Development of Model Cured Fermented Fish Sausage from Albacore Tuna

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Abstract

This project was carried out to explore how to create an export opportunity for Tonga that would have a measure of geographical exclusivity. The product is a model cured fermented tuna sausage that could be produced in Tonga from the commercial species albacore tuna (*Thunnus alalunga).* The motive for this model is that certain features of Tonga, namely a hot climate, expensive electricity for alternative tuna preservation methods and long shipping times to foreign markets, can be turned from a disadvantage to an advantage. The fish meat sausage was, salted, nitrite cured and bacillus-fermented at 32°C with a Tongan carbohydrate source, taro to create a product stable that requires little or no refrigeration. The research was carried out in two phases. The first was to determine the effect of microwave cooking on the fermentable sugar formation in tubers (taro, potato, kumara), and having defined a useful hydrolysed source, the second phase was sausage production to define its physiochemical properties.

Of the three potential cooked carbohydrate sources, kumara showed the highest glucose concentration followed by taro and then potato after hydrolysis. Taro, which is particularly important to Tonga as a staple food, generated potentially useful concentrations of glucose and was chosen for further work. The concentrations of taro used in this study were 0, 10, 20 and 30%, a likely range of concentrations to fulfill one criteria; a fermented tuna product but produced at minimal cost. Tuna is much more expensive than taro.

After four days of fermentation at 32ºC, the pH of each treatment was between 4.1 and 3.9, very low by fermented meat standards. Hardness and springiness increased on Day 4 while cohesiveness and adhesiveness decreased. The product was increasingly hard, sticky and brittle, probably due to staling, more formally called retrogradation of taro starch. The colour changes showed an increase in L* (light reflectance) while the a* (redness) values decreased in relative to b* (yellowness) values confirming that a brown fermented fish mince (FFM) sausage was produced.

Proteolysis in sausage containing 20% taro, measured colorimetrically by soluble peptide bonds, was greatest for uncured FFM ($P < 0.001$) sausage compared to cured FFM sausage. Lipid oxidation measured by thiobarbituric acid method was unvarying with curing and with time providing there is no important difference between curing and uncuring. Histamine being the sole biogenic amine decreased in concentration with a statistically

significant but unimportant difference between cured and uncured FFM sausage. These values were lower than the maximum allowable limit of 200 ppm in New Zealand as well as those reported for fermented fish sausage in Southeast Asia.

The results point to commercial opportunities and further research in which sensory analysis should be performed before the products can be tested in the market.

Statement of Originality

"I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the qualification of any other degree or diploma of a university or other institutions of higher learning, except where due acknowledgement is made in the acknowledgement".

Signed _____

Date _____

Chapter 1

Introduction

Geographical context

Historically, Pacific nations have always had a strong affinity with the sea and fish stocks are one of the most important resources in the Pacific. This is not surprising considering the ratio of sea area to land area in this vast region. In modern times, nutrition, welfare, culture, recreation, government revenue and employment of the Pacific Islands countries are linked to fish stocks, especially tuna. Twenty million square kilometers of the South Pacific is the home to the world's largest tuna fishery. Deep-sea fishing, mostly for tuna, is conducted throughout the Pacific, mainly by Korean, Taiwanese, Japanese and American fishing fleets. The tuna harvest is worth around US$3 billion annually and is a major source of employment for Pacific Island nationals, and Tonga plays a part on this by supplying labour to fishing fleets and canneries. Canning is the major means of fish preservation, a process that relies on a once-only increase in temperature that is cheaper in capital and energy costs than maintaining a cold chain to export markets. Energy is very expensive in the Pacific, based as it is on imported oil. Canneries employing islanders are operated in American Samoa, Fiji, and the Solomon Islands, but not in the Kingdom of Tonga, where I – Rosely Bourke – live. This thesis examines another way of preserving fish that could be applied in Tonga. The method is fermentation, which has an inherent low energy cost and indeed would capitalise on the high average ambient temperature in Tonga, thus turning a disadvantage to an advantage.

The chapter examines the history and current fishing practice in the South Pacific, and then examines preservation of muscle foods, followed by an examination of fermentation in the context of Tonga that leads to a description of the aims of this thesis.

Fishing history

Fishing is an ancient practice that dates back to, at least, the beginning of the Paleolithic period about 40,000 years ago when hunter-gatherers roamed the earth. Archaeology features such as shell middens, discarded fish bones and cave paintings show that sea foods were important for survival and consumed in significant quantities in the earlier days (Bailey & Parkington, 1988; Schiffer, 1972). During this period, hunter-gatherers were

limited to catching small quantities of fish from shallow lakes and along the seashore as they did not have the equipment or expertise with which to harvest large quantities of fish from the oceans, or even large lakes (Jackson , Kirby, Berger, Bjomdal, Botsford, Bourque, & Warner, 2001; Erlandson, 2001).

Before large civilisations or settlements developed, most food was consumed almost immediately after capture or harvest. However, as the population grew, survival required the preservation and storage of foodstuffs. During the Mesolithic Period (12,000 to 8,000 BP), the first civilisations developed relied heavily on fish for sustenance. These societies developed primitive fishing tools, such as stone-tipped fishing spears, fishhooks, fish lines, and nets (Yesner, 1987). As a result of the increased harvests, techniques of preservation such as salting, drying, smoking, and fermentation were developed on a large scale. For the first time the harvest of large quantities of foodstuffs, including fish, could be harvested without wasting a significant portion of the harvest due to deterioration (Erlandson, 2001). As the fishing techniques developed in these societies, larger and larger fishing craft were developed which could venture further out to sea and harvest more fish per venture. It was about this time that fishing developed into a defined vocation and the fishing industry was born (Erlandson, 2001).

International fish catch

Fish is a vital source of food for people according to the Food and Agriculture Organization (1997). It is man's most important single source of high-quality protein, providing 20% of the animal protein consumed by the world's population (Food and Agriculture Organization, 2010). It is a particularly important protein source in regions where livestock is relatively scarce. Fish supplies less than 10% of animal protein consumed in North America and Europe, but 17% in Africa, and 26% in Asia (Food and Agriculture Organization, 2000). The Food and Agriculture Organization (2010) estimates that about 6 to 7 billion people world-wide rely on fish as their primary source of animal protein.

Fish obviously has substantial social and economic importance. The Food and Agriculture Organization estimates the value of fish traded internationally to be US$94 billion per year (Food and Agriculture Organization, 2010). Over 45 million people are employed directly either full time or, more frequently, part time in capture fisheries or in aquaculture. At least 12% of these are women (Food and Agriculture Organization, 2010). It is also estimated that, for each person employed in capture fisheries and aquaculture production,

about three jobs are produced in secondary activities, including post-harvest, for a total of more than 180 million jobs in the whole entire fish industry (Food and Agriculture Organization, 2010).

According to Food and Agriculture Organization (2010), a review of the total world fisheries and aquaculture catch on the past six years shows an increase from 134 million tonnes in 2004 to 145 million tonnes in total for 2009 (

Figure 1). Consumption of food fish is increasing in parallel, having risen from 40 million tonnes in 1970 to 86 million tonnes in 1998 (Food and Agriculture Organization, 2000), and is expected to reach 110 million tonnes by 2010 (Food and Agriculture Organization, 1997). The remaining 27 to 29 million tonnes is used for non-food purposes such as fishmeal and fish oil, culture, bait, and pharmaceutical uses as well as for direct feeding in aquaculture and for fur animals. As of the updated review of the world fisheries by Food and Agriculture Organization (2010), it is estimated that using fish for human consumption has increased from 104 million tonnes in 2004 to about 118 million tonnes in 2009 as shown in

Figure 1.

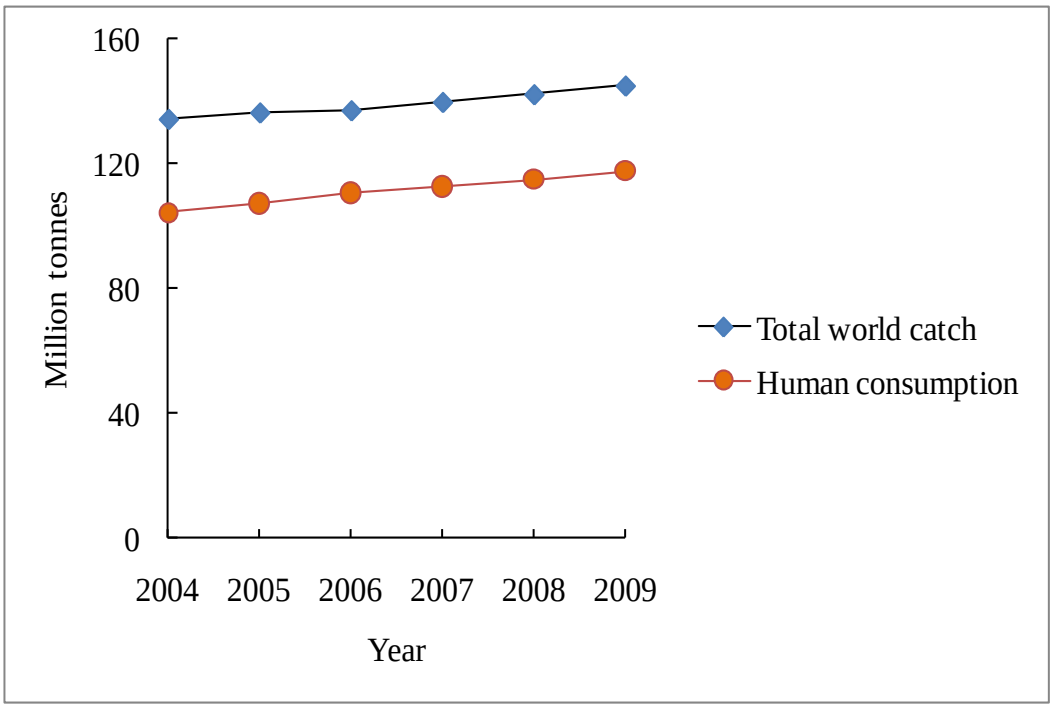

Figure 1. Graph of total world fish catch and human consumption from 2004 to 2009 in million tonnes (Food and Agriculture Organization, 2010)

Fish catch is marketed fresh, frozen, cured, canned or otherwise prepared for direct human consumption. Since the mid-1990s, the proportion of fish used for direct human consumption has grown as more fish is used as food and less for producing fishmeal and fish oil (not shown in

Figure 1). Of the fish destined for direct human consumption, fish in live or fresh form was the most important product, with a share of 49%, followed by frozen (25%), prepared or preserved fish (26%). Live and fresh fish consumption grew from 45 million tonnes in 1998 to 57 million tonnes by 2008. Fish for human consumption in all other forms increased from 47 million tonnes in 1998 to 59 million tonnes by 2008 (Food and Agriculture Organization, 2010).

The increases in per capital consumption and fish production account for only a small portion of the growth. The most significant point is the growing human population in many countries. This includes Asia, Africa and South America that are primarily responsible for this steadily growing demand for fish food. The reasons has now illustrate that a consistent source of fish is essential for the nutritional and financial health of a large segment of the world's population (Food and Agriculture Organization, 2010).

Fish catch in the South Pacific

The Pacific is the world's largest ocean and one of the global centres of marine biodiversity. The people of the Pacific islands have relied on fish to provide the bulk of their protein requirements, particular on fish from coral reefs, lagoons and even deep marine fish. Although the diet of Pacific islanders has become more varied during this century, reef fisheries continue to be a major protein source and remain a key element in the food security of the Pacific islands. Pacific islands societies have increasingly become more urbanised, with in many cases over 50% of the national populations residing in the capital cities. Pressure for usable land resources to generate other sources or protein is constrained by the mountainous terrains and seasonal flooding. Prices of imported food especially high-fat meat products like mutton flaps have increased greatly in recent years, thus seafoods continue to play a major role in the nutrition of Pacific islanders.

Tuna fisheries of the tropical South Pacific are among the most valuable in the world. Industrial fisheries for tunas and associated species extend over most of the tropical and temperate Pacific ocean and currently produce over 2.5 million tons or about 64% of the 2004

global tuna catch (Food and Agriculture Organization, 2010). The fishery targets four primary temperate and tropical tuna species: skipjack (*Katsuwonus pelamis*), yellowfin (*Thunnus albacores*), bigeye (*T. obesus*), and albacore (*T. alalunga*) tuna. Other species, bluefin tuna (*T. orientalis*), billfishes, and oceanic sharks are also taken, but the primary tuna species make up more than 90% of the total catch by weight (Food and Agriculture Organization, 2010).

Catches of skipjack tuna (*Katsuwonus pelamis*) have more than doubled since the beginning of the 1980s. They reached a peak of about 1 million tonnes in 1991 and have remained between 800,000 to 1 million tonnes annually since (Asian Development Bank, 2010). The yellowfin (*Thunnus albacores*) catches have also increased up to 430,000 tonnes in 1993. Only bigeye (*Thunnus obesus*) catches have remained fairly stable, at about 75,000 tonnes annually (Food and Agriculture Organization, 1999). Most of the increase in the catch has been due to the development of the purse seine fishery, which in 1994 accounted for 65% of the total skipjack catch, and 50% of the total yellowfin catch (Asian Development Bank, 2010).

The tuna fishery is conducted by fleets from many different nations. Traditionally fishing in this area was mainly done by the Japanese, who fished with longline or pole and line. Over time, new nations, especially Taiwan and Korea, emerged in the longline fishery. From the 1970s on, fishing by purse seine increased rapidly (McCoy & Gillett, 2005).

Fishery in Tonga

The geography of Tonga exerts a large influence on fishing in the country. Tonga is made up of some 150 islands of which 36 are inhabited, as well as many smaller islets and reefs with a water area of about 700,000 km^2. Tonga's ocean floor contains the second deepest ocean in the world, known as the Tonga trench. The islands, whose collective land area is about 747 km^2, are distributed in three main groups - Tongatapu (location of the capital and administrative centre, Nuku'alofa) and neighbouring islands in the south, the Ha'apai group located centrally, and the Vava'u group to the north. Other islands extend the archipelago further north and south beyond the main groups.

Fishing has always been an important subsistence activity in Tonga, having played an important role for Tonga in traditional food production. Up to the early 1960s, domestic demand for fish was almost wholly met through catches from the country's reefs and lagoons.

The industry grew steadily since then to become an important source of foreign exchange earnings (Rohorua, 2004).

Tonga's major local commodities exports are fish, root crops, vanilla, kava and squash (Figure 2). In 1993, fisheries exports became the second largest foreign exchange earner after squash, exceeding vanilla for the first time through to 2003 (Asian Development Bank, 2004). The same happens in 2004 to 2005 (Lui, Fa'anunu, & Koloa, 2005). In 2006 to 2010, fish exports have become the biggest foreign exchange earner creating more than 4.1% increase in the country's gross domestic product (GDP) (Tonga Department of Statistics, 2010).

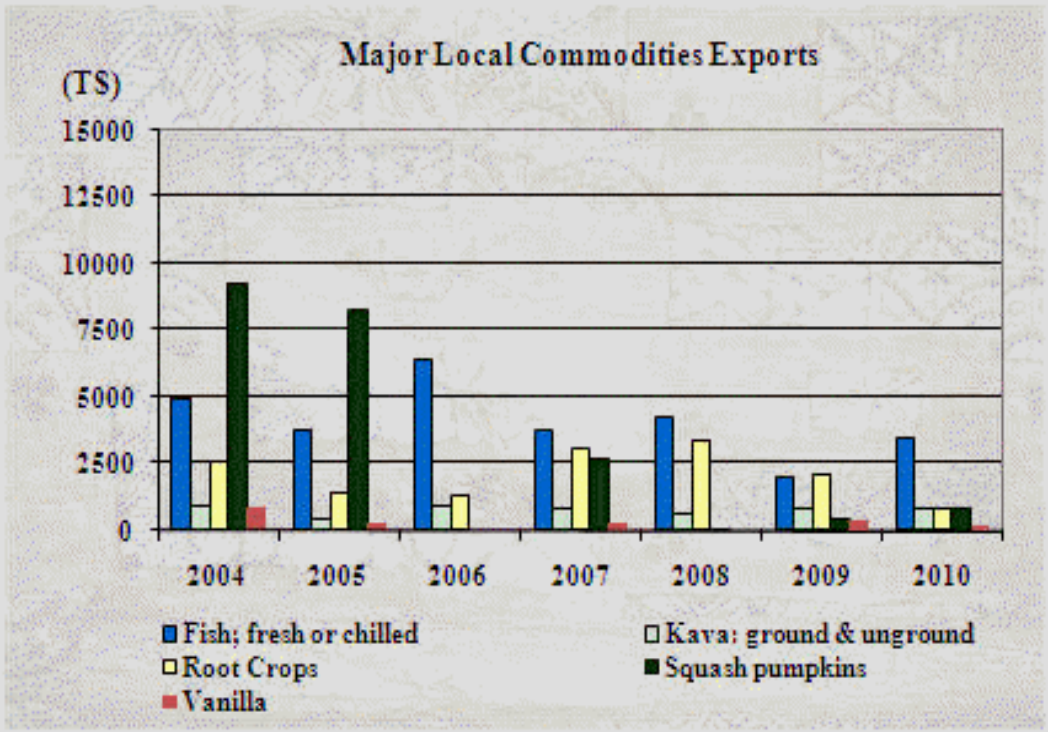

Figure 2. Tonga's major export from 2004 to 2010 in (T$) (Tonga Department of Statistics, 2010)

Three percent of the total area of the Pacific exclusive economic zone (EEZ) belongs to Tonga, although Tongan fishers only take about 0.3% of the total fish catch in the region. The highest catch was in 2001 with a total of 1,920 million tonnes of all species. The steady increase related to an increase in number of fishing fleets from 16 in 1999 to 26 at the end of 2002 (Secretariat of the Pacific Community (SPC), 1999-2002). Albacore tuna dominates the entire catch. However, after 2002 there was a decline in the catch purportedly due to a weather effect (McCoy & Gillett, 2005). The catch volume has not recovered and the volume

of tuna catches in Tonga has remained at or below 900 tonne per year (Figure 3), but other species have partly compensated for this lower catch (Lui, Fa'anunu, & Koloa, 2005).

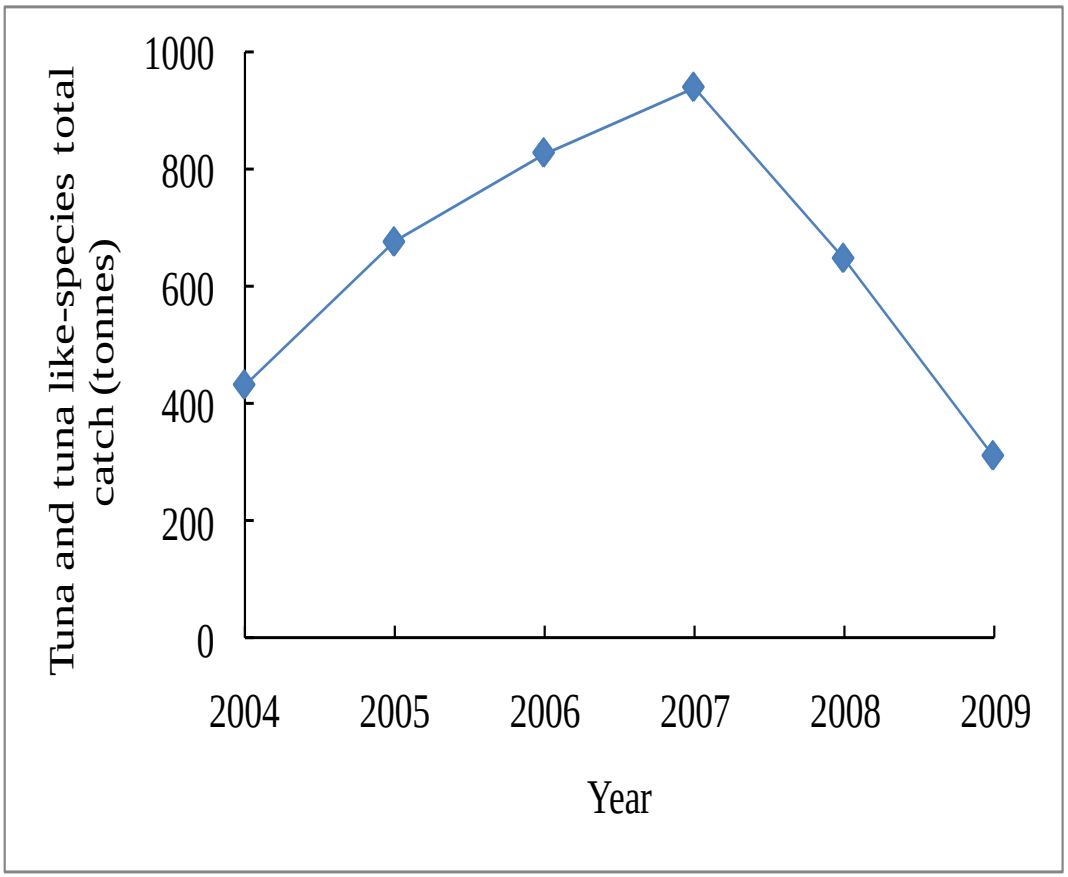

Figure 3. Total tuna catch by the Tonga longline fleet (tuna and tuna-like species) in volume (tonnes) (United Nations Food and Agriculture Organization, 2010)

Tonga's fish exports are mainly to Hawaii, Japan, East Asia, American Samoa, Australia and New Zealand. This business is dominated by the private sector, but 24% of the export business is through a quasi-government organisation, the Sea Star Fishing Company. The export markets are segmented according to demand: crustaceans and molluscs to East Asia; tuna to American Samoa for canning; fresh and frozen fish to a range of markets(Tonga Department of Statistics, 1995-2000). The export of fish to each of these diverse markets is either in the form of fresh, frozen or live, by air or sea freight. Fresh and frozen fish dominates fish exports from Tonga, and only a small fraction is for live export. For Tonga, the remoteness from major markets and trading partners is a problem for two reasons. First is

the cost of transport and the second is the time to market. A successful export market in fresh fish demands speed to market, and this requires expensive air freight. Frozen fish overcomes the time-to-market problem, but the eating quality and thus the value of frozen fish is less. Thus the cost of freight and time works against the competitiveness of the Tongan industry. What is missing is an alternative preservation model, one that overcomes these two problems and also fits well with the constraints of a hot climate.

Food preservation

Preservation of food requires the control of microorganisms throughout the food chain, and proper monitoring to ensure the safety and stability of the product during its shelf life (Prange, Birzele, Hormes, & Modrow, 2005). The ability to store large quantities of food has played a pivotal role in mankind's development. Evidence of food preservation dates back to the postglacial era, from 17,000 to 12,000 BP, and the first use of biological methods have been traced back to 8,000 to 3,000 BP, with evidence of fermentation processes used in producing beer, wine, vinegar, bread, cheese, butter, and yogurt (Masud, Kiran, & Anwar, 2002).

The cause of food spoilage was first declared by Louis Pasteur in 1864 showing that microorganisms in foods were the cause of such contamination. He subsequently found that microbes were killed by heating and that sealed containers could be used to preserve food by preventing recontamination from atmospheric air. However, spoilage of food is not only due to microorganisms, but rather to a various combinations of exogenous (microbiological) and endogenous factors, fat oxidation, and hydrolytic reactions in general (Gould, 2000). But there is no doubt that spoilage linked to pathological conditions is caused by microorganisms. Thus much of the aim of preservation is to prevent microbial growth. Many techniques have been designed to achieve this goal (Table 1) and some are discussed below.

Table 1. Categorization of procedures used to preserve foods

Technique	Factor influencing growth or survival of microorganisms
Cooling, chill distribution and storage	Low temperature to retard growth
Freezing, frozen distribution and storage	Low temperature and reduction of water activity to prevent growth
Drying, curing, and conserving	Reduction in water activity sufficient to delay or prevent growth
Vacuum and oxygen-free packaging	Low oxygen tension to inhibit strict aerobes and delay growth of facultative anaerobes
Modified atmosphere packaging	Carbon dioxide, in combination with other gases, to further inhibit growth
Addition of acids	Reduction of pH value and sometimes additional inhibition by the particular acid
Lactic and acetic fermentation	Reduction of pH value in food by microbial action and sometimes additional inhibition by the lactic and acetic acids formed and by other products of microbial growth
Alcoholic fermentation	Increase in concentration of ethanol
Emulsification	Compartmentalization and nutrient limitation within the aqueous droplets in water-in-oil emulsion foods
Addition of preservatives	Inhibition of specific groups of microorganisms
Pasteurization and sterilization	Delivery of heat sufficient to inactivate target microorganisms to the desired extent
Radurization, radicidation and radappertization	Delivery of ionizing radiation at a close sufficient to inactivate target microorganisms to the desired extent
Aseptic processing	Packaging sterilized foods without recontamination
Decontamination	Treatment of packaging materials and food ingredients with heat, irradiation, or chemical agents to reduce microbial contamination

From Gould (1989)

Low temperature

Lowering the temperature is the most common method of preserving almost any foodstuff. As the temperature of food storage is lowered, growth rates are slowed and fewer types of microorganisms are able to grow and multiply (Herbert, 1989; Russell, 1990). However, freezing to well below the freezing point of meat at -10ºC and less is required for truly longer term protection against multiplication of almost all bacteria provided the cellular water within the food to remain frozen (Franks, 1985).

Reduction in water activity

Water activity (a_w) are widely used to predict the stability of foods with respect to the potential growth of microorganism, as well as the chemical, enzymic, and physical changes that lead to loss of quality (Christian, 2000; Hardman, 1989; Seouw, 1988; Simatos & Multon, 1985; Troller & Christian, 1978). Lowering water activity can be achieved by curing (addition of salt and/sugar) or by drying (partial or complete). Different microorganisms grow best at different water activities – higher for bacteria than for yeast and moulds – but for complete security water activities below 0.60 are required. Dried foods are formulated and stored so as to maintain a water activity value well below this, commonly near 0.30. Other chemical, enzymic and physical changes that can affect quality are also minimal at this point (Seouw, 1988; Simatos & Multon, 1985).

Curing as a means of lowering water activity will be discussed in more details later.

Vacuum and modified-atmosphere packaging

This technique requires removal of oxygen, such as in vacuum or nitrogen-flushed packs, with consequent inhibition of microbial growth that absolutely requires oxygen – obligate aerobes. Facultative and obligate anaerobes may, however, continue to grow but generally have fewer unpleasant effects on food quality.

The using of carbon dioxide (CO_2) in modified-atmosphere packs replaces oxygen and has a specific antimicrobial effect of its own by acting as a removable preservative. This further extends safe, high quality and shelf-life for the product (Davies, 1995).

Equilibrium-modified atmosphere packaging is used primarily for fruit and vegetables. The food packaging plastics has a specific permeability property (Rooney, 1994). The package is sealed with or without a modified atmosphere, where subsequent respiration and

selective gas exchange across the packaging material establish an equilibrium-modified atmosphere.

Acidification

Bacteria generally do not grow well under acid conditions. For example, below pH 4.5, food-poisoning organisms such as *Clostridium botulinum* are well controlled and unable to grow (Riebroy, Benjakul, Visessanguan, & Tanaka, 2005). Reduction in pH can be achieved in two ways. First by direct addition of edible food acids like propanoic, acetic, lactic, citric, and malic acids. Second is fermentation that results in the accumulation of food acids from carbohydrate sources. This latter way is the topic of this research and will be described in more detail later.

Preservatives

Preservatives may be antimicrobial preservatives or antioxidants, each present in low concentrations. Antimicrobial preservatives inhibit the growth of bacteria or fungi including moulds, and antioxidants such as oxygen absorbers inhibit the oxidation of food constituents. Common antimicrobial preservatives include calcium propionate, sodium nitrate, sodium nitrite, sorbic and benzoic acids, sulphites and disodium EDTA. Antioxidants include phenolic compounds like butyl hydroxy-anisol and toluene, which are added to very many fatty foods to prevent fat oxidation.

Heating

The use of heat to preserve food is based on the loss of homeostasis of a microorganism, through for example inactivation or over stimulation of one or more metabolic enzymes, as temperatures rises above the temperature range to which each microorganism is adapted. Two temperature preservation categories in common use are pasteurisation and sterilisation. Pasteurisation is designed to destroy the vegetative cell of all disease-producing organisms as in the case of milk. Sterilization is used to destroy all viable organisms, such as yeasts, moulds, vegetative bacteria and spores, which are commonly measured by plating or enumerating techniques (Rahman, 2007). Heat remains the only substantial means for killing microorganism in foods while the other preservation techniques summarized in Table 1 only act primarily by inhibiting the growth of microorganism rather than by inactivating them.

Several preservation methods such as alcohol fermentation, radurization, radicidation and radappertization, aseptic processing and decontamination process mentioned in Table 1 are not discussed here as they are minor interests to this thesis. However, curing and fermentation are the two relevant technologies often combined in preservation techniques. These are the topics of the present study, and will be discussed in more details on the next section.

Curing

The preservation of meat by way of curing is based in an art that has been practiced for millennia (Binkerd & Kolari, 1975). Prior to the availability of refrigeration, foods, particularly fish and meat, were preserved by salting and marinating, also known as pickling. It was speculated that curing with salt was first practiced in saline desert or the coastal regions near the Dead Sea (Binkerd & Kolari, 1975). They noted that desert salt contains nitrate as an impurity and that saltpetre, or nitre (potassium nitrate), or wall saltpetre (calcium nitrate) from cave walls were used by ancient people for their preservative quality. The earliest specific mention of the characteristic pink colour of cured meat did not appear until late Roman literature. References to the flavour generated by saltpetre as part of the curing mixture were made in the early 18th century (Binkerd & Kolari, 1975). This effect was scientifically investigated when saltpetre was specifically recommended as an ingredient in curing recipes to promote the development of cured red colour (Brooks, Hainer, Morgan, & Pace, 1940).

Role of salt (NaCl)

Salt serves many purposes during the curing process also known to be the leading ingredient used in all types of meat curing. By way of solubilisation, osmosis, and diffusion, salt lowers water activity at the surface and throughout meat. As a result of this, growth of harmful non-salt tolerant microorganisms and bacteria that cause food deterioration are either killed or inhibited (Pearson & Gillett, 1996).

The presence of salt not only adds flavour to meat products, but it is also important in gelation of whole meat and meat emulsion products when they are cooked. Salt is said to 'extract' the myofibrillar proteins like myosin and actin. This is a form of limited solubilisation that on heating results in a strong and elastic gel that consumers associate with cured products (Shackelford, Reagan, Mann, Lyon, & Miller, 1989). Salt is, however, a prooxidant, as shown for example by Andersen and Skibsted (1991). As a prooxidant, salt

promotes the browning due to the formation of metmyoglobin, which on the face of it conflicts with fact that cured meats are often pink. Historically cured meats were pink because of the nitrate that was an impurity in salt available at the time. Nitrate indirectly creates the characteristic pink colour (Smith, 1873), and is discussed in more detail later. Another common addition to cured meats is sugar or other osmotically active carbohydrate, which can counteract some of the harshness and hardening effects of salt (Townsend & Olson, 1987). As the art progressed, spices and other flavourings were added to achieve distinct flavours amenable to commercial branding.

Historically salt was added as dry ingredient, which in the case of whole meat took days to diffuse evenly throughout the product. Modern curing practices are much faster relying as they do on brine injection or even vascular distribution (Faustman & Cassens, 1990; Holland, 1983).

Recently, researchers in many industrialised countries have closely studied the effect of salt, more accurately sodium, in our diet. In the USA, for example, the sodium intake from salt is approximately three times the recommended daily allowance (3 g of sodium) (Doyle & Glass, 2010). This excessive intake of sodium leads to hypertension, also known as high blood pressure (Dahl & Love, 1954), which increases the risk of stroke and premature death from cardiovascular diseases in the adult population (Rydén, Standl, Bartnik, Van den Berghe, Betteridge, de Boer, & Wood, 2007). The food industry throughout the Western world has responded voluntarily to the calls for reduced salt. For example, in the USA, the sodium content of 100 studies food products reduced by 10 to 15% between 1985 and 1997 (Liem, Miremadi, & Keast, 2011).

Role of sugar

The original reason for adding sugar to meat products was to provide a nutrient source for the lactic acid-producing bacteria used in fermented meat products. The unfermented excess creates a sour-sweet taste in cured meat products. At the same time it counteracts the harshness and toughening effect of salt in cured meat (Martin, 2001), and provide roundness and enrichment of flavour. Sugars are added at defined concentration depending on the taste required and the sweetness intensity of the particular sugars. The sugars most frequently used are sucrose, cane sugar, corn syrup, dextrose, and invert sugar (Martin, 2001).

Role of nitrate and/or nitrite

The use of nitrate in meat preservation was accidental in the sense that nitrate was a naturally occurring contaminant of salt (Binkerd & Kolari, 1975). But as a contaminant, its concentration in salt was variable and unpredictable, so the product colour was also variable and unpredictable. In the late 19th century, the technology of curing was greatly improved as the chemistry of the process became better understood (Martin, 2001). Studies showed that the active colour-fixing agent was, in fact, nitrite from the reduction of nitrate by bacterial action. This offered the possibility that the substitution of nitrite for nitrate could provide more consistent colour results. The first use of nitrate as a legal food additive substance was in 1908 in the USA, and was later extended to nitrite (Kerr, Marsh, Schroeder, & Boyer, 1926; Martin, 2001).

Except at the surface, raw meat is in an anaerobic state. This is because mitochondria in muscle cells continue to respire, and no further oxygen can be supplied by the vascular system. Micrococci bacteria present within the meat reduced nitrate to nitrite that will start the curing process. In time, nitrite is further reduced to produce nitric oxide and water. Nitric oxide bonds to the myoglobin, the main pigment in red meat to form mostly nitric oxide myoglobin that in the absence of air creates an even dark red colour. Upon cooking, the nitric oxide myoglobin turns into nitrosylhaemochromogen, which is that final pink colour typical of all cured meat (Pearson & Gillett, 1996).

Nitrite offered numerous functions such as imparting a bright reddish pink color, which is desirable and helps improve acceptability of cured meat product appearance for consumers (Wasserman & Talley, 1972). Nitrite is also involved in the characteristic cured meat flavour development (Macdougall, Mottram, & Rhodes, 1975). In addition to the color and flavour role, it acts as powerful antioxidants by preventing the development of oxidative rancidity, which could lessen the keeping quality thus, creating rancid flavour (Cross & Ziegler, 1965; Watts, 1954). However, it turns out that one of the most important outcomes of adding nitrite is to inhibit the growth of certain bacteria such as putrefactive anaerobes that causes spoilage of meats, and pathogenic bacteria such as *Clostridium botulinum* that causes foodborne intoxication and serious neuroparalytic disease (Tompkin, Christiansen & Shaparis, 1978).

Rapid curing using nitrites is today's preferred processing method, where nitrite is added directly into red-meat and poultry products, however, a number of observations have

led to concern about potential risks to human health. At present, primary concern is focused on the possibility of carcinogenic effects, especially nitrite interacting with substrates such as amines or amides to produce N-nitrosamine compounds, which can be generated in nitrite-preserved foods with high temperatures. This is experienced during frying in cured meats such as bacon (Martin, 2001). Many of these N-nitrosamine compounds are known to cause cancer in many animal species (Magee & Barnes, 1967). However, these adverse properties of nitrite can be negated by the use of ascorbic acid to minimise nitrosamine formation (Toldrá & Reig, 2006).

Researchers have found that both leafy and tuberous vegetables are rich in nitrate (Richardson, 1907). These vegetables include spinach, lettuce and radishes, all of which have high concentrations of nitrate compared to cured meats. The sodium nitrate is converted to sodium nitrite due to interactions during digestion where bacteria in the mouth reduce nitrate to nitrite (Rubin, 1977). According to White (1975), vegetable sources accounted for up to 86% of our sodium nitrite intake and the rest is from cured meat.

Excess nitrite in the diet affects the ability of the liver to store vitamin A, thus disturbing the thyroid function (Emerick, Mandigo, & Olson, 1963). In addition, nitrite can bind to haemoglobin in the blood and to myoglobin in the muscle which therefore, restraining the oxygen carrying capacity of blood (Martin, 2001). Thus, the maximum allowable level of nitrate/nitrite in cured meats limited to a maximum of 150 to 200 mg/kg in Western legislations (Bailey & Swain, 1973), a level adequate to ensure thorough development of the pink nitrosylhaemochromogen. Although the use nitrite in meat has some potential health issues, there is no substitute to replace the unique properties of nitrite in the role of colour development and bacterial inhibition in cured meat products.

Fermentation

Fermentation is the conversion of a carbohydrate such as sugar into an acid or an alcohol. More specifically, fermentation refers to the use of yeast to convert sugar into alcohol or to the use of bacteria to create lactic acid in foods. Fermentation occurs naturally in many different foods given the right conditions, and humans have intentionally made use of it for many thousands of years. Such food products play an important role in cultural uniqueness, local economy and gastronomic delight (Hui, Meunier-Goddik, Hansen, Josephen, Nip, Stanfield, & Toldrá , 2004).

In early societies, the transformation of basic food materials into fermented foods was a mystery and a miracle, for they had no idea what caused the usually sudden, dramatic, and welcomed transformation. Campbell (1987) and Bamforth (2005) estimated that the earliest uses of fermentation were most likely to create alcoholic beverages such as mead, wine and beer. These beverages may have been created as far back as 10,000 to15,000 BP in parts of the Middle East. The Egyptians manufactured cheese and butter around 3000 BP. The making of soy sauce and miso production in China goes back to around 3000 BP, and estimated that the transfer of knowledge for these production processes to Japan was around 1500 BP (Campbell-Platt, 1987). With increasing population concentrations, the devising of methods to preserve food was crucial to meet nutritional demands, and currently practised by every ethnic group world-wide (Hui, Meunier-Goddik, Hansen, Josephen, Nip, Stanfield & Toldrá , 2004).

As a technique, fermentation was developed as a low energy way in which to preserve foods, featuring alongside drying and salting in the days before the development of refrigeration, freezing and canning (Campbell-Platt, 1987). The most common examples have been the use of lactic acid bacteria to lower the pH and the employment of yeast to effect alcoholic fermentations. Preservation occurs by the generation of end products such as acids, alcohols and carbon dioxide. The anions, acetate and lactate are themselves effective antimicrobial agents, and the associated hydrogen ions lower the pH in the foods to prevent the growth of most hazardous food microorganisms. As edible acids, they thus enhance shelf life. They also improve texture, and thus contribute to the pleasant sensory profile of the food (Lee, Yu, Wong, & Saddler, 1994).

The methodologies and knowledge associated with manufacturing these products were handed down from generation to generation within local communities, monasteries and feudal estates. The science of food fermentation began after the microbiological work of Louis Pasteur in the 18th century, where he demonstrated that microorganisms are the common cause of foods deterioration or spoilage (Gould, 2000). He then proceeded in theorising that in fermentation, different microorganisms enzyme-catalyzed action are responsible for the changes of the food substrate (Lin, Bolsen, Brent, & Fung, 1992). Around the same time, the essential role of bacteria, yeasts and moulds in the generation of fermented foods came to be understood and this ultimately resulted in more controlled and efficient fermentations.

Over the centuries, fermentation techniques have been refined and diversified from local food production communities to larger scale of food production, necessary to meet the requirements of expanding and more distant markets. This in turn led to the development of larger scale fermentation processes for commercial production of fermented foods and alcoholic beverages, with the most widely used microorganisms including yeast for the production of beer, wine and spirits, and lactic acid bacteria (LAB) for a variety of dairy, vegetable and meat fermentations (Table 2).

Table 2. Types of fermented food

Group	Examples of food	Region of production
Dairy	Cheese, yoghurt, kefir, kurut	Europe, North America, Middle East, Africa, South-East Asia
Beverages	Beer, spirits, arak, coffee, tea, cocoa, sake, wines, cider	All countries
Cereals	Bread, rolls, pancakes, doughnuts, tape ketan, kenkey, injera, orgi, pozol, chicha, mirin, idli, dosa, papadam	All countries
Meat	Jerky, ham, salami, pickled meat, pepperoni	Europe, North America, India,
Fish	Fish sauce, bagoong, paak	South-East Asia, Europe, North America
Legumes	Soy sauce, miso	South-East Asia, India, Middle East
Miscellaneous	Mushrooms, nata, dage, ragi, vinegar	South-East Asia
Starch crops	Gari, chicouangue, tape ketella, poi, bwiru, chuno	Africa
Fruits and vegetables	Pickled fruits and vegetables, olives, kimchi, hum-choy, sauerkraut	All countries

From Campbell-Platt (1987)

Fermented foods form a significant proportion of human food intake. Fermented dairy products are of great importance in the Middle East, and cereals and legumes are fermented in large quantities in the Indian subcontinent (Table 2). In East and South-East Asia, fish and legumes are the most important fermented foods produced both contributing major protein

sources. Cereal products may be co-fermented with legumes, as in the use of rice or barley with soybeans in miso production, and wheat used with soybeans in soy sauce production (Table 2). Starch crops are of major importance in Africa as their main staple foods, particularly in poor communities. While many fermented foods are consumed in their areas of production, increasing quantities are found in world trade, ranging from wines, beers and spirits through cheeses to soy sauce and miso (Campbell-Platt, 1987).

Food fermentations around the world are classified based on the major products of the fermentation process. These include alcoholic fermentation, acetic acid fermentation, alkaline fermentation, lactic acid (non-alcoholic) fermentation, and amino acid/peptide sauce fermentations. Since this thesis is based on developing a cured fermented fish sausage containing a carbohydrate source with the aid of lactic acid bacteria (LAB) culture, discussion will be limited to lactic acid fermentation.

Lactic acid fermentation

Lactic acid bacteria (LAB) are the most commonly used microorganisms for preservation of foods. They are gram-positive, catalase- and oxidase-negative, and nonspore-forming bacteria (Aguirre & Collins, 1993). Their importance is associated mainly with their safe metabolic activity while growing in foods using available sugars for the production of organic acids and other metabolites without the need for oxygen. Lactic acid bacteria produce lactate and acetate, and the associated hydrogen ions, which lower the pH of the food and inhibit other pathogenic organisms. Ethanol, hydrogen peroxide, and bacteriocins can also be produced in smaller quantities and these also aid in preservation and safety (Table 3). Their common occurrence in foods and feeds coupled with their long-lived use contributes to their natural acceptance as safe for human consumption (Aguirre & Collins, 1993).

Table 3. Metabolites of lactic acid bacteria and its main target pathogenic and food spoilage organisms

Lactic acid bacteria end products and metabolites	Main target organisms
Organic acids	
Lactic acid	Putrefactive and Gram-negative bacteria, some fungi
Acetic acid	Putrefactive bacteria, clostridia, some yeasts and some fungi
Hydrogen peroxide	Pathogens and spoilage organisms, especially in protein-rich foods
Enzymes	
Lactoperoxidase system with Hydrogen peroxide	Pathogens and spoilage bacteria (milk and dairy products)
Lysozyme	Undesired Gram-positive bacteria
Low-molecular-weight metabolites	
Reuterin	Wide spectrum of bacteria, yeasts and molds
Diacetyl	Gram-negative bacteria
Fatty acids	Different bacteria
Bacteriocins	
Nisin	Some LAB and Gram-positive bacteria, notably endospore-formers
Lacticin	Gram-positive bacteria, inhibitory spectrum according to producer strain and bacteriocin type

From Breidt and Fleming (1997); Breidt, Hayes and Fleming (2000)

A familiar example of fermented products produced by lactic acid fermentation of milk is yoghurt and cheese. Sauerkraut, kimchi and other pickled vegetables are examples of lactic acid fermented vegetables. All alcoholic beverage industries use alcohol fermentations to produce the complete range of wines, beers etc. and distilled spirits. Other foods that can be fermented with lactic acid bacteria are cereals, meat and fish (Guizani, 2010).

Lactic acid fermentations of meat and fish

Meat and meat products are consumed world-wide, and is an excellent source of protein and nutritionally important amino acids by virtue of the amino acid profile (Lücke, 2000). However, meat is easily contaminated by pathogenic microorganisms from very many potential sources like hides, viscera, knives, hands and contact surfaces. It is therefore important to make meat safe for consumers in terms of stability, transportation and storage. One of the preferred methods used to achieve these qualities is meat fermentation, traditionally in the form of sausage (Hugas, Garriga, & Aymerich, 2003; Hugas & Monfort, 1997).

The history of fermented sausage has a long tradition and is believed to originate from Mediterranean countries at the time of the Romans (Lücke, 1985). The sausage was developed as a means of making the most of leftovers of meat and entrails. The same is true today. Fermented sausages are produced as a result of the lactic fermentation of a mixture of comminuted meat mixed with fat, salt, curing agents (nitrate/nitrite), sugar and spices. Most sausages are subsequently air dried and stored with little or no refrigeration.

The fermentation of meat and fish sausages, for example, using selected LAB strains strongly inhibited the spoilage bacterial growth but left the organoleptic properties of the products intact (Metaxopoulos, Mataragas, & Drosinos, 2002). Thus, LAB strains can be effectively used to preserve meat products for quality purposes in terms of texture, colour and shelf-life.

Fermented meat sausages are classified as either dry or semidry according to Campbell & Cook (1995), and Farnworth (2003). Dried sausages are made from coarsely chopped meat, typically pork and beef. They are fermented at 10 to 24°C for 3 to 6 weeks creating a product with pH 5.2 to 5.8 with a moisture level of about 35%. The shelf lives of dry sausages are excellent, which attributed to the high salt-to-moisture ratio, and they are less tangy than semidry sausages. These sausages are normally kept without refrigeration. Common examples of such fermented sausages are salchichon, chorizo, saucisson and salami.

Semidry sausages on the other hand are different from dry sausages by their pronounced tangy flavour. They are generally smoked during fermentation, and usually packaged after fermentation, and often require little refrigeration. The pH of semidry sausages is similar to that of dry sausages except they have higher moisture content, about 30

to 50%. Their shelf life is surprisingly good taking into account their slightly higher water activity of about 0.94 than for dry sausages, around 0.85. This stability is presumably due to the accumulation of acids and smoke compounds. Common examples are summer sausages, holsteiner, cervelat, tuhringer and chorizos with a range of different varieties in many countries.

Fish is an extremely perishable food, and it becomes inedible in less than a day at tropical temperatures. Spoilage begins as soon as the fish dies, and processing should therefore be done quickly to prevent the growth of spoilage bacteria. Fish is a low acid food and therefore very susceptible to the growth of food poisoning bacteria (Campbell-Platt, 1987). Preservation by lactic acid fermentation is a way of making changes to the flavour and texture of the fish to create range of different products. These fermented fish products in terms of fish sauce, fish paste and lacto fermented fish product with added carbohydrates contribute significantly to the protein intake of a large number of the world's population. These products have become a staple part of the diet in South East Asia especially Thailand, Kampuchea, Malaysia, Cambodia, Philippines and Indonesia (Campbell-Platt, 1987).

Fish sauce is made from small fish that would otherwise have little value for consumption, and can either be freshwater or saltwater fish. The fish is salted in a container for long period of time. The enzymes in the gut and from the halophilic microorganism grown in the system decompose fish meats, and the excluded liquid is fish sauce (Gildberg, Espejo-Hermes, & Magno-Orejana, 1984; Saisithi, 1994). Fish sauce consists of amino acids and peptides that are responsible for its characteristic meaty flavor and classified as a high-salt product. It is called nam pla in Thailand, nuoc mam in Vietnam, jeotkal in Korean, patis in the Philippines, shottsuru in Japan, ngan-pya-ye in Burma, tuk trey in Cambodia, nam pa in Laos, and yeesui in China. The name basically means fish water (Campbell-Platt, 1987).

Common example of fermented products with lower salt content to which fermentable carbohydrates such as cooked rice are added is shown in Table 4. The ingredients formulation is different for each product as shown in Table 4. Generally, pla-ra, pla-jao, and pla-paeng-daeng have longer shelf-life as compared to the 2 to 3 weeks self-life of som-fak, plaa-som and pla-jom (Table 4). Greater longevity is clearly due to high salt contents, although according to Phithakpol, Varanyanond, Reungmaneepaitoon, & Wood (1995), all products are best consumed within few days of fermentation. Other examples of this low salt

fermented fish product are sikhae in Korea, narezushi in Japan and Burong-isda in the Philippines (Lee, Yu, Wong, & Saddler, 1994).

Table 4. Fish/salt/carbohydrate products of Thailand

Name	Composition	Preparation time	Shelf-life
Pla-ra	Fish (whole or pieces): salt: roasted rice (ratio 3:1:0.2-4)	6-12 months	1-3 years
Pla-jao	Fish (pieces): salt: fermented rice (ratio 3:1:1-3)	10-20 days	2-3 months
Pla-som	Fish: salt: boiled rice: garlic (ratio 10:2:1:0.25-1)	5-12 days	3 weeks
Pla-jom	Fish: salt: roasted rice: garlic (ratio 10:1:3:1)	3-7 days	2 weeks
Som-fak	Fish (minced): salt: rice: garlic (ratio 10:0.5-1.5:2-3:1) Fish: salt: boiled rice: ang-kak (ratio 3:1:3:0.03)	5-10 days	2 weeks
Pla-paeng-daeng		5 days	6-12 months

From Adams, Cooke, & Rattagool (1985)

The formulations in Table 4 contain boiled, roasted or fermented rice along with garlic which also acts as carbohydrate substrates for the fermentation. In addition to a substrate role, garlic is believed to act as an antimicrobial agent particularly against Gram-negative bacteria due to garlic's allicin content (Feldberg, Chang, Kotik, Nadler, Neuwirth, Sundstrom, & Thompson, 1988). Garlic is also reported to promote the growth of lactic acid bacteria (Paludan-Müller, Valyasevi, Huss, & Gram, 2002; Zaika & Kissinger, 1984).

Fat oxidation and proteolysis are two of the important biochemical changes occurring during fermentation of fish mince. These affect nutritive value, texture, and flavour due to the formation of low molecular weight end products such as peptides, amino acids, aldehydes, organic acids and amines (Riebroy, Benjakul, Visessanguan, Kijrongrojana, & Tanaka, 2004). The products are typically slightly sour and salty and have a firm and springy texture. It is of

great importance that fat oxidation is controlled (by anaerobic conditions) because excessive fat oxidation in fish is undesirable due to the unpleasant odours and flavours that develop. It is also important to avoid excessive formation of biogenic amines.

Biogenic amines

Biogenic amines are basic nitrogenous compounds formed mainly by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones in fermented food (Hu, Xia, & Liu, 2007). In non-fermented foods, the presence of biogenic amines is indicative only of undesired microbial activity (Askar & Treptow, 1986). Biogenic amines are present in a wide range of food products, including fish products, meat products, dairy products, wine, beer, vegetables, fruits, and nuts. The amine content of these foods varies greatly due to differences in processing, age, ripeness, handling, storage, and variety of produce, cooking method, and many other factors. Some of them play a major role in human and animal physiological functions, such as regulation of body temperature, and brain activity. However, when these compounds are consumed in high quantities they can cause intoxications (Dana, Pavla, Tomas, Borivoj, & Vlastimil, 2003; Hu, Xia, & Liu, 2007).

Common examples of biogenic amines produced by microbial decarboxylation of amino acids are histidine, tyrosine, and tryptophan to generate the biogenic amines histamine, tyramine and tryptamine respectively (Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994). Histamine is heterocyclic in structure and tyramine together with tryptamine is aromatic. However, histamine and tyramine are the most studied biogenic amines due to their toxicological effects. Most of the intoxications produced by biogenic amines are related to histamine, because this amine may lead in a dilation of blood vessels, capillaries and arteries, causing headaches, hypotension, gastrointestinal distress and edemas. Tyramine on the other hand causes the increase of the noradrenaline concentration in blood as an indirect effect, acting like a vasoconstrictor, provoking headache, hypertension and migraine (Dana, Pavla, Tomas, Borivoj, & Vlastimil, 2003; Hu, Xia, & Liu, 2007). Furthermore, biogenic amines in general are considered precursors of carcinogenic amines, such as N-nitrosamines (Scanlan, 1983).

Reports of extremely high concentrations of histamine in some salted and dried fermented products were found in Asia (Lehane & Olley, 2000). Several cases have been reported related to scrombroid food poisoning from consumption of smoked kahawai in New Zealand (New Zealand Food Safety Authority, 2001; New Zealand Food Safety Authority,

2003). Although these and many other studies have shown an increase in the concentration of biogenic amines in fermented meat products, there have been no reports of death or any severe health problems cause by biogenic amines in these products (Moore, 2004; Reig & Toldrá, 2008). However, biogenic amine poisoning, especially histamine poisoning from the ingestion of fish is very common (Mines, Stahmer, & Shepherd, 1997). Main source of histamine poisoning is from the Scrombridae family of fish (Lehane & Olley, 2000). This family includes species such as mackerel, tuna, anchovies, sardines, marlin, and the salmonids.

The concern over food poisoning related to the consumption of foods containing biogenic amines has urged the regulatory authorities to set standards for foods. The United States Food and Drug Administration has set the upper histamine limit for tuna, mahi-mahi and related fish species at 500 ppm (Food and Drug Administration , 1998). The Canadian Food Authority similarly set a histamine limit of 500 ppm for seafood (Gill, 2005), whereas Food Standards Australia New Zealand (2006) has set an upper limit of histamine in fish at 200 ppm.

Fish preservation in Tonga

Tongan people rely on fish to provide the bulk of their protein requirements. Of the fish landed in Tonga, what is not consumed quickly is exported. Chilled and frozen fish dominate fish exports from Tonga, and only a small fraction is exported live (to high priced markets like Japan). For Tonga, the remoteness from major markets and trading partners is a problem for two reasons. First is the cost of transport and second is the time to market. A successful export market in chilled (often called 'fresh') fish demands speed to market, and this requires expensive air freight. Frozen fish overcomes the time-to-market problem, but the eating quality and thus the value of frozen fish is less. Thus the cost of freight and time works against the competitiveness of the Tongan industry. What is missing is an alternative preservation model, one that overcomes these two problems and also fits well with the constraints of a hot climate.

Experimental plan

Tonga is well placed as a site to develop fermented fish products, based on models in Table 4. Tonga has fish in abundance, particularly tuna, a carbohydrate source (e.g. taro, kumala [kumara]), temperatures that favour fermentation, and minimal requirement for

refrigeration. Importantly, electricity is very expensive in Tonga and therefore so is refrigeration. Along with salt, a further ingredient could be copra, the desiccated white fatty tissue of the coconut. Arguably coconut could add a fat flavour-carrying dimension to the product. Another ingredient will be pyrophosphate, which is routinely added to salted meat products to improve flow properties during sausage blending and extrusion. Lactic acid bacteria culture would be included to control fermentation. Finally, the formulation will include nitrite at concentrations suitable for curing. Tuna flesh is dark red, indication of high haem iron content. Oxidative rancidity, which is an obvious potential problem in fish products, is minimised when haem iron is present in a food. The incubation temperature would set at 30 to 32°C, achievable at minimal cost in Tonga.

An obvious question at this time is who in New Zealand, for example, would buy such a product? If there were no market there would be no reason to develop it. While New Zealand consumers have no experience of fermented fish products, novel foods and new gourmet items have a significant following particularly in some affluent market segments. For example, the upmarket magazine *Cuisine*, as just one example of a dedicated food appreciation magazine which has an audited monthly circulation of prestigious food and wines often associated with a specific culture. At the time of writing the proposed route to market would be through restaurants and gourmet food outlets, avoiding supermarkets entirely in the early stages of market development. A key feature of promotion would be that the product was sourced from Tonga, using only ingredients, including spices and sourced in that country. Thus, there would be an appeal to 'geographical exclusivity', although realising that none of the ingredients is exclusive to Tonga.

Prior work in New Zealand on fermented New Zealand marine fish species was done by Khem (2009). In that work, three species common in New Zealand waters fermenting with previously steamed rice was unsuccessful, probably because the endogenous bacteria used for fermentation could not hydrolyse and ultimately ferment the starch in cooked rice. Glucose was substituted for rice, with successful results. In the present work, it is proposed to avoid the use of glucose if possible, thus enabling an 'all-Tongan ingredients' claim. Another reason for using carbohydrate source is that it is cheaper than meat and it will produce a product that is cheap plus, if used judiciously, it can contribute to a desirable textural quality.

Kumara, which originated in South America, has a long history of cultivation in Tonga, along with taro. These two are obvious choices. Potato will also be included because

of its importance in New Zealand, a country likely to be an export target. These carbohydrates will be initially hydrolysed to create fermentable sugars by microwave cooking and whichever one demonstrate the outstanding end result in terms of quality fermentable sugars (glucose) will be used for the second phase of the research which is the sausage production.

Sucrose is the main oligosaccharide present in these tubers with lesser concentrations of glucose and fructose (Picha, 1986). However, the bulk of carbohydrate is present as starch. When these tubers are cooked, the amylose and amylopectin polymers are made available for enzyme-catalysed hydrolysis by endogenous amylolytic enzymes, when these are active, and spontaneous hydrolysis due to prolonged cooking in an aqueous environment. These processes yield sweet fragments of reducing sugar, mostly maltose and is fermentable (Jenkins , Wolever,Thorn, Lee, & Kalmusky, 1983; Valetudie, Colonna, Bouchet, & Gallant, 1993).

Maltose is a disaccharide of glucose and when hydrolysed, it yield glucose that can be conveniently measured with a glucose meter, otherwise designed for determination of glucose concentration in blood. Thus, these meters could indicate the general progress of hydrolysis, but would always underestimate the concentration of fermentable sugar because they are specific for only β-D-glucose. Moreover, the meters are calibrated for blood, so for glucose determination in raw and cooked tubers, and it was necessary to calibrate for glucose in the aqueous extract of tubers. This was done by the method of standard additions.

The proposed study is therefore to produce a model cured fermented tuna (*Thunnus alalunga*) sausage that will be able to claim a measure of geographical exclusivity to Tonga and could provide commercial opportunities for Tonga, specifically a relatively stable export item. The fermentation outcomes for the cured FFM sausage will be analysed for textural appearance, colour, pH, soluble protein, fat oxidation, and biogenic amines, most of which have been outlined in the context of the next chapter. The results are to be compared with equivalent published values for fermented fish products from Southeast Asia with the aim of making preliminary commercial assessment in New Zealand market.

Chapter 2

Materials and Methods

Fish and other food components

Tuna fish meat was freshly bought from the Auckland CBD Fish Market (Figure 4). The species on sale was often claimed to be albacore (*Thunnus alalunga*), but on other occasions no specific name was claimed beyond the name, tuna. Red skin potatoes (Figure 5), red skin kumara (Figure 6) and desiccated coconut flakes were purchased from the local Auckland supermarket. Taros (Figure 7) were bought from Otahuhu's local fruit shop.

Figure 4. Typical tuna flesh, with its high haemoglobin content

Figure 5. Red skin potato and its flesh

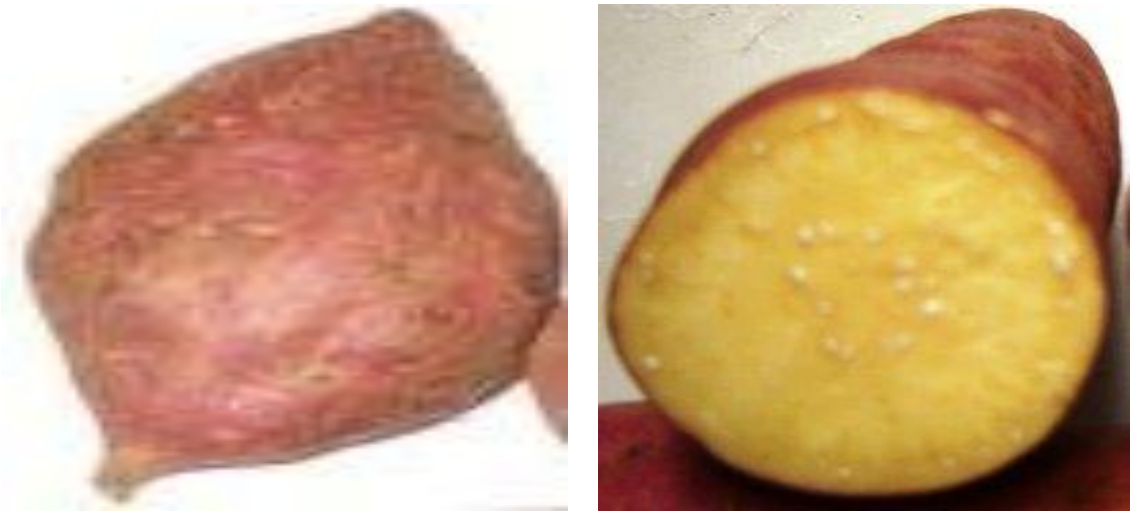

Figure 6. Red skin kumara and its flesh

Figure 7. Taro and its flesh

Chemicals

Trichloroacetic acid (TCA) and thiobarbituric acid (TBA) were obtained from B.D.H Laboratory Chemicals. Malonaldehyde was obtained from Fluka (Buchs, Switzerland). Free base histamine, tryptamine and tyramine, also 1, 1-dimethylbiguanide hydrochloride was purchased from Sigma (St.Louis, USA). Bovine serum albumin (BSA) was obtained from Serva Feinbiochimica (GmbH, Germany). Folin-Ciocalteau reagent was obtained from Scharlau Chemie S. A. (Barcelona, Spain). Sodium dodecyl sulphate (SDS), sodium hydroxide, sodium carbonate and sodium potassium tartrate, potassium dihydrogen phosphate and dipotassium hydrogen phosphate were obtained from B.D.H Laboratory Chemicals, as

were pyrophosphate, cupric sulphate, acetonitrile, sodium nitrite, methanol, hydrochloric and perchloric acids.

Fermentation culture mixture (*Pediococcus pentosaceus* and *Staphylococcus carnosus*, BFL-F02 BactoFlavor®) was donated by Chr. Hansen Pty. Ltd. (Melbourne, Australia). Salt (NaCl) and glucose were obtained from AUT Chemistry laboratory. Filler (Orafti Raftiline) is a non -starch polysaccharide that was purchased from Terry Holdings Limited.

Processing Equipment

An Ultra-Turrax disperser with a T18 dispersing element (IKA-Wereke, Germany) was used for the dispersion of samples immersed in water or other solutions depending on the type of analysis. Generally, material is drawn up into the apparatus by a rapidly rotating rotor or blade positioned within a static head or tube containing slots or holes. The material is centrifugally thrown outward in a pump-like fashion to exit through the slots or holes. Since rotor turns at speeds up to 12,000 rpm, the tissue is rapidly reduced in size by a combination of extreme turbulence, cavitations and scissor-like mechanical shearing occurring within the narrow gap between the rotor and the stator.

In a microwave cooker, foods containing high concentrations of moisture are cooked by the heat generated by the absorption of microwave energy from water molecules in the food. The prepared beakers containing 5 mL of deionized water and tubers were cooked by microwave heating (Figure 9) for 0, 1, 3, 5, 7, 9, 11 and 15 min at lowest heat setting that was equivalent to about 100 W.

A glucometer or glucose meter (Figure 11) is a medical device for determining the approximate concentration of glucose in the blood, commonly used by people with diabetes or hypoglycemia. This was obtained from the AUT Microbiology Laboratory, and was the proposed method for determining the glucose content in raw tubers such as potato, kumara and taros. A small drop obtained from filtered supernatant solution of tuber is placed on a disposable test stripthat the meter reads and uses to calculate the glucose level of raw tubers and expressed $mmolL^{-1}$.

A domestic Kenwood food mixer model number KM210 (Figure 8) was used to prepare FFM mixture by passing ingredients twice through a conventional worm-drive mincer fitted with a 4-mm plate, flowed by mixing in the bowl attachment for 5 min. The extruder

nozzle was later attached to the mincer so that the FFM mixture could be extruded into the syringe barrels and subsequently covered to keep air out.

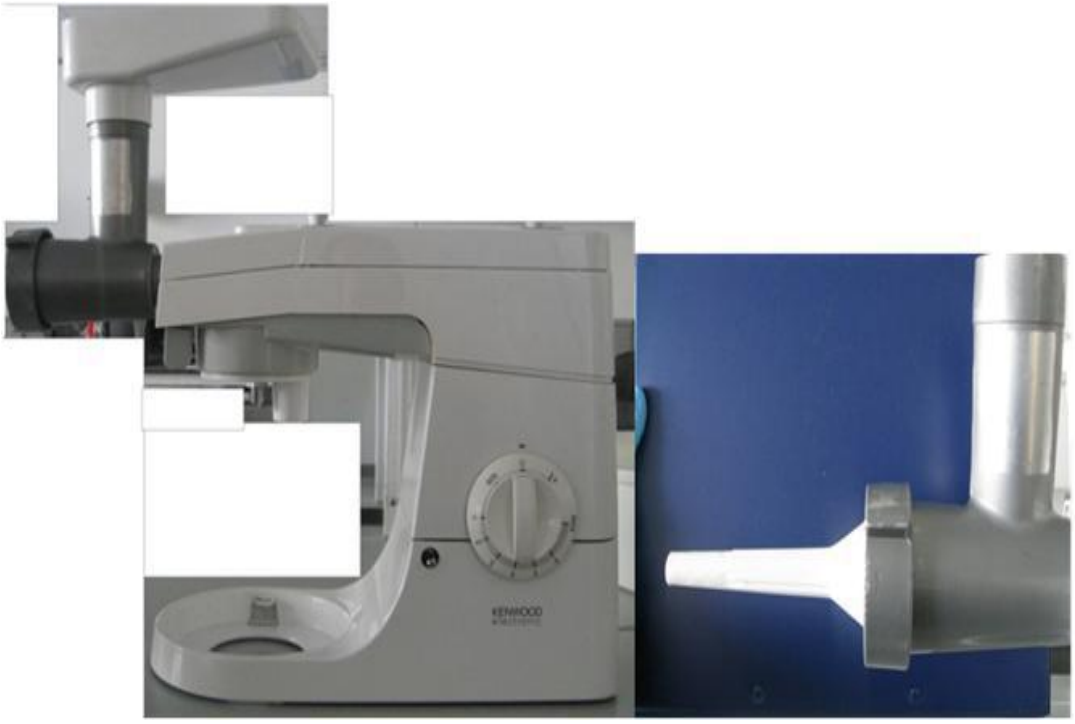

Figure 8. The domestic Kenwood food processer was used to prepare minced raw sausage (left) and the extruder nozzle (right) was used to fill the syringe barrels.

Hydrolysis methods for tubers

The tubers, taro, kumara and potato, were hydrolysed in two ways, first by microwave cooking (Figure 9), and second according to the normal way of cooking taro in Tongan culture. The microwave cooking involved peeling of the tubers source and cutting into cubes measuring 2 x 2 and 2 cm. The cubes were placed in a 50 mL beaker, one cube per beaker containing 5 mL of deionised water to moisten, and prevent carbohydrate source from drying and hardening. The beaker was covered with Parafilm^{TM} that was covered with a watch glass. The aim was to allow minimize water loss during heating but to avoid pressure buildup. The prepared beakers were cooked by microwave heating for 0, 1, 3, 5, 7, 9, 11 and 15 min at lowest heat setting that was probably equivalent to about 100 W. The beakers were then cooled at room temperature.

For the Tongan cooking (Figure 10), the taro was cut into four pieces and cooked fully immersed in water in a pot on a stove top at a steady boil for 40 min. After cooking, the liquid was decanted and the cooked pieces were cooled at room temperature.

Figure 9. Beaker containing tuber with a fitted layer of Parafilm™ and a watch glass resting on top ready for cooking by microwave heating

Figure 10. Taro pieces covered with water and cooked in the Tongan way on a stove top

Glucose analysis

Tuber hydrolysis and glucose readings

The cooked tuber cubes were prepared as described in previous section. The cubes were thinly sliced, weighed and homogenization at 9,500 rpm using an Ultra-Turrax homogeniser (IKA-Wereke Germany) with 45 mL of deionized water for 2 min. The mixture was centrifuged at 3000 gravities for 15 min, and then the supernatant is removed and made up to 50 mL. The precipitate was not washed and recentrifuged because the volume was negligible compared with the volume of supernatant. Aliquotes of the 50 mL volumes were

dilute as required to bring the glucose concentration into the dynamic range of the Accu-check glucometer (Figure 11). The values returned by the meter in mmol L^{-1} were used to calculate the concentration of glucose in tuber pieces expressed as mg of glucose g^{-1} of raw tuber. There were three replicate homogenizations at each of eight time points, and data are the mean of a single glucose determination at each time.

Figure 11. Insertion of a test strips in the base of the glucometer. The glucose concentration is reported in mmol L^{-1}

Calibration of the glucose meter

Meters designed to measure glucose concentration in blood are calibrated for that matrix, and it was uncertain that testing an aqueous extract of cooked tuber would return accurate results. Properties of the new matrix, both physical and chemical, may affect the calibration, where physical properties include viscosity for example. This uncertainty of accuracy can be overcome by the method of standard additions. In this method known quantities of the analyte in question, in this case glucose, are added to the matrix containing the unknown concentration of that analyte. This is also known as spiking. In the present case, the responses of the meter when the known quantities of glucose are added can be used to calculate the true concentration of the glucose. These two values – the true value and the value returned by the meter – are used to derive a correction factor with which readings have to be multiplied. The cooked tuber were prepared and analysed for glucose content as

described in the previous section. For each tuber, there were three spiked replicates at each of eight time points, and data are the mean of a single glucose determination at each time point.

Novel equipment

Sixty millilitre (60 mL) syringes with a diameter of 25 mm was used as the casing for the FFM sausage. The plunger is taken out, and the needle end of the syringe was excised on a lathe. The interior surface of the syringe barrel was lubricated with petroleum jelly to make extruding of FFM sausage for analyses easy. The plunger was drawn to the 60-mL point, and the barrel was filled with FFM. The open end of the filled syringe barrel was then tightly covered with one layer of Parafilm^{TM} overlaid with another layer of aluminium foil followed by a rubber band to shield the cover, and to keep air out (Figure 12).

Figure 12. Syringes filled with FFM mixture, fitted with a layer of Parafilm^{TM} and aluminium foil also sealed with a rubber band

Fermented fish mince (FFM) preparation

The albacore tuna (*Thunnus alalunga*) was bought chilled and as fresh as possible from the New Zealand Auckland CBD Fish Market, and maintained on ice in the laboratory. However, the choice of tuna species was dictated by availability throughout year 2010 to 2011. Using cold equipment in an ambient temperature laboratory, the fillets and taros which was the selected carbohydrate source, cooked in the Tongan way were minced through a 4-mm plate twice, and blended for 5 min in a domestic Kenwood mixer bowl together with

glucose, desiccated coconut flakes, pyrophosphate, nitrite, and lactic acid bacteria culture at varying amounts. The mixtures were extruded into the 60-mL syringe barrels as described in Novel equipment above. The extruder was attached to the mincer so that the minced fish could be extruded into the syringe barrels and subsequently covered to keep air out. After sealing with Parafilm^{TM}, aluminium foil and rubber band, the syringes were incubated horizontally at 32°C for 4 days in a Qualtex oven (Andrew Thom Limited, Sydney, Australia) (Figure 13). Each day, flat cylinders of FFM sausage with range of heights and weights depending on the type of test analysis were extruded and cut for analyses.

Figure 13. Syringes of FFM mixture ready to be fermented in a Qualtex oven at a controlled temperature of 32ºC

Ingredient concentration

The ingredient concentrations of FFM sausages were prepared according to Table 5 and Table 6. Table 5 show four treatments based on the taro concentration ranging from 0 to 30%. The FFM sausages made from these four treatments were used for determination of pH, colour and texture. Results obtained from these three analyses led to a change in ingredient concentration where glucose was not included (Table 6). The treatments narrowed to two treatments instead of four, cured and uncured with fixed concentration of taro. This table was used for preparing FFM sausages for determination of TCA, TBARS and biogenic amines concentrations.

Table 5. The ingredient concentration of FFM sausages for pH, colour and texture analysis, using Tonga-cooked taro at four concentrations

Ingredient	Quantity (g 100 g^{-1})			
	0%	10%	20%	30%
Fish meat	88	78	68	58
Taro	0	10	20	30
Glucose	3	3	3	3
Desiccated coconut	5	5	5	5
Salt	3.8	3.8	3.8	3.8
Sodium pyrophosphate	0.2	0.2	0.2	0.2
Sodium nitrite	0.01	0.01	0.01	0.01
Lactic acid bacteria	0.01	0.01	0.01	0.01

Table 6. The ingredient concentration of FFM sausages for pH, TBARS, TCA and biogenic amines analysis

Ingredient	Quantity (g 100 g^{-1})	
	Uncured	Cured
Fish meat	71	71
Carbohydrate source (taros)	20	20
Glucose	0	0
Desiccated coconut	5	5
Salt	3.8	3.8
Sodium pyrophosphate	0.2	0.2
Sodium nitrite	0	0.01
Lactic acid bacteria	0.01	0.01

Physical analysis of FFM

Texture profile analysis

Texture profile analysis was performed on extruded FFM discs, 25 mm high, only at Day 0 and Day 4. The instrument used was a TAXT plus Texture Analyser (Stable Microsystems, U.K.) with a load cell of 50 kg, and fitted with a cylindrical aluminium probe whose 50 mm diameter flat surface contacted and completely covered the upper flat surface of the discs (25 mm diameter). The extruded FFM sausage samples were placed flat on a glass plate overlaid the instrument's metal base and two compression cycles were performed at room temperature. The textural parameters were measured according to Riebroy, Benjakul, Visessanguan, Kijrongrojana & Tanaka (2007), with the following operating conditions: crosshead speed 5 mm s^{-1}, 50 % strain, surface sensing force 0.971 N, threshold 0.294 N, pre-

test speed 1 mm s^{-1}, post-test speed 10 mm s^{-1}, and the time interval between the first and second strokes was 1 sec. The Texture Exponent 32 software (Stable Micro Systems, U.K.) was used to collect and process the data. Analyses were defined and calculated as described by Bourne (1978) where hardness, adhesiveness, springiness and cohesiveness were calculated from the force-time curves generated for each sample. There were three replicate fermentations for each of the different treatments, and a single determination was made for each replicate and each time. Those data are the basis of statistical analysis.

Colour measurement

The colour of the samples was measured in *L**, *a**, *b** space using a ColorFlex® system (Model 45/0 Hunterlab ColorFlex, Reston, Virginia, U.S.A.) with illuminant D 65 as the light source for a 10° standard observer. *L**, *a** and *b** values indicate lightness, redness/greenness, and yellowness/blueness, respectively. The instrument was calibrated against a black and white reference tile prior to use. Extruded samples of the triplicated FFM sausage treatments were cut (20 mm high × 25 mm diameter), and placed centrally on the base of a clear crystallising dish that sat beneath a black shroud. The individual measured values were corrected for the colour of the empty crystallising clear dish (mean of five readings), and then averaged to get the blank-corrected colour reading for a treatment.

Chemical analysis of FFM

Determination of pH

The pH of the sample was determined according to the method of Benjakul, Seymour, Morrissey & An (1997). A 5 g sample of FFM sausage was homogenized for 2 min with 50 mL of deionised water using an Ultra-Turrax homogenizer (IKA-Wereke, Germany). The pH was measured using a portable pH meter (Meterlab, U.K.) which was initially calibrated against two authenticated buffer solutions, pH 7 and pH 4, prior to use. There were three replicates for each treatment, and data are the mean of a single pH analysis at each time.

Determination of trichloroacetic acid-soluble peptides

Trichloroacetic acid-soluble peptides were determined by the method of Green and Babbitt (1990) with slightly modifications. The FFM sausage sample (1 g) was homogenized at 9,500 rpm min^{-1} using an Ultra-Turrax homogeniser (IKA-Wereke, Germany) with 9 mL of 15% (w/v) trichloroacetic acid for 2 min. The homogenate was kept on ice for 1 hour then centrifuged at 3000 gravities for 10 min. The supernatant was retained while the residue is

extracted once more following the same procedure. The supernatants are combined and made to 20 mL with 15% (w/v) TCA solution. The soluble peptides in the supernatant were measured by the method of Lowry, (Lowry, Rosebrough, Farr, & Randall, 1951). Bovine serum albumin (BSA) standards were made with a concentrations series from 0 to 2500 ppm in deionised water. The copper reagent responsible for colour development was made by mixing 26 mL of 0.8% sodium carbonate with 2 mL of 0.2% $CuSO_4.5H_20$ and 2 mL of 0.1% sodium potassium tartrate. This copper reagent was in turn used to make the Lowry solution; this comprised 3 mL of copper reagent, 1 mL 0.1% sodium dodecyl sulphate, and 1 mL of 1 M sodium hydroxide.

For each sample or standard BSA solution, 400 µL of sample, 200 µL of 0.2 M Folin reagent and 400 µL of Lowry solution were mixed in 1 mL plastic cuvettes and absorbances read at 532 nm in a spectrophotometer (Ultraspec 2100 pro, U.K.). Results are expressed mg of bovine serum albumin equivalents kg^{-1} FFM sausage. There were three replicate for treatment, and results are the mean of three TCA analyses at each time point.

Determination of thiobarbituric acid reactive substances (TBARS)

Lipid oxidation was determined using the TBAR method according to Buege & Aust (1978) with modifications. A 2.5 g FFM sausage sample or an equivalent 2 mL of a known malonaldehyde solution was homogenized with 12.5 mL TBARS solution (0.375% thiobarbituric acid, 15% trichloroacetic acid and 0.25 M HCl) at 9,500 rpm min^{-1} using an Ultra-Turrax homogenizer. The mixture was heated in a boiling water bath (100 °C) for 10 min to develop the pink colour. The mixture was cooled with running cold water and centrifuged at 3,000 gravities for 30 min. The absorbance of the supernatant was measured at 535 nm in a spectrophotometer. The TBARS value was calculated from a standard curve of malonaldehyde with a concentration ranging from 0 to 2500 ppm, and expressed as mg of malonaldehyde equivalents kg^{-1} of FFM sausage. There were three replicate at each of two different treatments, and data are the mean of three TBARS analysis at each time.

Determination of biogenic amines

Histamine, tyramine and tryptamine stock solutions were made by dissolving 1.656 g of each in 1 L volumes of 0.1 M HCl. Each solution was diluted with 0.1 M HCl to a working concentration of 100 mg L^{-1}, and was kept at 4°C. Samples of FFM sausage were extracted according to the method of (Cinquina, Longo, CalÄ±Ì€, De Santis, Baccelliere, & Cozzani,

2004) with a slight modification. A 5 g sample was added to 20 mL of 1 M perchloric acid and the mixture was homogenised at 9,500 rpm using the Ultraturrax and centrifuged at 3,000 gravities for 15 min at 4ºC. The supernatant was filtered through a Whatman paper No. 1. The procedure was repeated twice, and the extracts were then combined to the final volume of 50 mL in a volumetric flask. Aliquots were filtered through a 0.45 µm PTFE syringe filter before injection into a liquid chromatograph for analysis.

Quantitation of biogenic amines was determined from a dilution series of the standard biogenic amines. The chromatographic separation of amines was performed with a Shimadzu LC-10AD (Tokyo, Japan) fitted with a Luna 5µ C18 (2) 150 × 3.90 mm column and a LC 1200 UV/VIS detector set to measure absorbance at 217 nm. The mobile phase was a mixture of 70 mL of acetonitrile, 30 mL of methanol, 1500 mL of deionised water and 100 mL of an ammonium acetate solution. This was made by dissolving 7.7 g of ammonium acetate in 1 L of water and the pH was adjusted to 4.5 with phosphoric acid. The flow rate of the mobile phase was 0.5 mL min^{-1}. There were ten replicate at each of two different treatments, and data are the mean of ten biogenic amine analyses at each time.

Data analysis

Data were first marshalled by routines within Microsoft Excel. Data were analysed using One-way Anova in Minitab 15 (Minitab Inc., State College, Pennsylvania). Comparisons between individual means were done with the Tukey test in that routine.

Chapter 3

Results and Discussion

Calibration of the glucose meter

In the method of standard additions, a known quantity of the analyte in question, glucose in this case, is added to a matrix containing an unknown concentration of glucose. From the response of the meter it is possible to calculate a correction factor, which can be unity, for each matrix. Factors used for correction of glucose concentration values returned by the meter are shown in Table.

Table 7. Correction factor for three tubers

	Kumara	Taro	Potato
Correction factor	1.02	1.13	1.08

The correction factors were all close to unity, with little variation between tubers. Kumara has the lowest correction factor and taro is the highest.

Concentration of glucose during cooking of tuber pieces

Figure 14 shows the concentration of glucose generated by microwave cooking of kumara, potato and taro. Clearly, the graph shows that kumara generated the highest concentration and potato the lowest. Close inspection of Figure 14 shows that the standard deviations were surprisingly low at all times in all tubers. Review of the data confirmed the accuracy of the low standard deviations.

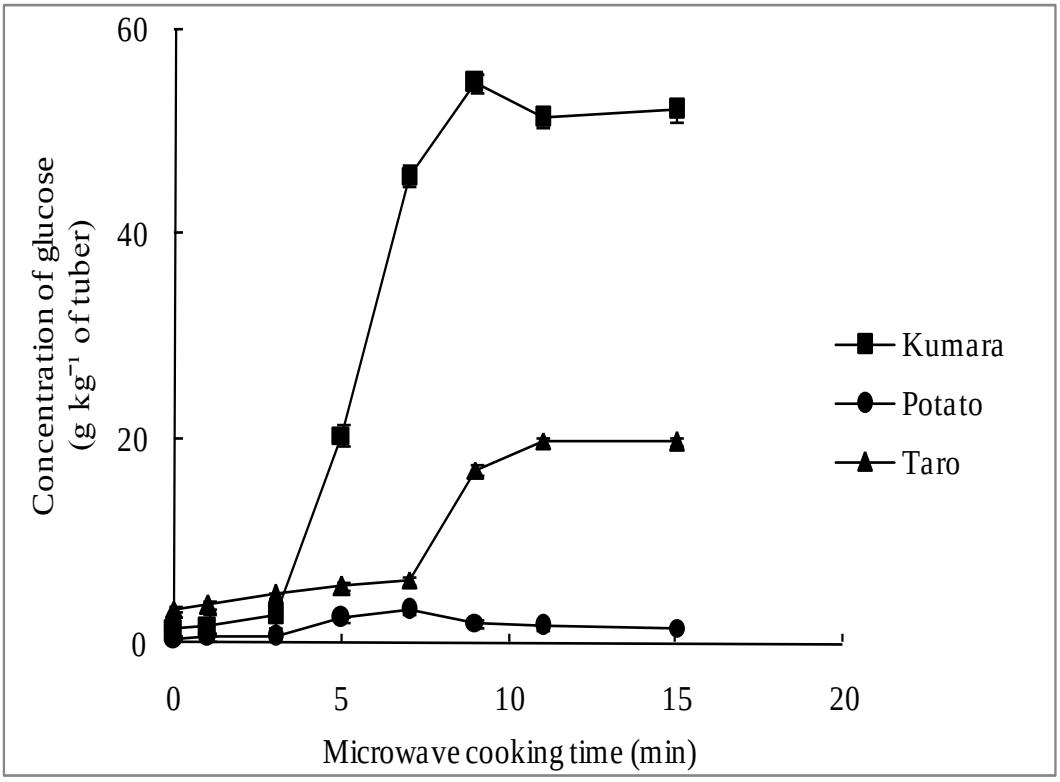

Figure 14. Changes in concentration of glucose produced by kumara, potato and taro at each microwave cooking time. There were three replicate homogenisations at each of eight time points, and data are the mean of a single glucose determination at each time. The scarcely visible standard deviations are shown as vertical bars.

In the first 3 min, there was little change in any tuber, but was followed by a major increase in glucose concentration in kumara at 5 min. A useful increase was not observed in taro until 9 min. Potato showed the least change at all times. It is reemphasised that Figure 14 reports only glucose concentration and that other fermentable carbohydrates will be generated on cooking but were undetected. Such fermentable carbohydrates included fructose, sucrose and maltose. This is certainly true for kumara as Table shows. Beyond 9 min, there was little change in any of the tubers.

Table 8. Composition of sugars in raw and cooked roots of seven different sweet potato cultivars

Cultivar	Sugar concentration (%, w/w)							
	Glucose		Fructose		Sucrose		Maltose	
	Raw	Baked	Raw	Baked	Raw	Baked	Raw	Baked
Centennial	0.24	0.27	0.30	0.43	4.10	5.17	0	9.33
Jasper	0.44	0.42	0.43	0.41	3.63	5.14	0	7.75
Travis	1.50	2.73	1.15	1.99	2.87	3.26	0	4.02
Jewel	1.22	1.29	1.01	1.20	2.78	3.98	0	7.55
White Star	0.40	0.39	0.39	0.40	2.25	3.35	0	14.12
Rojo Blanco	0.95	1.22	0.65	0.97	1.30	1.59	0	10.77
Tongan	0.45	0.37	0.33	0.26	2.03	2.43	0.64	7.09

From Picha (1986)

It is clear from Figure 14 that taro generates potentially useful concentrations of glucose and almost certainly other fermentable sugars. Whereas taro is cultivated throughout the world, it has a strong cultural link to the Pacific Islands, Tonga included. It was chosen for further work because it could confer a degree geographical exclusivity in a fermented tuna product. The concentrations chosen were 0, 10, 20 and 30% taro, a likely range of concentrations to fulfill two criteria; a fermented tuna product, and produced at minimal cost. Taro is a lot cheaper than tuna.

Changes in pH during incubation

Four taro concentrations were chosen, 0, 10, 20 and 30% such that the proportion of tuna was reduced as required. All other ingredients shown in Table 1 remained at the same concentrations. However, in the treatments examined here, 3% glucose was added to all. This was done because the 0% taro treatment would otherwise have nothing to support fermentation.

Figure 15 shows the mean pH values of FFM sausage for all treatments. Clearly, a lactic fermentation process takes place, causing a decrease in pH values with time. Falls in pH value are caused by an accumulation of organic acids, mainly lactic, present in these types of sausages as a result of carbohydrate breakdown yielding sugars during fermentation (Bloukas, Paneras, & Fournitzis, 1997). The results confirm the effectiveness of the LAB in the starter cultures for sausage.

The pH of the 0% taro treatment was numerically the highest to Day 4, and the 10% taro treatment usually had the lowest pH. All pH values were between 4.1 and 3.9 at Day 4, and were little different from values at Days 2 and 3.

The pH of the FFM sausage for all treatments decreased markedly from Day 0 to 1 except for the 0% taro treatment. Glucose was not limiting so it is likely that taro contributed some growth factor that accelerated fermentation.

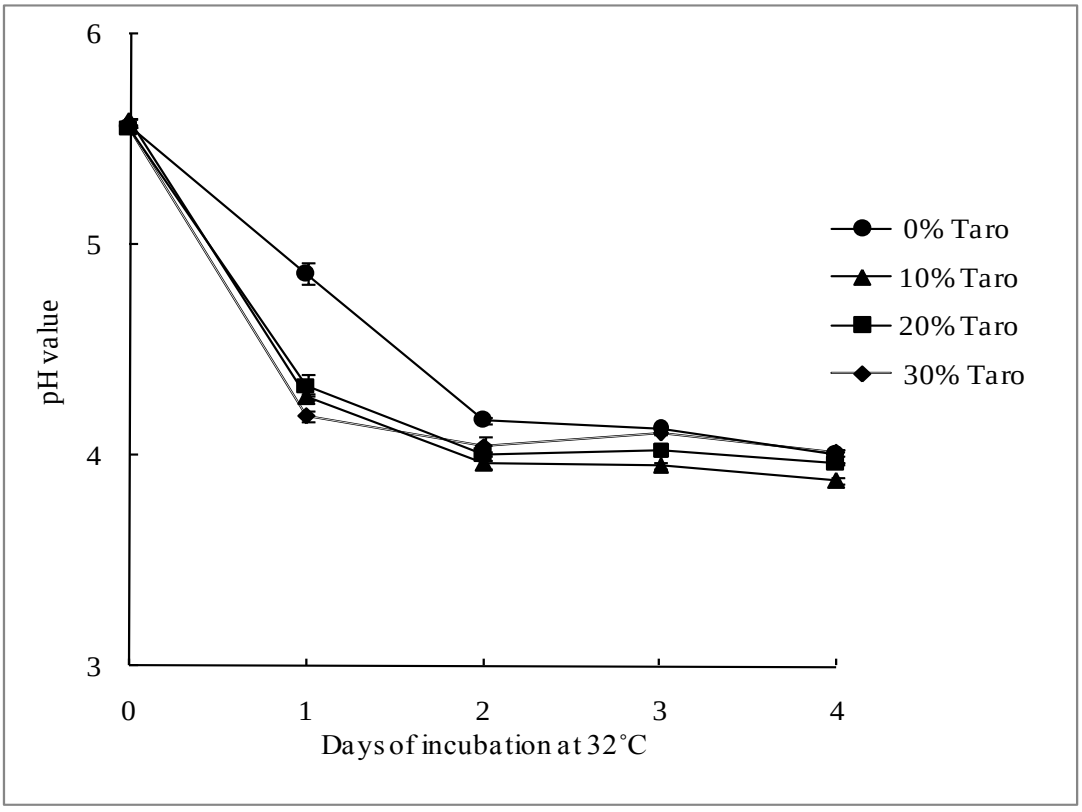

Figure 15. The pH changing curve of cured FFM sausage for the four different treatments during incubation. Data points are means and vertical bars are standard deviations of three replicates. The variation between replicates was low.

A taro concentration of 20% was subsequently chosen as the working concentration for a commercial model. Higher concentrations could be used, as is clear from Figure 15, but it was considered that the essential nature of the FFM sausage was that tuna, not taro, had to be the defining ingredient.

Curing is optional in the formulation of a FFM sausage, but as discussed in Chapter 1, is useful to prevent oxidative rancidity from creating rancid flavour which would lessen the keeping quality. It also imparts a red hue, which is asthetically desirable to consumers

(Wasserman & Talley, 1972). Curing can also inhibit the growth of certain bacteria such as putrefactive anaerobes that causes spoilage of meats, and pathogenic bacteria such as *Clostridium botulinum* which causes food borne illness (Tompkin, Christiansen, & Shaparis, 1978).

The effect of uncured on pH was thus examined with 20% taro, and no added glucose. All other ingredients shown in Table 2 remained at the same concentrations and time of incubation was up to 16 days. Figure 16 show that uncuring affected the pH of the FFM sausage, such that the pH with uncuring was appreciably higher at all times ($P < 0.001$). From Day 4 right through to Day 12, uncured FFM sausage continue to fall slightly and no change through to Day 16. In the case of cured FFM sausage, little change happens after Day 4 to Day 16. A short review of the literature has failed to show why uncuring resulted in a higher pH.

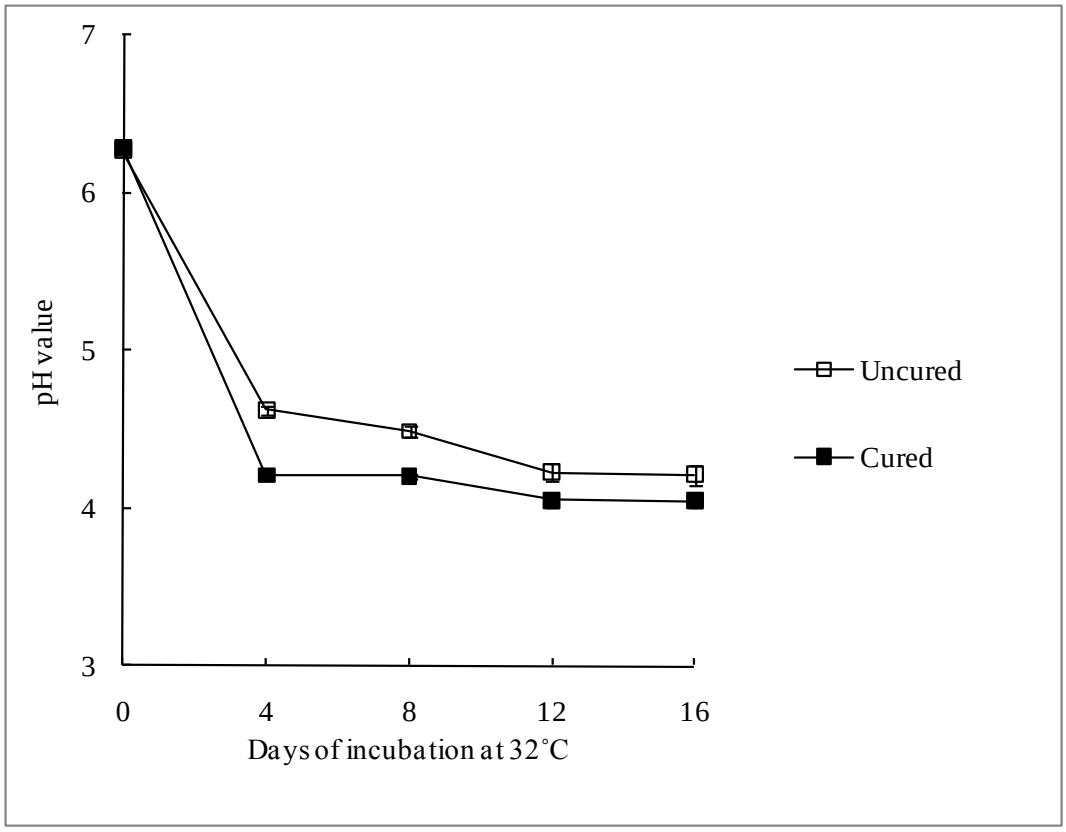

Figure 16. The pH changing curve of cured and uncured FFM sausage without glucose during incubation. The taro concentration was 20%. Data points are means and vertical bars are standard deviations of three replicates. The variation between replicates was low

The pH of fermented foods is an important safety factor, and pH 4.6 is considered desirable because almost no food pathogenic and spoilage bacteria grow below 4.6 (Riebroy, Benjakul, Visessanguan, & Tanaka, 2005). The pH value in the present study for all treatments ranged between pH 4.6 and 3.9 on Day 4 as shown in Figure 15Figure 16. The positive technological aspects of acidification is the assurance that product is safe to consume, and exhibits faster drying and improved texture through the denaturation and coagulation of proteins (Hugas & Monfort, 1997). However, excessive acidification such as seen here adversely alters the aroma and flavour and can leads to colour defects due to inhibition of the LAB (Buckenhüskes, 1993). The ultimate FFM product should provide a balance between desirable acidity, reddening and flavour, but these sensory attributes were not assessed in this project.

Textural properties of FFM during fermentation

Although 20% taro was chosen as the working commercial model, it was of interest to find out how a range of taro concentrations affected texture. Texture was assessed by texture profile analysis (TPA) where the mechanical properties of uncured fermented tuna sausages was determined on Day 0 and Day 4 using Tongan-cooked taros at four different concentrations. The mechanical properties were hardness, springiness, adhesiveness and cohesiveness (Bourne, 1978). These are key parameters that can be used to characterise the textural properties of any sausage (Li, Carpenter, & Cheney, 1998). An obvious question is: why are the properties measures at Day 0, a time where FFM sausage would never be eaten? The answer is that mechanical properties at Day 0 are important in respect of materials handling, so basic data have been collected here.

Figure 17 shows a typical TPA force-time curve of an extruded FFM sausage disc at Day 4. Hardness is the maximum force required to compress the sample on the first compression. According to Figure 17, the first major peak determines the hardness of the sample, where force has units of grams (1 g = 0.00981 N). Springiness relates to the reshaping ability of the sample after the compressive force has been removed which is not directly shown in Figure 17. It is calculated by the distance of the detected height of the product on the second compression (Distance 2) divided by the original compression distance (Distance 1). Cohesiveness on the other hand is how well the sample withstands a second deformation relative to how it behaved under the first deformation. It is measured as the area of work during the second compression divided by the area of work during the first

compression (A2/A1) as shown below in Figure 17. Adhesiveness is a measure of the stickiness of sample to surfaces, thus clearly relies on the nature of contacted surfaces. In Figure 17, the negative area for the first compression cycle is the measure of the adhesiveness which represents the work needed to overcome the attractive forces between the surfaces of the probe and the food sample tested.

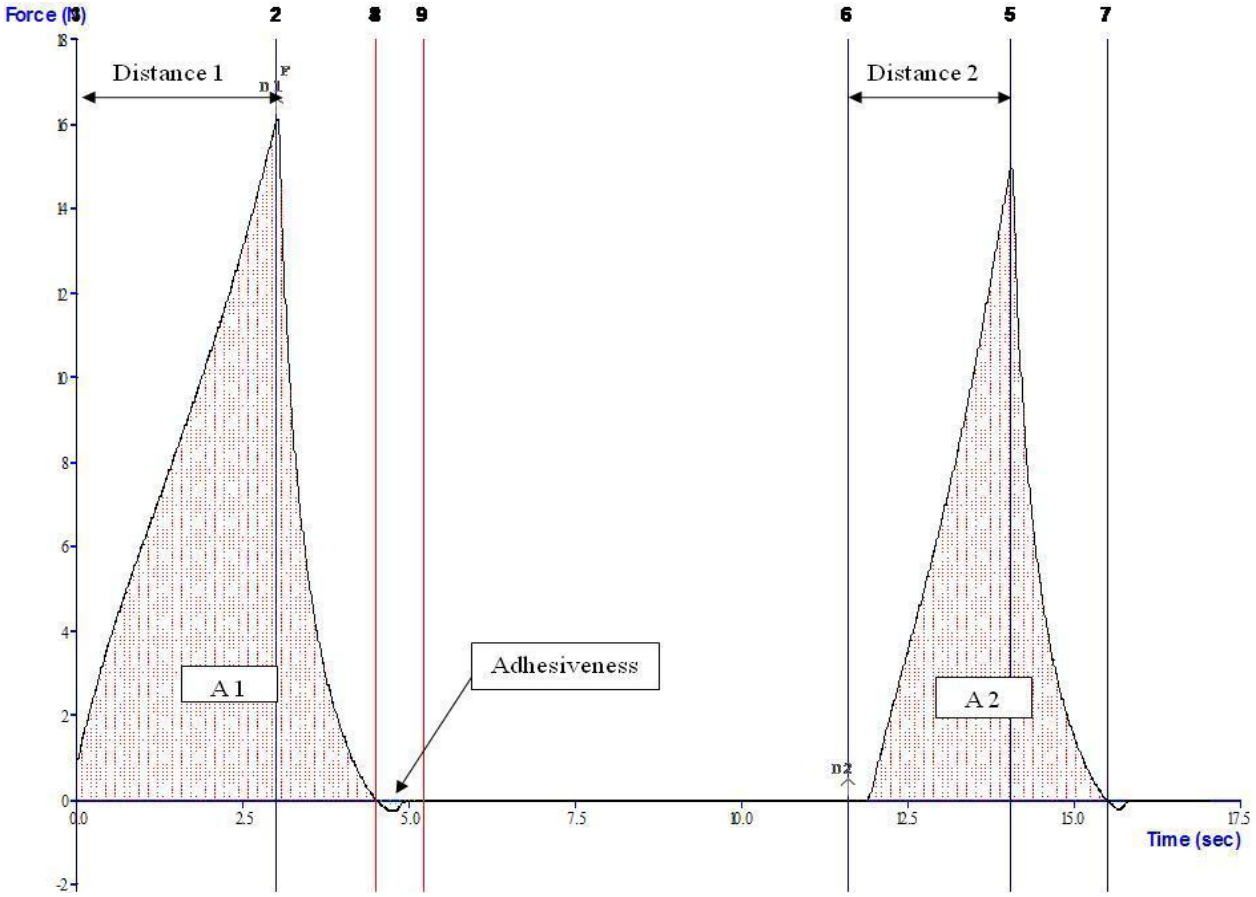

Figure 17. Example of a force-time curve of a 25-mm extruded cured FFM sausage disc compressed twice in one TPA analysis. The curve was drawn by the TAXT Plus Texture Analyser by computer

The TPA results are shown in Figure 18, Figure 19, Figure 20 and Figure 21. Hardness values on Day 4 were about four times the hardness values in Day 0 for all treatments (Figure 18) ($P < 0.001$). Hardness was thus strongly affected by fermentation. A Day 0, there was a significant difference ($P < 0.05$) between FFM sausage with 0% and the other treatments. At Day 4, the 0% and 30% taro treatments were lower and higher in hardness than the 10 and 20% treatments ($P < 0.001$). It is clear that more taros is added to FFM sausage, and the higher the hardness value. Thus, the hardness data show that the

increase in hardness can be accounted for by the denaturation and gelation of the meat protein in the tuna flesh together with the high starch and fiber effect from taro.

Among texture attributes, hardness is regarded as the most important to consumers as it determine the commercial value of a fermented meat (Dingstad, KubberÃ¸ NÃ¦s, & Egelandsdal, 2005), but how this applies to a completely new product made from fermented fish is unknown.

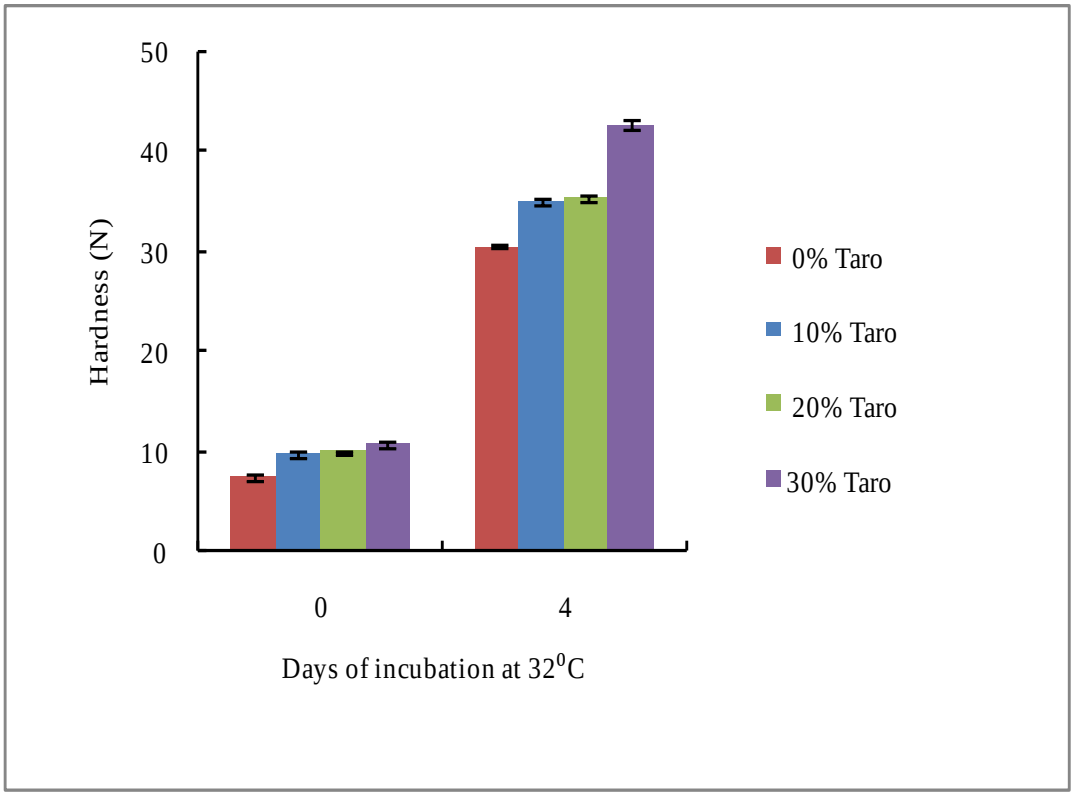

Figure 18. Changes in hardness of cured FFM sausage extruded from syringes at Day 0 and Day 4 for the four taro treatments. Data values are means and vertical bars are standard deviations of three replicates

The springiness of FFM sausage is shown in Figure 19. It shows that springiness measured on Day 4 was slightly higher than on Day 0 for all treatments except for FFM sausage with 0% taro showing no difference between days. The fermented sausages were all similarly springy, returning to nearly 80% of the original height on Day 4. FFM sausage with 10% taro has the highest springiness value of about 90% of the original height ($P < 0.05$ compared with other Day 4 treatments).

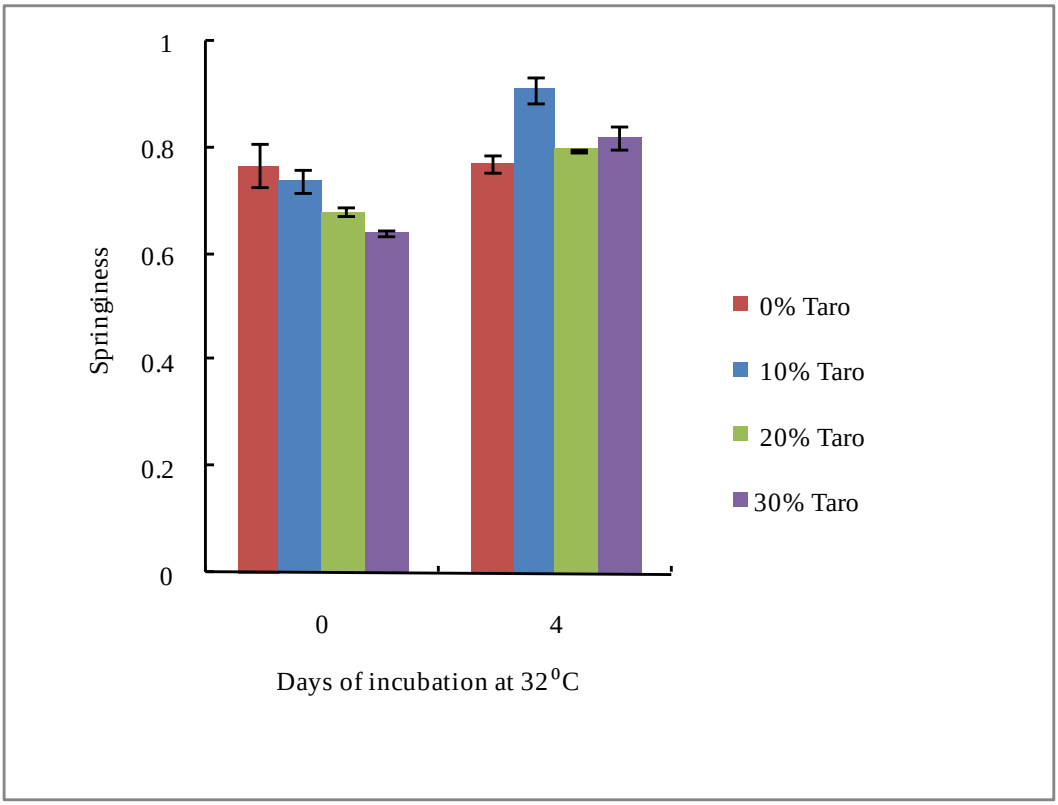

Figure 19. Changes in springiness of cured FFM sausage extruded from syringes at Day 0 and Day 4 for the four different treatments. Data values are means and vertical bars are standard deviations of three replicates.

Figure 20 shows the cohesiveness of FFM sausage on Days 0 and 4 for the four different treatments. Clearly, the cohesiveness of the fermented sausage reduces on Day 4 for all four treatments ($P < 0.001$) indicating a structure that is more brittle on Day 4. Put simply, the FFM sausage is harder on Day 4 but does not hold together as well. Closer inspection of Day 4 data in Figure 18 and Figure 20 strongly suggests that as taro concentration increases, the FFM sausage becomes increasingly brittle (hard, not cohesive), which may be a problem in slicing the FFM sausage for presentation and consumption. (Logically a FFM extruded in a sausage shape would be sliced laterally in the manner of conventional salami.) Thus the choice of taro concentration is likely to be a tradeoff between cost and texture.

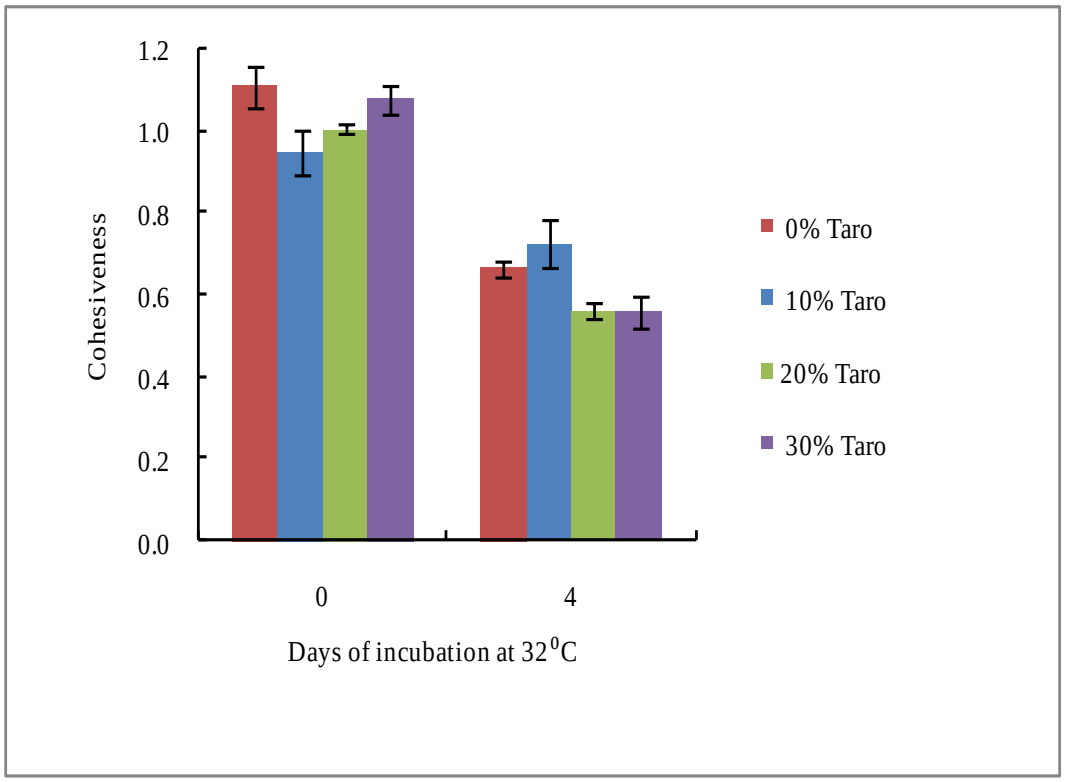

Figure 20. Changes in cohesiveness of cured FFM sausage extruded from syringes at Day 0 and Day 4 for the four different treatments. Data values are means and vertical bars are standard deviations of three replicates.

Adhesiveness is a measure of the stickiness of samples to surfaces, thus clearly relies on the nature of contacted surfaces as discussed previously. Adhesiveness results are shown in Figure 21 where high negative values indicate high adhesiveness. The adhesion force is a combination of an adhesive force and a cohesive force in most food systems (Hoseney & Smewing, 1999). A food material is perceived as being sticky when the adhesive force is high and the cohesive force is low (Hoseney & Smewing, 1999). At 0% taro, the FFM sausage was less adhesive on Day 4 than on Day 0 ($P < 0.001$), but the other treatments presented a more complicated treatment (Figure 21). Adhesiveness should be low on Day 4, because each slice of FFM sausage should be discrete for ease of consumption. After slicing, the pieces should stick to a plate. The 20% taro treatment was a failure in this respect, because it was the most adhesive and equally the least cohesive (Figure 20). However, it must be noted that the adhesiveness is surface-specific, in this case a flat sheet of glass and an adonised aluminium probe. But one of these surfaces is like a domestic plate in that crockery is always glazed. Thus, the choice of 20% taro may be flawed for reasons of adhesiveness, and is suggested in the literature. Adhesiveness may be considered a negative character for

some meat products (Fiszman & Damasio, 2000), where it could affect their sliceability (Bozkurt & Bayram, 2006). But whatever the reason for the differences, Riebroy, Benjakul, Visessanguan, & Tanaka (2005) maintained that adhesiveness is probably less important than hardness as a quality attribute. Sausage dry matter and pH have been found to play an extremely significant role in adhesiveness (Herrero, OrdÃ³Ã±ez, de Avila,Herranz, de la Hoz, & Cambero, 2007), and the very low pH recorded for these FFM products (Figure 15) may be intimately involved in adhesiveness.

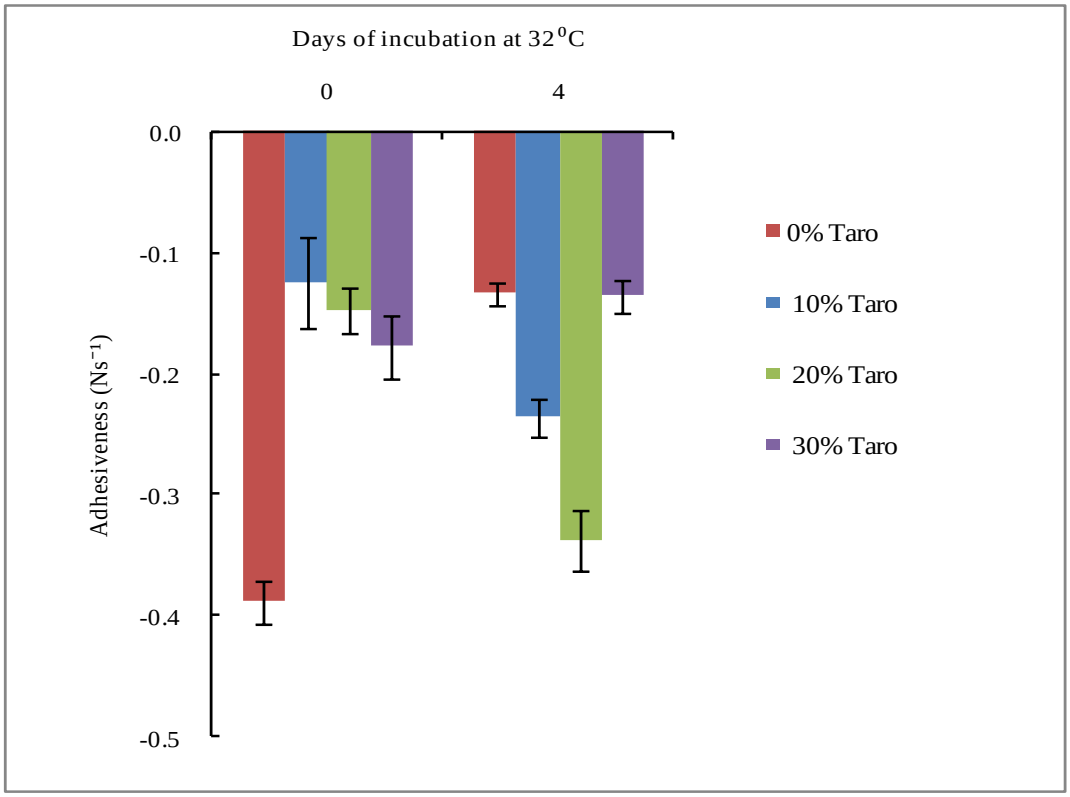

Figure 21. Changes in adhesiveness of cured FFM sausage extruded from syringes at Day 0 and Day 4 for the four different treatments. Data values are means and vertical bars are standard deviations of three replicates.

Addition of taro undoubtedly had a major, but complex effect on textural properties and much work needs to be done to decide on a final proportion for taro. Moreover, texture may well be affected by taro variety and of course the extent of cooking. Equally, different tuna species may have different textural properties when very fresh and on storage (Saisithi, Wongkhalaung, Boonyaratanakornkit, Yongmanitchai, Chimanage, & Maleehuan, 1986). Typically, fish muscle proteins undergo hydrolysis by endogenous proteolytic enzymes at a rate ten times higher than those of mammalian muscle (Dunajski, 1980), and this will lead to

softer textures. Importantly, fermentation was done at 32˚C, and is possible that by endogenous proteolytic enzymes may be very active at that temperature, leading to deterioration in gel structure through damage to collagen and myosin (Bremner & Hallett, 1985). This possibility suggests that experiments should be repeated at a range of fermentation temperatures.

Changes in colour of FFM

The L* values are shown in Figure 22. Clearly, the graph shows that FFM sausage with 10% taro had the highest L* values (light reflectance), and FFM sausage with 0% is the lowest on Day 4. The FFM sausage made with 0% taro had a markedly different reflectance from the other three treatments ($P < 0.001$). The plus taro treatments were numerically similar to each other. At earlier times, there was a major increase in reflectance at Day 2 for the 0% taro treatment, probably indicating gelation as the pH fell to pH 4.2 (Bremner & Hallett, 1985) (Figure 22). Changes in the plus taro treatments were not so marked, indicating that reflectance was dominated by the presence of taro rather than gelation. According to Riebroy, Benjakul, Visessanguan, Kijrongrojana, & Tanaka, (2004), whiter FFM sausages are more acceptable to consumers in the Cambodian market. It is unknown whether consumers in other countries would respond in the same way. While whiteness as mental construct is not necessarily the same as L* values, it is highly likely that fermented tuna sausage made with 10, 20 or 30% taro would be the acceptable due to higher L* values.

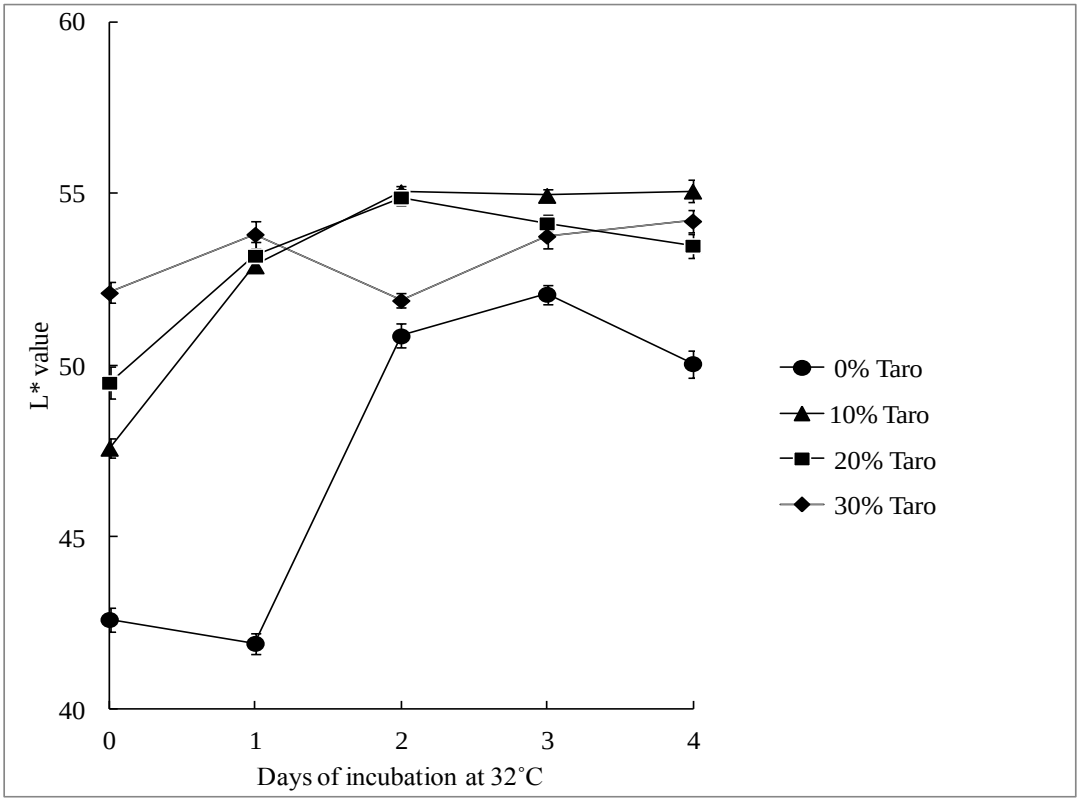

Figure 22. The reflectance changes of cured FFM sausage for the four different treatments during fermentation. Data points are means and vertical bars are standard deviations of three replicates

The a* values are shown in Figure 23 and were positive over the four days of fermentation. Redness peaked at Day 2 for reasons that will be discussed later. Tuna muscle is red pigmented due to its myoglobin content. Figure 23 shows that FFM sausage made with 0% taro had the highest a* values at all times followed by 10, 20 and 30% taros were the lowest, usually in that order. This indicates a dilution of myoglobin by increasing taro concentration. Overall, the greater reflectance in Figure 22 and reduced redness in Figure 23 due to increasing taro concentration would generate a potentially attractive light pink FFM sausage.

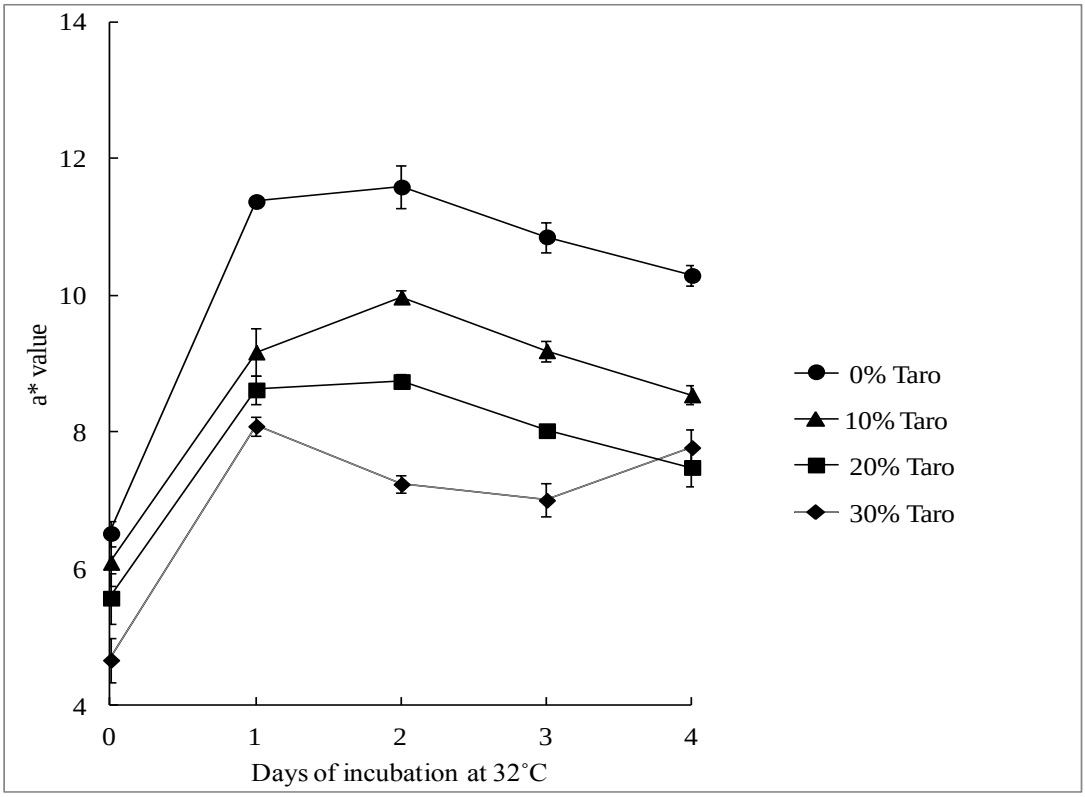

Figure 23. Development of redness colour of cured FFM sausage for the four different treatments during fermentation. Data points are means and vertical bars are standard deviations of three replicates

The b* values indicate yellowness (+ values)/blueness (– values). Figure 24 shows that yellowness largely declined over time for all treatments with some complexity at Day 4. A sequence parallel to that for redness (Figure 23) was observed here, where FFM sausage made with 0% taro had the highest b* values followed 10, 20 and 30% in that order. This is a dilution effect where the pigmented tuna is diluted by the relatively colourless taro.

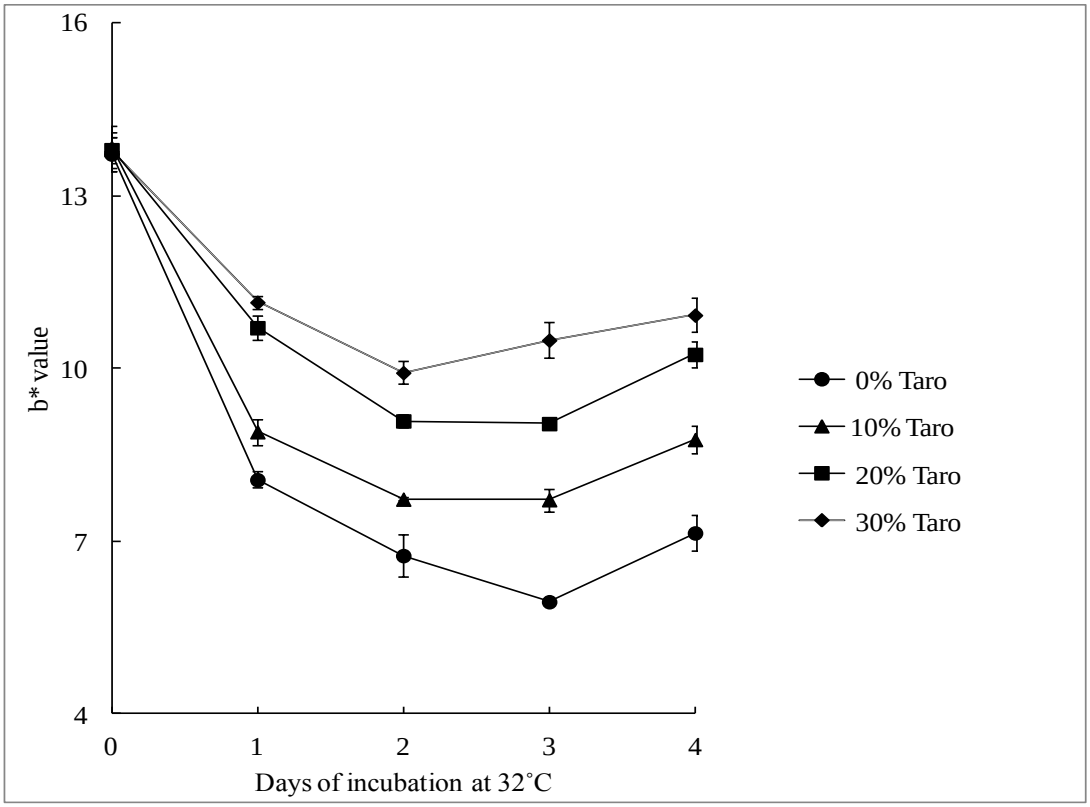

Figure 24. Development of yellowness colour of cured FFM sausage for the four different treatments during fermentation. Data points are means and vertical bars are standard deviations of three replicates

In comparing a* and b* values over time, it is clear that as the contents of the syringes increased in redness with time (Figure 23), yellowness tended to decrease (Figure 24). That is because FFM sausage on Day 0 was brown. In ruminant meat, an increase in positive b* values relative to positive a* values is a good indicator of browning (Young & West, 2001) due to metmyoglobin formation. The converse applies here where an initially brown mixture of cured fish and taro becomes pink/red.

Under the deteriorative conditions of a comminuted fish muscle, the original myoglobin and oxymyoglobin in the fish muscle initially tend to oxidise to the brown pigment metmyoglobin with the oxygen trapped in the sausage mix (Young & West, 2001). This oxygen is rapidly consumed by the LABs and the meat mitochondria resulting in an anaerobic environment. Under these conditions, metmyglobin reductase in the meat is able to reduce metmyoglobin back to myoglobin (the deoxy form), utilising NADPH from glycolysis. Subsequently, the NO-myoglobin is formed, which is a stable typical pinkish cured colour (Pearson & Gillett, 1996). NO-myoglobin is the pink colour that dominates Hunter a* values in this fermentation study as shown below. This explains the increase in redness after twenty

four hours during fermentation. Interestingly, metmyoglobin formation is favoured by acidic conditions as is clear from the following equation as reported by Brown & Mebine (1969).

$$H^{+} + Mb^{2+}O_2 \rightarrow Mb^{3+} + 0.5H_2O + 0.75O_2$$

Oxymyoglobin Metmyglobin

As pH falls during fermentation, metmyglobin should increase, but cannot because oxygen is unavailable. The pink colour thus develops and in theory persists. However, Garcia-Marcos, Rosmini, Pérez-Alvarez, Gago, López-Santoveña, & Aranda (1996) and Fernandez-Lopez (1998) maintain that very acid conditions (pH < 7) such as were encountered here (Figure 15) can denature nitrosomyoglobin and reduce pinkness. This may explain the reversal of colour values after Day 2, where brownness begins to develop (a* declines, b* increases). But there is another possible cause for the reversal in colour after Day 2.

The browning effect may be due to the Maillard reaction (Maillard, 1915) between sugars and amino groups, which in the case of muscle foods are free amino acids, peptides, and proteins. These combine through Maillard reactions at high temperatures and low water activity to ultimately yield brown melanoidins (Bozkurt & Erkmen, 2004; Kayaardı & Gök, 2004; Vural, 1998). In the present study, sugars from taro and amino acids/peptides/proteins from the fish could react. Figure 25 summarizes the very complex Maillard reaction.

Conditions of temperature, pH and water activity critically influences the Maillard reaction. Temperature and duration of high temperature were studied by Maillard himself (1912), who reported that the rate of the reaction increases with temperature. Many workers have confirmed this observation (Labuza, Reineccius, Monnier, O'Brien, & Baynes, 1994; O'Brien, Nursten, Crabbe, & Ames, 1998). The Maillard reaction can also occur at room temperature but proceed much more slowly (Hodge, 1967; Shahidi, Rubin, & D'Souza, 1986). The reactivity of the sugar and amino group is also affected, influenced by the pH. The open chain form of the sugar and the unprotonated form of the amino group, considered to be the reactive forms, are favoured at higher pH (Hodge, 1953). The lower the pH, the more protonated amino group is present in the equilibrium and therefore, less reactive with the sugar and the longer it takes for the process to occur. Low water activities also accelerate the reaction. This is because like caramel- compounds like hydroxymethyl furfural (HMF) (Figure 25) that are the intermediates in the progression to melanoidins are dehydration

products of glucose, with six carbon atoms and missing three H_2O molecules. The law of mass action demands that as water activity decreases (but not to the point of the glassy state (Fennema, 1976), the Maillard reaction accelerates. The Maillard reaction can still occur with water present but comparatively slowly.

In the present study, the conditions of fermentation were lowered water activity, lowered pH (Figure 15), heat at 32°C, adequate supply of carbohydrates (added glucose and taro), and possibly amino acids/peptides/proteins from tuna. Reducing sugars such as maltose and glucose obtained from hydrolysis of taro have an aldehyde group which is free to react with the free amino group of amino acids, peptides and proteins from tuna. In proteins, most free amino groups will be ε-amino group of lysine, but also the α-amino groups of terminal amino acids. The condensation product is a N-substituted glycosylamine, which rearranges to form the Amadori rearrangement product (ARP) (Figure 25). According to Figure 15, with pH < 7, the ARP rearranges to form furfural when pentoses are involved, or hydroxymethylfurfural when hexoses are involved (Figure 25). This is the proposed pathway here because the pH of the FFM sausage was less than 7 (Figure 15). Furfural and hydroxymethylfurfural are also reactive aldehydes and take part in further reactions that eventually lead to melanoidins (Figure 25), which, it is proposed, are responsible for the browning observed after Day 2 (Figure 23 and Figure 24).

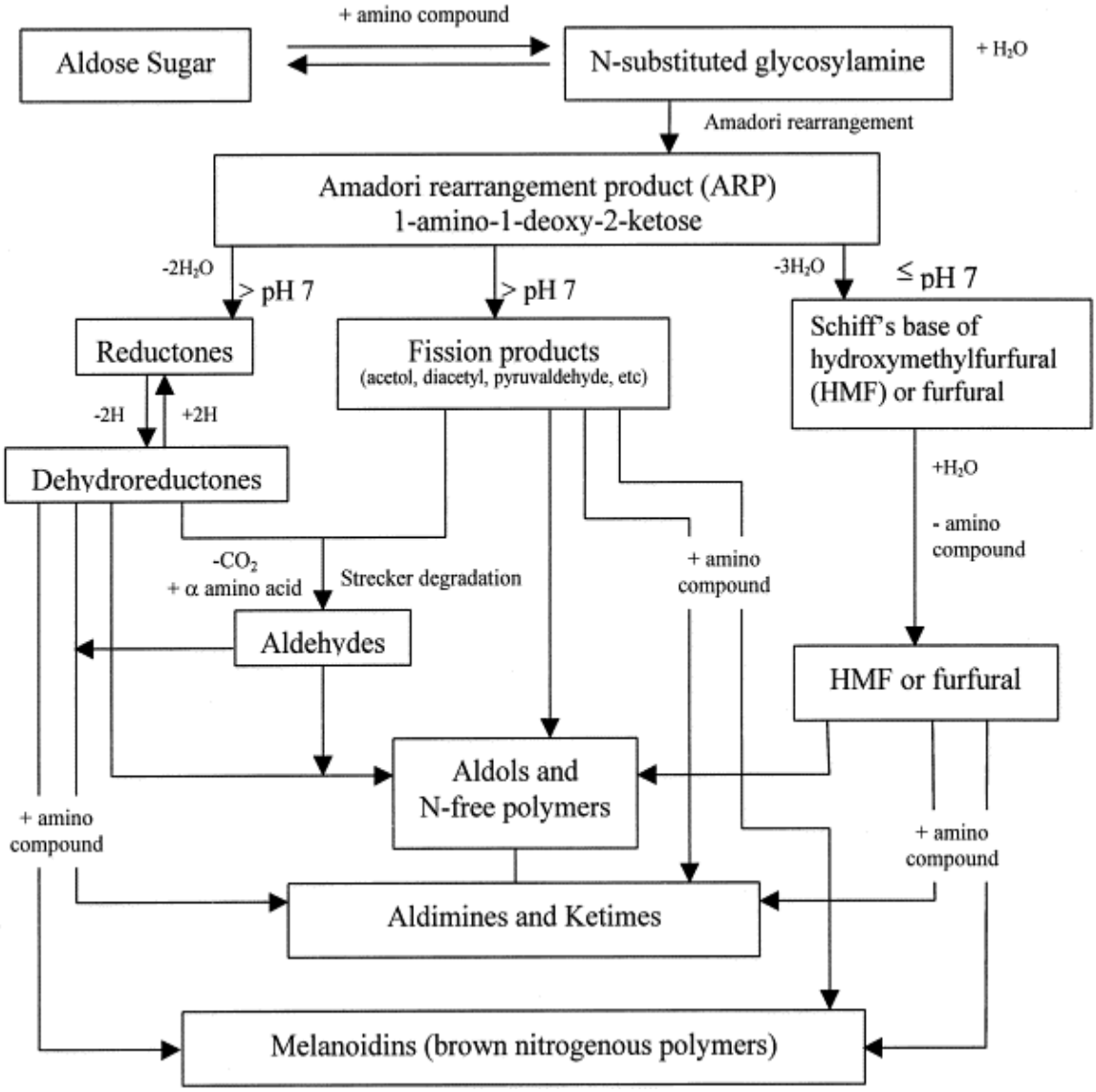

Figure 25. Maillard reaction scheme adapted from Hodge (1953)

The idea that the browning is caused by the Maillard reaction between carbohydrates and proteins was a result of the increase in positive b* values relative to positive a* values. The ε-NH_2 of protein would be reactive if the reducing sugar could access those groups in proteins. That may not be the case. What could be happening is an increase in concentration of amino acids and peptides due to proteolytic activity by bacteria in the fermentation mix and by acid-active proteases in the fish muscle. This is the subject of the next section.

Proteolysis of FFM during fermentation

Proteolysis of meat products can be determined as tricholoroacetic acid (TCA)-soluble peptides and amino acids. Figure 26 shows the calibration curve based on bovine serum

albumin (BSA). The data obtained was fitted with a quadratic equation. The equation was later used to quantify TCA-soluble peptides and amino acids, and plotted in Figure 27.

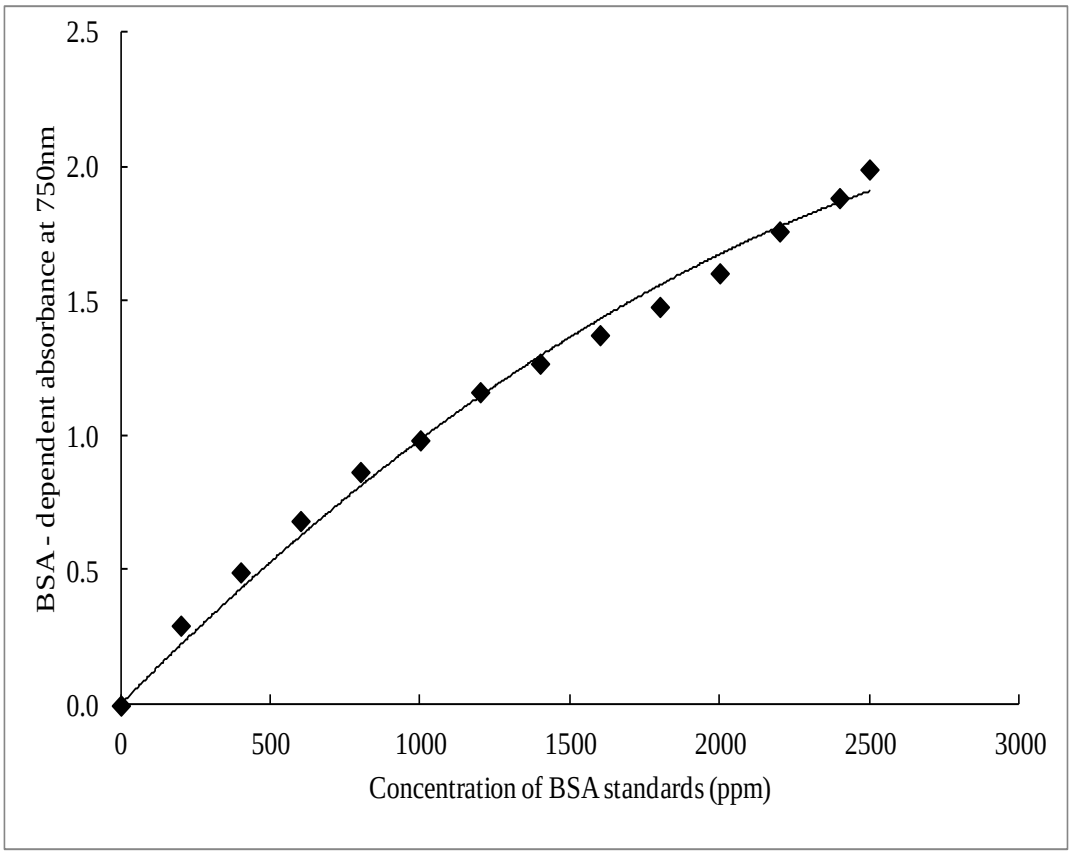

Figure 26. Calibration curve used to quantify TCA-soluble peptides and amino acids. The absorbance at 750 nm was described by the equation that passes through the origin: Absorbance = $-1E-07x^2 + 0.0011x$ where x represents the concentration of proteinaceous matter. The R^2 value was 0.992, and after solving for x, TCA-soluble peptides and amino acids were quantified

Generally, protein hydrolysis increased with increasing fermentation time as shown in Figure 27. Proteolysis was higher in uncured sausage ($P < 0.001$), and was more variable as judged by the standard deviations with 20% taro showed the highest proteolysis and cured FFM sausage was the lowest.

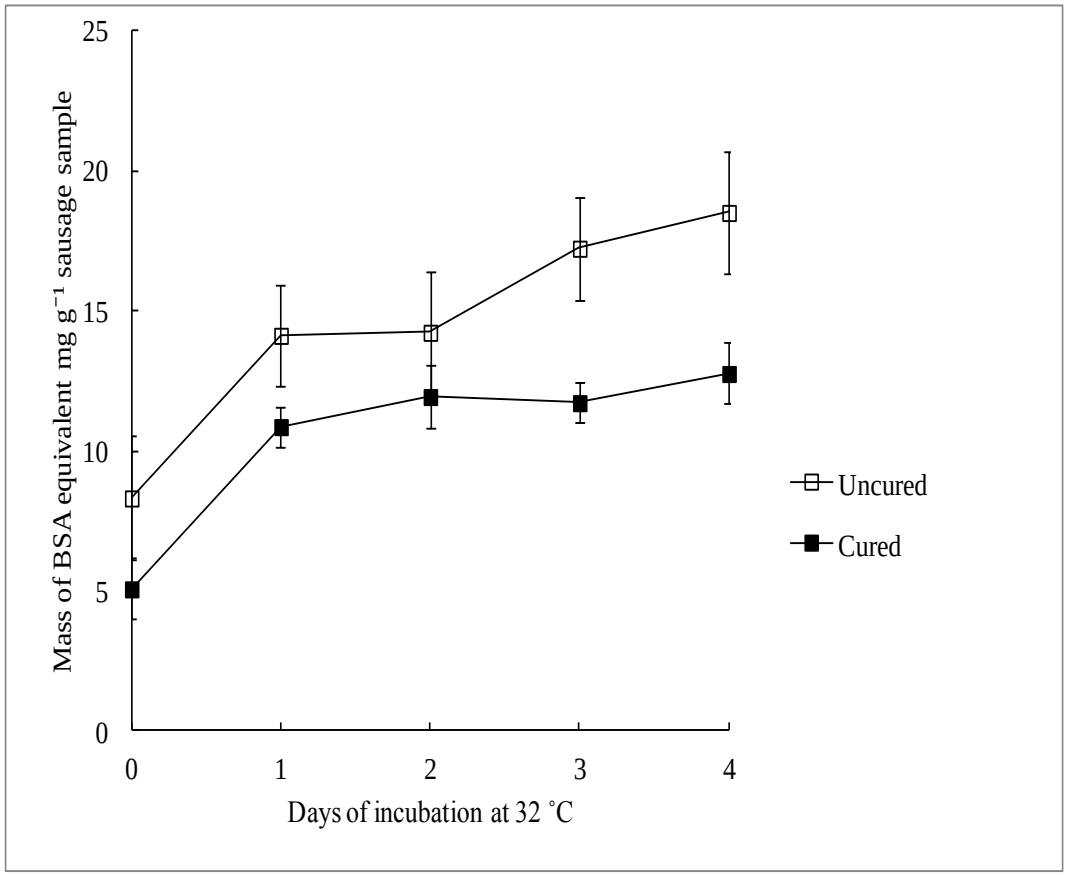

Figure 27. Protein hydrolysis during fermentation of uncured and cured FFM sausage produced with 20% taro. Data points are means and bars indicate standard deviation from three replicates.

The pattern of the proteolysis in FFM sausages is influenced by several variables such as product formulation, processing condition and starter culture (Hughes, Kerry, Arendt, Kenneally, McSweeney, & O'Neill, 2002). Differences in TCA-soluble peptide and amino acid contents can also be due to differences in muscle components and post mortem proteolysis (Benjakul, Visessanguan, Riebroy, Ishizaki, & Tanaka, 2002). The proteolysis due to the action of microbial peptidases degrades the protein fragments in the FFM sausage mainly to polypeptides, smaller peptides, free amino acids and amines. These are known as taste promoters and flavour precursors (Ordonez, Hierro, Bruna, & de la Hoz, 1999; Stahnke, Holck, Jensen, Nilsen, & Zanardi, 2002). However, study of protein degradation in fish by SDS-polyacrylamide gel electrophoresis done by Benjakul, Seymour, Morrissey, & An (1997), shows that heavy chains of myosin in particular was extremely susceptible to proteolysis among all the proteins and could be the case happens in this study. Thus, Valyasevi and Rolle (2002) reported strong proteinase activity from staphylococci and bacilli which is responsible for breakdown of fish protein into peptides and free amino acids. Nitrite

is known to affect bacteria in variable ways, and an effect on the fermentation microflora may be responsible for the difference in proteolysis between cured and uncured FFM sausage (Figure 27).

On the face of it, uncured FFM sausage could be the most flavourful from an amino acid/peptide perspective due to its high rate of protein hydrolysis despite the high variations shown by the standard deviations in contrast to the cured sausage. However, this is just an assumption until sensory trials are performed which did not happen at this stage of study.

Fat oxidation in FFM during fermentation

Based on calibration curve in Figure 28 for thiobarbituric acid, the changes in fat oxidation in FFM sausage over 16 days were quantified and plotted in Figure 29, which shows that TBAR values were between 37 to 39 mg kg^{-1} and were essentially unvarying with curing and with time. Four of the five times points in Figure 29 were from FFM sausage samples extruded from an air-impermeable syringe (Figure 12). Thus, all the fat oxidation occurred at the time of preparation, Day 0, where the mixture was comminuted and blended in ambient atmospheric conditions.

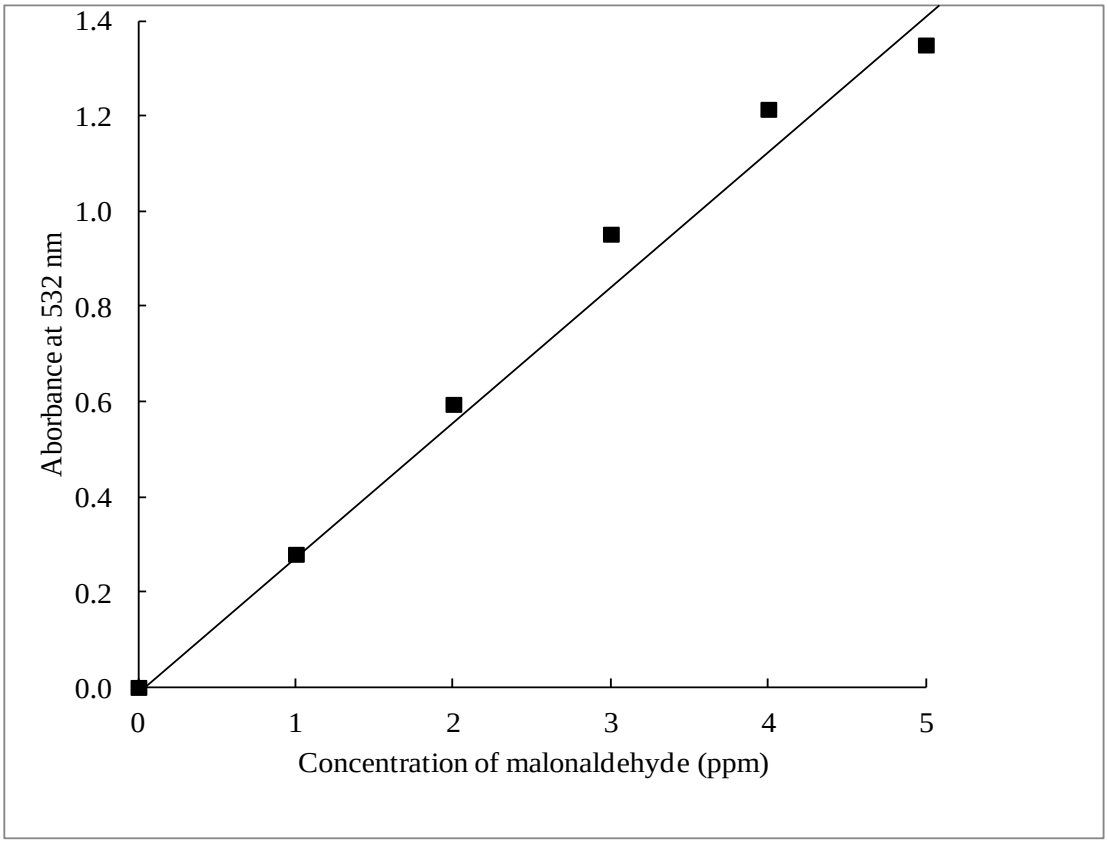

Figure 28. Calibration curve used to quantify TBARS values. The absorbance at 532 nm was described by the equation: Absorbance = 0.290x, where x represent the TBARS values. The R^2 value was 0.978. After solving for x, TBARS values were quantified

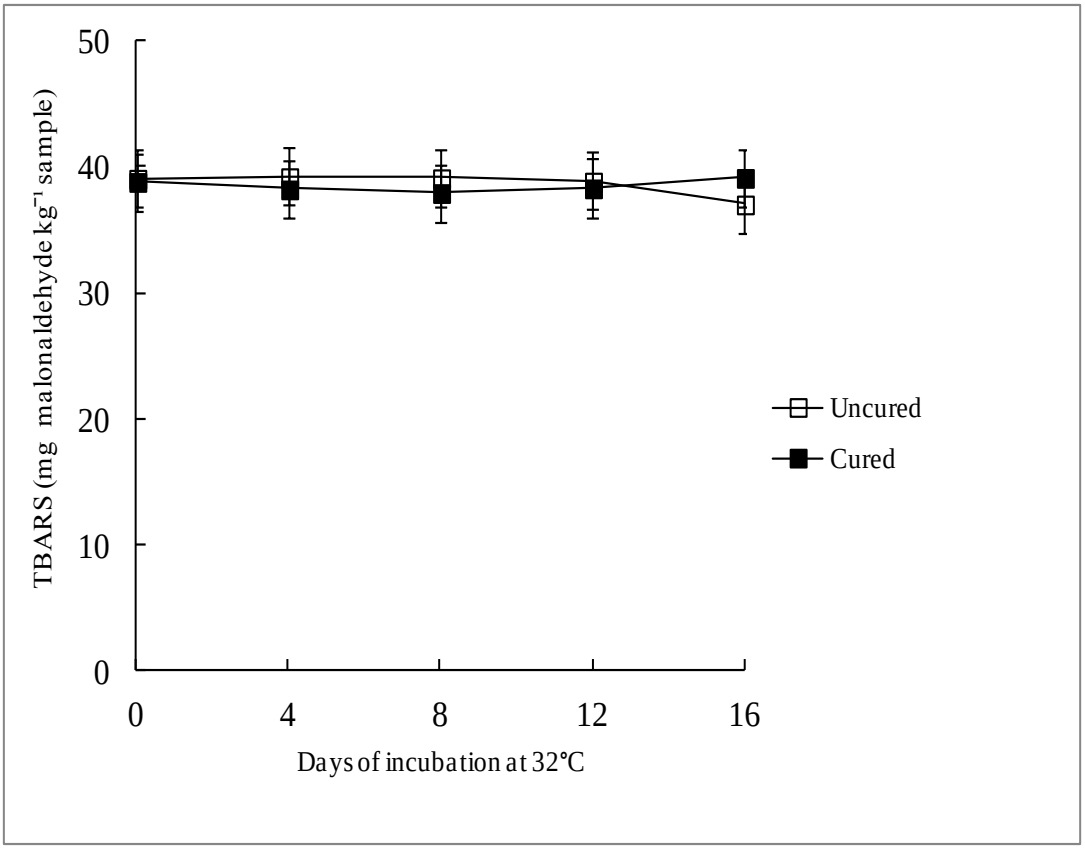

Figure 29. Fat oxidation over 16 days of uncured and cured FFM sausage produced with 20% taro. Data points are means and bars indicate standard deviation from three replicates

TBARS value is a widely used indicator for the assessment of degree of lipid oxidation. The highly unsaturated lipids in fat-rich fish are easily susceptible to oxidation. The greater the number of double bonds, the faster the oxidation reactions (Gurr, Harwood, & Frayn, 2002; Gurr, Harwood, & Frayn, 2008). These reactions produce alcohols, aldehydes and ketones which are necessary to develop desirable flavour (Manley & Mankoo 2005; Park, 2005), but when in excess are responsible for undesirable rancidity.

Riebroy, Benjakul, Visessanguan, & Tanaka (2007) reported fat oxidation in FFM sausage produced from six tropical marine species as having TBARS values from 10 to 40 mg kg^{-1}. Compared to commercial FFM sausage produced from tropical fresh water species with TBARS values from 5 to 14 mg kg^{-1} (Riebroy, Benjakul, Visessanguan, Kijrongrojana, &

Tanaka, 2004), FFM sausage produced from those six marine species thus showed more extensive oxidation, probably because tropical marine waters will be cooler on average than shallower tropical fresh waters, and likely to have a higher concentration of unsaturated fatty acids making up the muscle triacylglycerols (Riebroy, Benjakul, Visessanguan, Kijrongrojana, & Tanaka, 2004). Tuna is a red/pink-fleshed migratory deep marine species that roams into temperate zones. There are three reasons why tuna may be excessively prone to fat oxidation in FFM sausage. Because of tuna's cooler water habitat, its triacylglycerols fatty acid profile is thus likely to be dominated by unsaturated fats. Second, the pink/red colour is due to iron in myoglobin haem and it is well known that iron is a proxidant (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998) when released from haem as could happen in comminution and mixing. Third, the high concentration of haem iron means that tuna muscle is basically oxidative rather than glycolytic (as occurs in white muscle). Oxidative metabolism requires mitochondria, which is rich in phospholipids that are composed of unsaturated fats (Dillard & Tappel, 1971; Fletcher, Dillard, & Tappel, 1973). Thus the high TBARS values found here are not surprising.

A FFM sausage would never be produced commercially in a plastic syringe, but might be produced in an airtight plastic tube in the manner of pet food like dog roll (but with very different graphics). Thus although TBAR values were initially high, it appears they do not increase provided air is kept out. Moreover, the initially high value might be reduced by the inclusion of dedicated food-grade antioxidants like tertiary butyl hydroquinone or tocopherol, and/or spices which have well-known antioxidative properties (vanilla/vanillin). If the FFM sausage were to be produced in air-permeable packaging then added antioxidants would become very important, as would the nitrite cure as discussed in Chapter 1.

Fat oxidation is not the only problem that can occur with fish products, biogenic amines can also be a problem and this is examined in the next section.

Changes in biogenic amines in FFM during fermentation

The chromatogram of histamine, tyramine and tryptamine standards are shown in Figure 30. Histamine eluted at about 4.4 min, tyramine at 16 min and tryptamine at about 23.5 min at a flow rate of 0.5 ml min^{-1}. The proposed and tested internal standard, 1, 1-dimethylbiguanide hydrochloride, always coeluted with histamine irrespective of the many solvent variations tested. It was therefore not included in the definitive determinations. Histamine was the sole identified biogenic amine found in both uncured and cured FFM

sausage. Figure 31 does not show data beyond 15 min but very many trials never revealed peaks after that time to 30 min. The peaks other than histamine remain unidentified and at the moment are regarded as perchloric acid-soluble matter that absorbed at 217 nm.

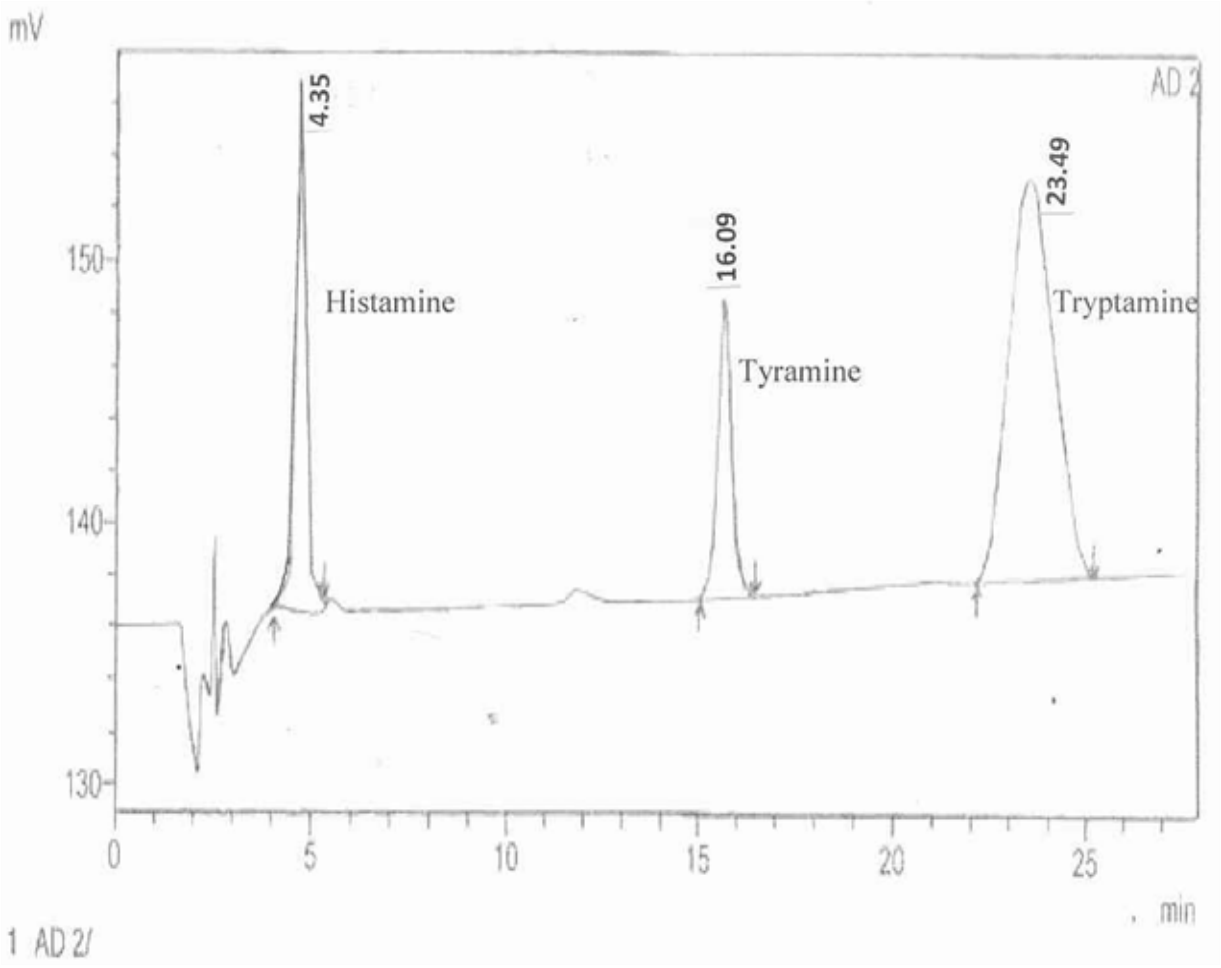

Figure 30. Chromatogram of biogenic amine standards. Identified amines from left to right are histamine, tyramine and tryptamin

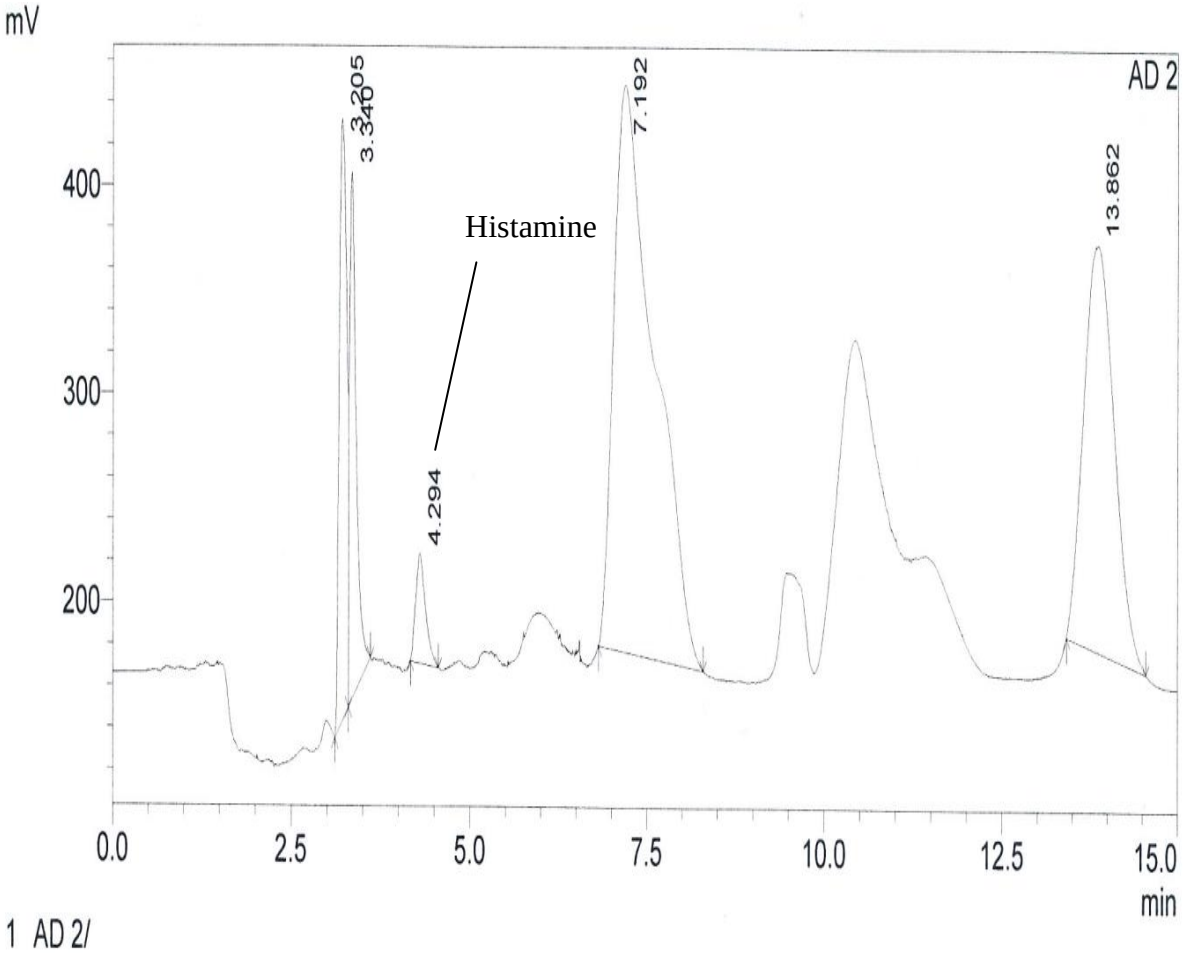

Figure 31. An example of a chromatogram of an FFM sausage sample made with 20% taro

Figure 32 shows that histamine concentration decreased. On Day 0, the uncured FFM sausage had a very similar concentration (98 mg kg^{-1}) to that of cured FFM (95 mg kg^{-1}). Both decreased marginally by Day 4, with a statistically significant ($p < 0.01$) but unimportant difference between cured and uncured.

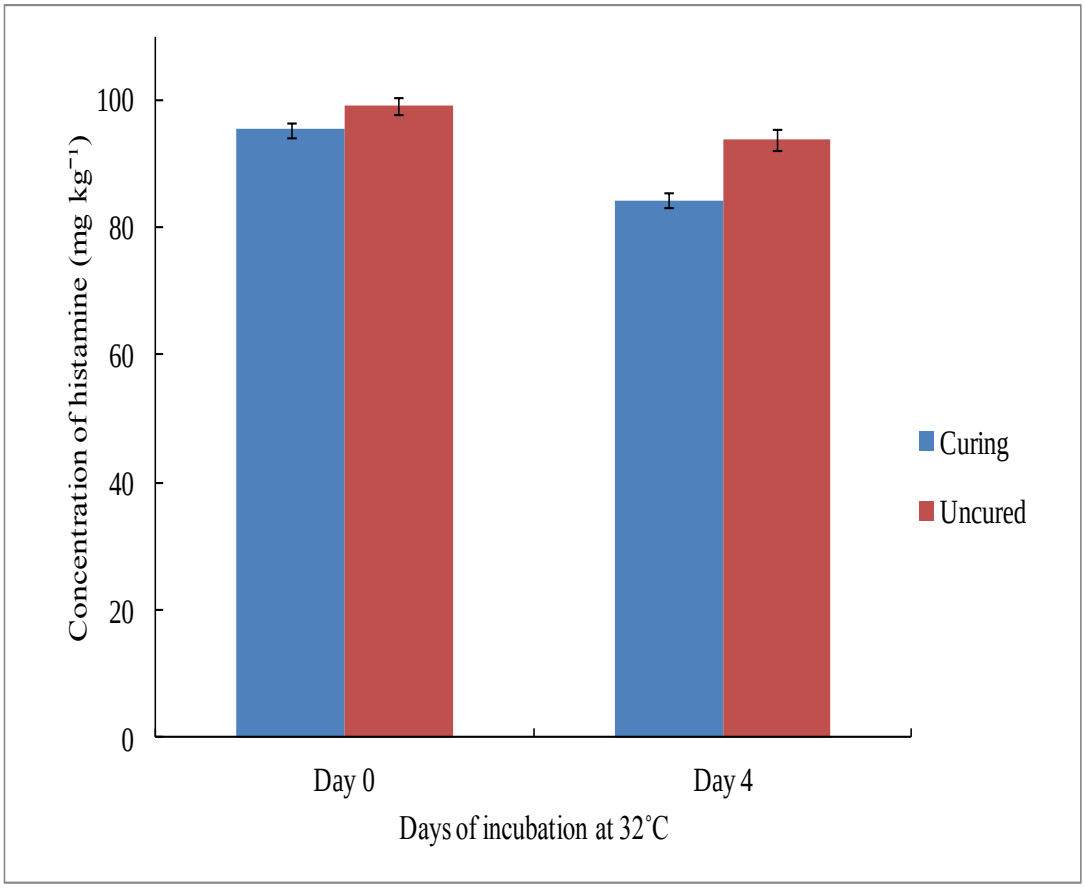

Figure 32. Histamine concentration in uncured and cured FFM sausage made with 20% taro. Data points are means and bars are standard deviation of 10 replicates per treatment

Biogenic amines are basic nitrogenous compounds formed mainly by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones (Askar & Treptow, 1986; Maijala & Eerola, 1993) and are synthesised by microbial metabolism (Brink, Damink, Joosten, & Veld, 1990). Biogenic amines are toxic in high concentrations. Although biogenic amines formations in foods are of concern, they also serve as indicators of food spoilage.

The differences in amine levels among different type of products and manufacturers can be due to the hygienic quality of raw materials, growth and activity of different kind of bacteria as well as to the different processing technologies used. The biogenic amine formation in fermented sausages has been often related to lactic acid bacteria (Bauer, Tschabrun, & Sick, 1989; Maijala & Eerola, 1993; Paulsen & Bauer, 1997). The same applies for FFM sausage in this current study.

Histamine contents of the FFM sausage in the current study varied between 84 to 98 mg kg^{-1}. According to Nout (1994), good manufacturing practice demands histamine contents in fermented sausage in the range of 50 to 100 mg kg^{-1}. The histamine content found in Turkish style sausage was between 6.72 and 362 mg kg^{-1} (Senoz, Isikli, & Coksoyler, 2000). Southeast Asia commercial FFM sausage has histamine, tryptamine and tyramine concentrations respectively from 55.1 to 291 mg kg^{-1}, 19 to 71 mg kg^{-1} and 19 to 225 mg kg^{-1} (Riebroy et al., 2004). The allowable maximum concentration of tyramine in foods is 800 mg kg^{-1} and above 1080 mg kg^{-1} tyramine is considered toxic (Shalaby, 1996). Food Standards Australia New Zealand (2006) has set upper limit of 200 mg kg^{-1} for histamine in fish. In the current study, both uncured and cured FFM sausages were below this limit at Day 4. However, the possibility of further histamine production beyond Day 4 remains an unexplored possibility.

Chapter 4

Overall Discussion

Plant growth and development are dependent upon the fixation of carbon dioxide and its reduction into carbohydrates. When sugars are produced in excess of the energy requirement of the plant for growth and development, they are converted into insoluble carbohydrates like starches and fructosans that are stored in tubers, which are underground stem. These carbohydrates are often called storage, or reserve carbohydrates that can be quickly broken down into sugars again if needed (Kozlowski, 1992). In this study, the tuber of taro was chosen as the source of carbohydrate in the development of FFM sausage. It generated enough glucose for the lactic acid fermentation (although not as much as kumara), and has a strong cultural link to the Pacific Islands including Tonga. Taro could confer a degree of geographical exclusivity in a newly formulated fermented tuna product.

Deriving from the fermentable sugar in taro, a purported lactic fermentation caused a decrease in pH values to between 4.5 to 3.9 over time for all the four cured treatments containing 0, 10, 20 and 30% taro, although where no taro was added, glucose served as the sugar source. The decrease in pH value was caused by an accumulation of organic acids, presumably lactic acid, from an anaerobic fermentation (Bloukas, Paneras, & Fournitzis, 1997). This suggests that the FFM sausages would safe to consume as almost no food pathogenic and spoilage bacteria can grow at pH values below 4.6 (Riebroy, Benjakul, Visessanguan, & Tanaka, 2005; Owen & Mendoza, 1985). However, excessive acidification as seen in the case of cured treatment with 20 and 30% taro concentration with pH values of 4 to 3.9 could adversely alter the aroma and flavour of the sausage. A study done by Buckenhüskes (1993), showed that dried fermented sausage with a pH lower than 4.5 contributed to extremely sour and tangy taste, and could lead to colour defects due to inhibition of the LAB. It must be noted that what applies to the dried fermented sausage may not apply to fish sausage. Its sensory attributes are unknown. Future sensory analysis is recommended to determine panelist preference of the products in terms of colour, taste and texture.

Addition of taro undoubtedly had a major, but complex effect on the objective textural properties. FFM sausage produced in this study became harder, stickier, with no change in springiness and brittleness with increasing concentration of taro. Hardness, springiness and

cohesiveness values in the present study showed comparable values to those produced by marine species in Southeast Asia by Riebroy, Benjakul, Visessanguan, & Tanaka (2005) and Riebroy, Benjakul, Visessanguan, & Tanaka (2007). In these models cooked rice was the carbohydrate source. Adhesiveness values were however, very different and higher than those for rice models. However, the difference may be related to the nature of the test surfaces of probe and base (not described by those authors). The differences could also be due to staling, more formally called retrogradation, of taro starch during fermentation. Retrogradation is very dependent of the proportions of amylose and amylopectin in the starches and again, information that is not available for the rice used. By extension, it is expected that texture will be affected by taro variety and the extent of cooking. Equally, different tuna species may have different textural properties when very fresh and when stored (Saisithi, Wongkhalaung, Boonyaratanakornkit, Yongmanitchai, Chimanage, & Maleehuan, 1986). Typically, fish muscle proteins undergo hydrolysis by endogenous proteolytic enzymes at a rate of ten times higher than those of mammalian muscle (Park, 2005), and this could possibly lead to softer textures. More importantly, fermentation was done at 32˚C in this study to simulate Tonga's high average ambient temperature so as to minimise the cost of temperature control. It is possible that the endogenous proteolytic enzymes may be very active at that temperature, and could lead to deterioration in gel structure through damage to collagen and myosin (Bremner & Hallett, 1985). This possibility suggests the need for further studies to examine the effect of other fermentation temperatures.

In this study, the colour of the FFM sausage was strongly influenced by the concentration of taro added and upon completion of fermentation yielded a brown FFM product. Studies done by Valyasevi and Rolle (2002), and Riebroy, Benjakul, Visessanguan, & Tanaka (2007) demonstrated that colour of a different range of FFM sausage from marine and fresh water species varied from white to brown. As of this stage, the consumer acceptability of the brown FFM sausage produced is unknown. Hence, further consumer sensory testing is recommended.

Further development of FFM sausage made from New Zealand marine species and taro

Sodium pyrophosphate and sodium nitrite were included in the sausage formulation described in this thesis (Table 1 and Table 2). Two of their roles that are particularly important will be discussed. Firstly, pyrophosphate ($P_2O_7^{4-}$) is a chemical analogue of adenosine triphosphate that can dissociate actomyosin into its component parts, actin and

myosin. This reduces viscosity because the rigor complex is broken, and because myosin by itself is more useful than actomyosin for gelation in sausage products (Knipe, 2004). This is important as the unfermented FFM mixture can be readily packed into casings. Secondly, phosphates bind to metal ions like Fe^{2+} that can otherwise catalyse fat oxidation. Nitrite on the other hand creates the characteristic cured red colour the consumers expect in a fermented sausage along with salt to induce inhibition of undesirable microbes. However, nitrite did not achieve its goal in this study as the FFM sausage after fermentation was brown. This may be due to the Maillard reaction between reducing sugars that probably remain in excess, and the ample amino groups from fish protein. If the concentration of residual glucose – as a representative fermentable sugar – were minimal at the end of fermentation, browning might be avoided. However, this possibility remains to be tested.

Spices and herbs have been added to meat foods for millenia (Chi & Wu, 2008). They are used in foods to impart flavour, pungency and colour. They also have antioxidant, antimicrobial, and nutritional properties leading to complex secondary effects such as salt and sugar reduction, improvement of texture and prevention of food spoilage ions (Aguirrezabal, Dominguez, & Zumalacarregui, 1998; Zaika & Kissinger, 1984). By analogy, in the Southeast Asian FFM sausage models, garlic is included as a basic ingredient. However, many other spices have been used in the production of fermented sausage at various concentrations depending on the sausage types (Chi & Wu, 2007). Spices that are commonly used in fermented meat sausages include pepper, chilli, paprika, garlic, mace, pimento and cardamom depending on culture of producing countries (Chi & Wu, 2007; Verluyten, Leroy, & Vuyst, 2004).

Lemon grass, pepper, galangal, garlic and ginger have all been tested as a means of reducing the fishy odour (Saisithi et al., 1986). Oregano contains butylhydroxyanisol (BHA) which is used in controlling oxidation of mackerel oil (Tsimidou, Papavergou and Boskou, 1995). Rosemary acts both a direct fat antioxidant and metal chelator (Yanishlieva, Marinova, & Pokorny, 2006). Sage is used in food flavouring and seasoning, and a combination with rosemary was found to be the best antioxidant activity among the numerous herbs and spices tested (Djarmati, Jankov, Schwirtlich, Djulinac, & Kjordjevic, 1991). Thyme on the other hand has been commonly used as culinary herb spices for adding flavour and eliminating unpleasant smell (Yanishlieva, Marinova, & Pokorný, 2006). It also possess

strong antioxidative effect which is associated with its high content of cavacrol and thymol (Schwarz, Ernst, & Ternes, 1996).

None of these herbs and spices are native to Tonga and vanilla bean is currently one of the biggest major exports of Tonga. Returning to the theme of geographical exclusivity, vanilla bean could be possibly included in the newly formulated FFM sausage. Vanilla is used traditionally as a sweet flavor in ice cream, yogurt, and other dairy products, confectionary, baking or beverages and many other food products. However, vanilla contains high amounts of phenolic compounds, which could function as antioxidant and anti-microbial agents. Vanilla bean addition may not only enhance flavour of the FFM product but could also reduce the high fat oxidation rate of the sausage. To sum up, further development of a commercial cured and fermented tuna meat sausage, using garlic, chilli and vanilla could be included to create a signature Tongan FFM sausage and be subjected to further chemical and sensory analysis.

Finally there is the matter of casings. Commercial FFM sold in Southeast Asia is normally packed with banana leaves or in small plastic bags. A FFM sausage would never be produced commercially in a plastic syringe as what was done in this study. However it may be an idea to pack the sausage in a substantially airtight plastic tube as it is anaerobically fermented. It is concluded from a study based on different packaging materials by Saisithi et al. (1986) that polypropylene casing was well suited with the current product requirement. This is because it is cheap compared to the other packaging materials and is convenient for printing which adds another benefit to the product presentation. In addition, they are mechanically strong, relatively heat resistant, and impermeable for smoke, gases and water vapour.

Food safety and sensory analysis

Each of the improvements listed in the previous section would require some research activity through comparison of various treatments. Many of these could be explored with the assays employed in the present study but sensory analysis must be done at the end of all this experimentation.

The first step will be to ensure the product is safe for consumers. Food products to be on sale must meet a certain standard by regulatory authority. In New Zealand, the body responsible for administering legislation covering food for sale is the New Zealand Food

Safety Authority (NZFSA). According to the draft guidelines for the production of uncooked comminuted fermented products, several pathogens need to be screened for as a verification procedure. These include coagulase positive staphylococci, *Escherichia coli* and *Salmonella* (New Zealand Food Safety Authority, 2008).

When it is clear that the products are free from pathogens and safe for consumption, consumer sensory testing should be conducted (Lawless & Heymann, 1998). This will be focused on liking of texture, colour and flavor. The intended consumer is much more likely to be a gourmet than a frequenter of fast foods. Thus, organising a consumer trial would involve more than just accessing students on the AUT campus. The comparisons that could be made may involve the current formulation for the basic FFM treatments against different formulation of increasing complexity and diversity in terms of spicing as mentioned above. From these techniques, a preferred formulation could be selected for a commercial launch.

Route to market

The proposed approach is to develop a market through delicatessen retailers who target gourmets, and through full service restaurants at the top end of the market. While New Zealand consumers have no experience of fermented fish products, novel foods and new gourmet items have a significant following particularly in some affluent market segments. This newly produced FFM sausage could probably be promoted in the upmarket New Zealand magazine *Cuisine*, a dedicated food appreciation magazine which has an audited monthly circulation of prestigious food and wines. At the time of writing the proposed route to market for my FFM product would be through restaurants and gourmet food outlets, avoiding supermarkets entirely in the early stages of market development. A key feature of promotion would be that the product was sourced from Tonga, using only ingredients and sourced in that country. Thus, there would be an appeal to 'geographical exclusivity', although realising that none of the ingredients is exclusive to Tonga.

Chapter 5

Conclusion

A fermented fish meat (FFM) sausage was produced from New Zealand albacore tuna (*Thunnus alalunga*) using taro as the carbohydrate source for fermentation. This was modelled after South East Asian equivalents, but here using ingredients characteristic of the South Pacific.

The fermentation was a success in terms of 20% taro formulation shows best texture, high rate of proteolysis reaction and lipid oxidation also histamine being the sole biogenic amine was lower than the maximum allowable limit. Despite of all these success, there were few remaining issues regarding the brown colour of the final FFM product, irrespective of nitrite curing along with pH values way too low by fermented meat standards.

Temperature plays a very important role in fermentation of fermented food products. Increasing the temperature will enhance the bacterial growth and therefore fermenting all the sugars in the FFM that causes the actual low pH values and brown colour of the FFM. It is suggested that the temperature should be lowered to around 10-12°C as in the traditional fermented sausages. In this case, pH will reaches 5 to 5.5 because it stops lactic acid bacteria from fermenting sugar. The remaining sugar will be utilized for flavor development and stronger colour. In addition, it is suggested that a different culture mixtures that does not hydrolyse gelatinized starch should be used other than *Pediococcus pentosaceus* and *Staphylococcus carnosus*. This might help improve the FFM colour.

The results from the current study point to commercial opportunities for production of FFM sausage from albacore tuna that has comparable properties with the commercial Southeast Asian FFM sausages. On the face of this, the model developed in this study is far from complete where further research could be carried out as suggested earlier to incorporate ingredients like garlic and vanilla to improve flavour and develop appropriate casings. Consumer sensory testing should also be conducted to identify the most preferred FFM sausage. In addition, a route to market through delicatessen retailers who target gourmets, and through full service restaurants at the top end of the market is recommended for this product. Ultimately, FFM sausage made from albacore tuna and taro could create an export opportunity for Tonga.

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