
9. Settings complete. Select run a test to conduct analysis.



Ensure base is clear of samples and position probe close to base.

Return Distance (mm): [illegible]
Return Speed (mm/Sec): 10
Contact Force (g): 1

Figure 10-1: Compression gauge and probe height settings used for TPA.



T.A. Settings:- TPA

Sequence (Click to see options)

Caption	Value	Units
Pre-Test Speed	1.00	mm/sec
Test Speed	5.00	mm/sec
Post-Test Speed	5.00	mm/sec
Target Mode	Strain	
Strain	30.0	%
Time	5.00	sec
Trigger Type	Auto (Force)	
Trigger Force	0.049	N
Advanced Options	Off	

Units

Distance: mm

Force: N

Time: sec

Figure 10-2: TA setting for TPA hardness.

10.3 Appendix B1 – Sample Raw TPA Data (Baseline TPA Raw Data)

Table 10-1: Factory produced dog roll TPA shear force.

Shear force (0FT)		
Test ID	Batch	Area F-T 1:2
Baseline Shear force		N.sec
		Area F-T 1:2
Start Batch Bc0	Beef & Beetroot	
Bc01	Bc0	160.438
Bc02	Bc0	186.212
Bc03	Bc0	113.475
Bc04	Bc0	174.389
Bc05	Bc0	150.517
Bc06	Bc0	147.111
Bc07	Bc0	140.147
Bc08	Bc0	193.520
Bc09	Bc0	132.856
Bc010	Bc0	106.362
Bc011	Bc0	142.584
Bc012	Bc0	221.020
Bc013	Bc0	112.000
End Batch Bc0	Bc0	
Average:	Bc0 (F)	**152.356**
S.D.	Bc0 (F)	34.178
Coef. of Variation	Bc0 (F)	22.433
standard Error		9.479
Start Batch pc0	Chicken & Pumpkin	
pc01	pc0	38.676
pc02	pc0	84.128
pc03	pc0	42.189
pc04	pc0	35.153
pc05	pc0	31.469
pc06	pc0	41.237
pc07	pc0	31.747
pc08	pc0	31.545
pc09	pc0	37.504
End Batch pc0	pc0	
Average:	pc0 (F)	**41.517**
S.D.	pc0 (F)	16.495
Coef. of Variation	pc0 (F)	39.732
standard Error		5.498

After 1 Freeze thaw cycle (1FT)		
Test ID	Batch	Area F-T 1:2
Baseline Shear force		N.sec
		Area F-T 1:2
BC11	BC1	45.390
BC12	BC1	59.907
BC13	BC1	83.109
BC15	BC1	103.061
BC16	BC1	59.407
BC17	BC1	119.759
BC18	BC1	83.043
BC19	BC1	49.726
BC110	BC1	92.274
BC111	BC1	89.102
End Batch BC1	BC1	
Average:	BC1 (F)	**78.478**
S.D.	BC1 (F)	24.230
Coef. of Variation	BC1 (F)	30.875
standard Error		7.662
Start Batch PC1	Chicken & Pumpkin	
PC11	PC1	33.602
PC12	PC1	49.799
PC13	PC1	48.073
PC14	PC1	34.393
PC15	PC1	29.958
PC16	PC1	34.904
PC17	PC1	33.234
PC18	PC1	42.200
PC19	PC1	31.650
PC110	PC1	29.742
End Batch PC1	PC1	
Average:	PC1 (F)	**36.756**
S.D.	PC1 (F)	7.308
Coef. of Variation	PC1 (F)	19.883
standard Error		2.311

After 2 Freeze thaw cycle (2FT)		
Test ID	Batch	Area F-T 1:2
Baseline Shear force		N.sec
		Area F-T 1:2
Start Batch Pc2s	Chicken & Pumpkin	
Pc2s1	Pc2s	41.402
Pc2s2	Pc2s	34.352
Pc2s3	Pc2s	30.315
Pc2s4	Pc2s	28.459
Pc2s5	Pc2s	41.254
Pc2s6	Pc2s	30.367
Pc2s7	Pc2s	36.463
Pc2s8	Pc2s	28.404
Pc2s9	Pc2s	35.808
Pc2s10	Pc2s	32.795
End Batch Bc0	Pc2s	
Average:	Pc2s (F)	**33.962**
S.D.	Pc2s (F)	4.797
Coef. of Variation	Pc2s (F)	14.124
standard Error		1.517
Start Batch Bc2s	**Beef & Beetroot**	
Bc2s1	Bc2s	98.714
Bc2s2	Bc2s	103.668
Bc2s3	Bc2s	124.803
Bc2s4	Bc2s	110.836
Bc2s5	Bc2s	116.910
Bc2s7	Bc2s	121.261
Bc2s8	Bc2s	113.574
Bc2s9	Bc2s	95.569
Bc2s10	Bc2s	83.089
End Batch Bc0	Bc2s	
Average:	Bc2s (F)	**107.603**
S.D.	Bc2s (F)	13.488
Coef. of Variation	Bc2s (F)	12.535
standard Error		4.496

Table 10-2: TPA results for Hardness and Cohesiveness of Factory dog rolls.

Hardness after N0 Freeze Thaw Treatment (0FT)					
Test ID	Force 1	**Hardness**	Area F-T 1:3	Area F-T 4:6	**Cohesiveness**
Baseline OFT	N	**N**	N.sec	N.sec	**Ratio**
	Force 1	**Force 2**	Area F-T 1:3	Area F-T 4:6	**Area F-T (4:6)/Area F-T (1:3)**
Start Batch PC0	**Chicken and pumpkin dog roll - Factory Product**				
PC02	18.276	22.746	37.178	4.944	0.133
PC03	17.531	22.050	39.008	4.965	0.127
PC04	16.888	20.738	35.837	4.472	0.125
PC05	17.684	22.254	38.386	4.612	0.120
PC06	16.283	22.130	38.048	4.733	0.124
PC07	17.910	23.186	39.817	5.160	0.130
PC08	15.931	20.143	38.727	4.293	0.111
PC09	16.797	21.692	39.418	4.710	0.119
PC010	17.354	21.781	36.547	4.130	0.113
PC011	18.966	23.517	38.132	5.086	0.133
PC012	18.893	23.412	38.280	5.022	0.131
PC013	17.409	22.884	39.120	4.819	0.123
PC014	14.295	18.144	32.292	3.589	0.111
Average:	16.786	**21.370**	35.956	4.766	**0.123**
S.D.	2.112	2.446	6.991	0.592	0.008
Coef. of Variation	12.583	11.445	19.442	12.426	6.417
Standard Error		0.416	0.552	0.123	0.002
Start Batch BC0	**Beef & beetroot dog roll - Factory Product**				
BC02	26.430	33.846	28.497	13.481	0.473
BC03	29.554	37.679	31.590	16.000	0.506
BC04	31.157	35.858	28.113	16.908	0.601
BC05	30.772	36.767	30.339	16.812	0.554
BC06	30.487	37.767	31.701	16.776	0.529
BC07	25.961	32.122	25.505	13.146	0.515
BC08	29.737	36.739	29.926	15.318	0.512
BC09	28.076	33.354	26.250	14.506	0.553
BC010	27.972	33.995	27.873	14.969	0.537
BC011	29.195	35.447	27.893	14.746	0.529
BC012	27.726	32.973	25.322	14.017	0.554
BC013	26.067	34.308	30.588	14.004	0.458
BC014	21.299	27.210	20.199	10.821	0.536
BC015	28.495	35.483	29.837	15.480	0.519
Average:	28.066	**34.539**	28.117	14.785	**0.527**
S.D.	2.575	2.742	3.079	1.670	0.035
Coef. of Variation	9.176	7.940	10.951	11.293	6.734
Standard Error		0.733	0.823	0.446	0.009

Hardness after 1 Freeze Thaw Treatment (1FT)

Test ID	Force 1	**Force 2**	Area F-T 1:3	Area F-T 4:6	**Cohesiveness**
Baseline 1FT	N	**N**	N.sec	N.sec	**Ratio**
	Force 1	**Force 2**	Area F-T 1:3	Area F-T 4:6	**Area F-T (4:6)/Area F-T (1:3)**
Start Batch PC1	**Chicken and pumpkin dog roll - Factory Product**				
PC11	11.016	11.900	8.025	5.842	0.728
PC12	12.795	13.921	9.196	6.643	0.722
PC13	9.609	10.762	7.428	5.004	0.674
PC14	11.376	12.608	8.364	5.878	0.703
PC15	11.821	12.985	8.491	6.043	0.712
PC16	11.229	12.547	8.793	6.009	0.683
PC17	11.416	12.536	8.078	5.750	0.712
PC18	11.296	12.725	8.476	5.701	0.673
PC19	9.441	10.442	6.828	4.641	0.680
PC110	9.116	9.878	5.792	4.207	0.726
PC111	12.215	13.298	8.043	5.903	0.734
PC112	10.457	11.371	6.448	4.735	0.734
PC113	11.026	12.278	8.479	5.815	0.686
PC114	13.420	14.783	9.885	6.988	0.707
PC115	12.356	13.722	9.162	6.332	0.691
PC116	11.235	12.225	7.348	5.354	0.729
PC117	11.573	12.707	8.141	5.805	0.713
PC118	12.235	13.405	8.903	6.391	0.718
PC119	10.891	11.859	7.444	5.419	0.728
PC120	12.692	14.144	9.755	6.747	0.692
PC121	12.256	13.387	8.794	6.339	0.721
PC122	11.532	12.725	8.873	6.236	0.703
PC123	8.267	9.068	5.181	3.556	0.686
PC124	11.186	12.215	7.586	5.352	0.705
Average:	11.269	**12.396**	8.063	5.695	**0.707**
S.D.	1.218	1.351	1.158	0.814	0.020
Coef. of Variation	10.812	10.897	14.364	14.288	2.772
Standard Error		0.276	0.236	0.166	0.004
Start Batch BC1	**Beef & beetroot dog roll - Factory Product**				
BC11	15.957	17.758	11.762	7.294	0.620
BC12	12.787	14.017	9.464	6.104	0.645
BC13	14.703	16.759	12.459	7.395	0.594
BC14	16.370	18.352	12.713	7.942	0.625
BC15	15.593	17.290	12.127	7.719	0.637
BC16	14.203	16.080	11.764	6.982	0.593
BC17	15.097	17.152	12.568	7.636	0.608
BC18	15.772	17.705	13.198	8.026	0.608
BC19	17.561	19.922	14.761	8.825	0.598
BC110	16.266	18.110	11.848	7.399	0.624
BC111	17.275	19.667	14.179	8.256	0.582
BC112	15.658	17.271	11.618	7.533	0.648
BC113	17.159	19.422	13.781	8.278	0.601
BC114	17.226	19.180	12.911	7.912	0.613
BC115	16.078	17.992	12.343	7.520	0.609
BC116	18.052	20.171	13.686	8.410	0.614
BC117	14.987	16.752	11.154	6.926	0.621
BC118	16.840	19.278	14.169	8.250	0.582
BC119	18.124	20.562	15.281	9.062	0.593
BC121	12.946	14.553	10.150	6.193	0.610
BC122	16.144	18.072	12.270	7.640	0.623
BC123	14.992	16.604	11.155	7.093	0.636
Average:	15.900	**17.849**	12.516	7.654	**0.613**
S.D.	1.449	1.702	1.432	0.738	0.019
Coef. of Variation	9.115	9.534	11.442	9.648	3.049
Standard Error		0.363	0.305	0.157	0.004

Hardness after 2 Freeze Thaw Treatment (2FT)					
Test ID	Force 1	Force 2	Area F-T 1:3	Area F-T 4:6	Cohesiveness
Baseline 2FT	N	N	N.sec	N.sec	Ratio
	Force 1	Force 2	Area F-T 1:3	Area F-T 4:6	Area F-T (4:6)/Area F-T (1:3)
Start Batch PC2	**Chicken and pumpkin dog roll - Factory Product**				
PC21	10.263	11.119	7.060	5.154	0.730
PC22	9.090	9.867	6.168	4.506	0.731
PC23	9.735	10.518	6.487	4.817	0.743
PC24	9.814	10.579	6.759	4.930	0.729
PC25	8.770	9.457	5.991	4.383	0.732
PC26	9.971	10.810	6.858	5.015	0.731
PC27	8.956	9.732	6.141	4.472	0.728
PC28	8.739	9.577	6.206	4.497	0.725
PC29	8.173	8.811	5.442	3.950	0.726
PC210	9.206	10.042	6.200	4.439	0.716
PC211	6.703	7.131	4.135	3.102	0.750
PC212	8.936	9.746	6.178	4.461	0.722
PC213	9.109	9.936	6.055	4.336	0.716
PC214	8.731	9.474	5.503	4.042	0.735
PC215	9.712	10.616	6.897	4.989	0.723
PC216	11.575	12.531	8.026	6.021	0.750
PC217	10.416	11.246	6.899	5.124	0.743
PC218	11.138	12.119	7.696	5.610	0.729
PC219	9.695	10.559	6.114	4.489	0.734
PC220	9.904	10.811	7.141	5.165	0.723
PC221	9.314	10.071	6.186	4.529	0.732
PC222	8.816	9.584	6.040	4.325	0.716
PC223	9.930	10.822	7.113	5.184	0.729
PC224	8.598	9.243	5.730	4.250	0.742
Average:	9.387	10.183	6.376	4.658	0.731
S.D.	0.990	1.093	0.800	0.592	0.010
Coef. of Variation	10.544	10.734	12.553	12.717	1.308
Standard Error		0.278	0.214	0.163	0.003
Start Batch BC2	**Beef & beetroot dog roll - Factory Product**				
BC21	16.017	18.118	13.057	7.999	0.613
BC22	14.000	15.525	9.086	5.980	0.658
BC23	12.102	13.520	8.889	5.721	0.644
BC24	13.190	14.515	9.423	6.289	0.667
BC25	13.812	15.205	9.450	6.222	0.658
BC26	13.392	15.116	10.491	6.390	0.609
BC27	13.128	14.688	10.167	6.459	0.635
BC28	13.779	15.464	10.997	6.959	0.633
BC29	12.377	13.659	9.484	6.106	0.644
BC210	13.802	15.359	10.151	6.459	0.636
BC211	12.873	14.236	9.117	5.972	0.655
BC212	11.968	13.178	7.775	5.161	0.664
BC213	13.541	14.955	9.748	6.261	0.642
BC214	14.598	16.216	10.593	6.844	0.646
BC215	12.073	13.479	9.124	5.756	0.631
BC216	12.404	13.694	9.023	5.984	0.663
BC217	14.385	16.165	10.590	6.698	0.632
BC218	14.403	16.200	11.191	6.963	0.622
BC219	11.925	13.359	8.997	5.633	0.626
BC220	11.980	13.474	9.205	5.662	0.615
BC221	11.035	12.082	8.006	5.335	0.666
BC222	14.106	15.695	10.384	6.706	0.646
BC223	13.660	15.241	10.754	6.849	0.637
BC224	11.132	12.360	7.177	4.700	0.655
Average:	13.153	14.646	9.703	6.213	0.642
S.D.	1.199	1.398	1.241	0.697	0.017
Coef. of Variation	9.118	9.545	12.790	11.223	2.676
Standard Error		0.285	0.253	0.142	0.004

Table 10-3: Baseline Raw Data Summary of TPA conducted for Factory produced dog rolls.

Shear Force (N.sec)	**Freeze thaw treatments**					
	BB 0FTT	**BB 1FFT**	**BB 2 FTT**	**PC 0FTT**	**PC 1FTT**	**PC 2FTT**
Average:	152.356	78.478	107.603	41.517	36.756	33.962
S.D.	34.178	24.230	13.488	16.495	7.308	4.797
Coef. of Variation	22.433	30.875	12.535	39.732	19.883	14.124
Standard Error	9.479	7.662	4.496	5.498	2.311	1.517
Cohesiveness	**BB (0FT)**	**BB (1FT)**	**BB (2FT)**	**CP (0FT)**	**CP (1FT)**	**CP (2FT)**
Average:	0.586	0.613	0.801	0.633	0.705	0.827
S.D.	0.034	0.019	0.039	0.024	0.020	0.029
Coef. of Variation	5.731	3.049	4.874	3.779	2.778	3.542
Standard Error	0.011	0.004	0.008	0.008	0.004	0.007
Hardness (N)	**BB (0FT)**	**BB (1FT)**	**BB (2FT)**	**CP (0FT)**	**CP (1FT)**	**CP (2FT)**
Average:	32.336	17.849	14.646	23.872	12.349	10.183
S.D.	1.056	1.702	1.398	3.550	1.369	1.093
Coef. of Variation	3.267	9.534	9.545	14.869	11.084	10.734
Standard Error	0.334	0.363	0.285	0.573	0.292	0.278

10.4 Appendix B2 – Baseline Thaw Drip Raw Data

Table 10-4: Thaw drip loss over 48 hours for baseline samples.

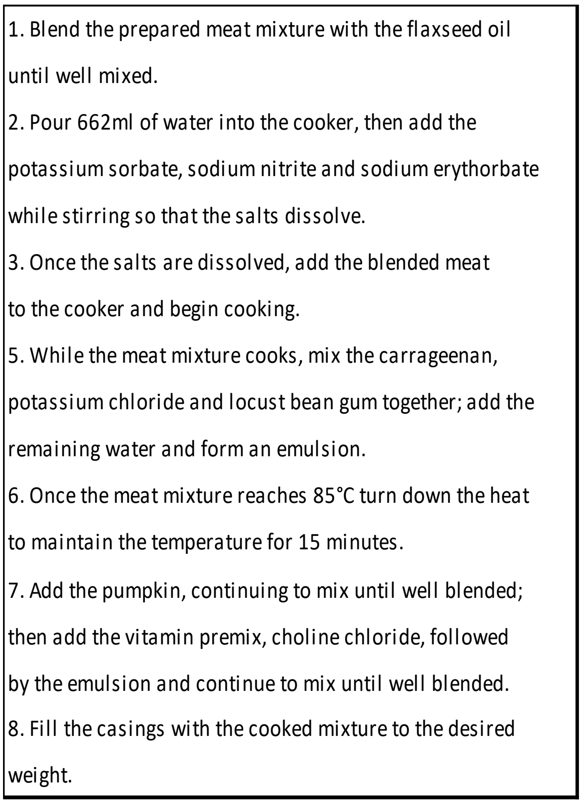
Sample	weight in (g)	weight casing+ clip (g)	weight out (g)	Difference (g)	Drip loss (%)
BB (0FTT)	2017.1	7.9	2008.4	0.8	0.04%
BB (1FTT)	1996.7	7.5	1974	15.2	0.76%
BB (2FTT)	1991.8	7.6	1951.6	32.6	1.64%
PC (0FTT)	2012.2	7.5	2003.8	0.9	0.04%
PC (1FTT)	1984.8	7.2	1963.5	14.1	0.71%
PC (2FTT)	1998.4	7.7	1941.6	49.1	2.47%

Difference = ((weight in – weight casing + clip) – weight out)

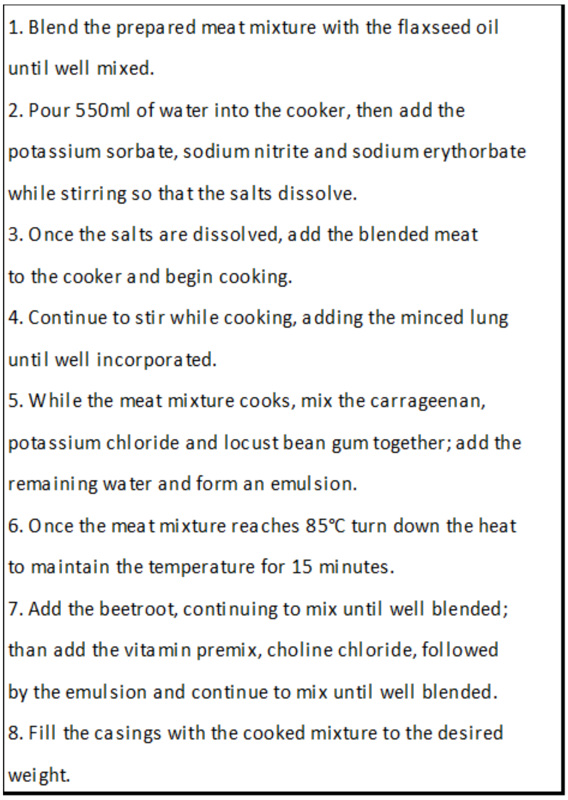
% Loss = (difference / (weight in – weight casing + clip)) x 100

10.5 Appendix C – Processing SOP Pilot Plant

Table 10-5: SOP Chicken and pumpkin and beef and beetroot dog roll production.



1. Blend the prepared meat mixture with the flaxseed oil until well mixed.
2. Pour 662ml of water into the cooker, then add the potassium sorbate, sodium nitrite and sodium erythorbate while stirring so that the salts dissolve.
3. Once the salts are dissolved, add the blended meat to the cooker and begin cooking.
5. While the meat mixture cooks, mix the carrageenan, potassium chloride and locust bean gum together; add the remaining water and form an emulsion.
6. Once the meat mixture reaches 85°C turn down the heat to maintain the temperature for 15 minutes.
7. Add the pumpkin, continuing to mix until well blended; then add the vitamin premix, choline chloride, followed by the emulsion and continue to mix until well blended.
8. Fill the casings with the cooked mixture to the desired weight.



1. Blend the prepared meat mixture with the flaxseed oil until well mixed.
2. Pour 550ml of water into the cooker, then add the potassium sorbate, sodium nitrite and sodium erythorbate while stirring so that the salts dissolve.
3. Once the salts are dissolved, add the blended meat to the cooker and begin cooking.
4. Continue to stir while cooking, adding the minced lung until well incorporated.
5. While the meat mixture cooks, mix the carrageenan, potassium chloride and locust bean gum together; add the remaining water and form an emulsion.
6. Once the meat mixture reaches 85°C turn down the heat to maintain the temperature for 15 minutes.
7. Add the beetroot, continuing to mix until well blended; than add the vitamin premix, choline chloride, followed by the emulsion and continue to mix until well blended.
8. Fill the casings with the cooked mixture to the desired weight.

Cleaning SOP:

1. Emergency stop is activated, and grate is opened to carry out manual cleaning
2. After manual cleaning, the grate is closed, and the vessel filled with hot water (60^0C). two (2) scoops of degreasing detergent.
3. The emergency stop is deactivated, and the operation mode switched to direct steam injection (DSI) mode.
4. The process is set to the following:
    a. Temperature – 90^0C
    b. Holding time – 15 minutes
    c. Agitation speed – 90 RPM
5. After process cleaning with detergent, a second treatment is carried out in the same manner but adding 1% caustic acid to aid final cleaning.
6. The vessel is rinsed after the second operation and grate is opened to aid drying.

A full procedure can be found on the Blentech SOP manual located with the equipment.

10.6 Appendix D – Table Summary of Differences in TPA Hardness and Thaw Drip Loss

Table 10-6: Differences between chicken and beef prototypes.

Product Base	Experiment Label	Variables: kappa CGN	LBG	Iota CGN (+) CaCO₃	Iota CGN (-) CaCO₃	Guar gum	Xanthan gum	Alginate (+) CaCO₃	Alginate (-) CaCO₃	STPP	TPA Hardness (N) 0FT	TPA Hardness (N) 1FT	Thaw drip 0FT	Thaw drip 1FT	% Difference in Treatments	TPA Hardness 0 -1FT		Thaw drip loss 0 -1FT
Chicken	Control 1	☑	☑								17.7	11.5	0.3%	1.4%	Δ (FT)	-35%	Δ (FT)	1.1%
	A4	☑	☑	☑			☑				9.8	7.2	0.1%	0.2%		-27%		0.1%
	D4	☑	☑				☑	☑			10.3	8.1	0.0%	0.1%		-21%		0.1%
	Control 2	☑	☑								14.5	7.6	0.1%	1.2%		-47%		1.2%
	A5	☑	☑	☑			☑			☑	9.7	6.9	0.0%	0.4%		-29%		0.4%
	D5	☑	☑				☑	☑			13.1	8.7	0.0%	0.2%		-33%		0.2%
	D6	☑	☑				☑	☑			11.1	8.9	0.0%	0.2%		-20%		0.2%
	D7		☑				☑	☑			2.7	3.4	0.1%	1.1%		29%		1.0%
Beef	Control 2	☑	☑								17.8	6.9	0.0%	2.2%		-61%		2.2%
	A4	☑	☑	☑			☑				6.5	5.0	0.1%	3.0%		-23%		2.9%
	D4	☑	☑				☑	☑			12.9	5.3	0.0%	2.1%		-59%		2.1%

Key:		
	☑	standard dog roll ingredient used
	☑	test ingredient applied
	Δ (FT)	difference between treatments

10.7 Appendix E – Selected Ingredient Costs and Specifications

Table 10-7: Prices and of selected ingredients used for the improvement of freeze thaw stability in dog rolls. (Personal communication, Alastair Haliburton, Medallion Pet Foods. 2020).

Ingredient	Mesh size	NZ Cost/kg
Kappa carrageenan	150	$23.85
Iota carrageenan	40	$15.00
Locust bean gum	200	$51.00
Sodium alginate	80	$42.00
Xanthan gum	80	$6.00
Calcium carbonate		$2.50