THE DESIGN OF A SHELF-STABLE SAUSAGE FOR THAILAND

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ABSTRACT

Sai Krok Prew is the naturally fermented sausage in Thailand made from pork, fat, cooked rice, sugar and spices. This sausage has a very short shelf life of three days, and inconsistent quality. Studies were carried out to find the basis for a process to increase the shelf life without using refrigeration, and to give a consistently high quality product. Simulated commercial conditions as found in Thailand were used in the laboratory, by mixing in a bowl chopper, mechanical stuffing, storage at 30-32°C, 75% R.H. for two days (factory conditions), and then further storage at 30-32°C, 97-99% R.H. (transport and retail sale).

Lactic acid and GDL were used to produce chemically acidified Sai Krok Prew. Lactic acid gave a completely unacceptable flavour and texture; GDL gave a satisfactory texture but a harsh flavour. There might be a good possibility of producing a low quality Sai Krok Prew by using GDL.

A satisfactory process for microbiologically fermented sausage was developed using starter culture, straight nitrite and sugar. The starter culture did not give a quicker rate of pH reduction than chance fermentation, as used commercially in Thailand, but suppressed the growth of other microorganisms.

An extreme vertices designs were used to study the effect of pork, fat and rice composition upon texture acceptability. Texture was improved with increased pork and fat and decreased with increased rice and an optimum composition was obtained. This would have to be related to cost before commercial production could be considered.

Sensory testing was used in an attempt to correlate the attributes of texture - firmness, rubberiness, juiciness, oiliness, smoothness, stickiness. Only rubberiness was correlated directly with fat content; a decrease in fat caused an increase in rubberiness. The other texture attributes appeared to have complex relationships with chemical composition. Generally, pork increased firmness and rice increased grittiness.

The shelf life was increased by dipping the sausage in 4% potassium sorbate solution for one minute and/or vacuum packaging. Over 14 days shelf life was obtained with potassium sorbate, and ten days with vacuum packaging.

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CHAPTER 1

INTRODUCTION

Fermented sausages can be classified as dried and semi-dried sausages. The meat mixture is allowed to ferment, either by naturally occurring bacteria, or by the addition of a culture of prepared bacteria. The major biochemical changes are due to the action of lactic acid producing bacteria, both homofermentative and heterofermentative lactic acid bacteria. The new approach is the use of chemical acidulant as a substitute or part substitute for the acid produced by lactic acid bacteria. Glucono-Delta-Lactone (GDL) is the most widespread chemical acidulant used in fermented sausage manufacturing. Straight lactic acid was also reported to be used as a chemical acidulant.

After fermentation or addition of chemical acidulant, the sausages are dried to the desired moisture content. Fermented and chemically acidulated sausages are normally consumed in an uncooked form.

Generally, there are three types of fermented sausages in Thailand. These sausages are named Nam, Sai Krok Prew and Mum. Nam is fermented from pork, pork skin (sliced), rice, salt, potassium nitrate and various seasonings. Nam can be classified as moist fermented sausage because a drying period is not required. The components when mixed together are wrapped with plastic film. Around this plastic film, the sausage is wrapped with many layers of banana leaves. The ripening period at room temperature (30-32°C) for Nam is usually about three to four days. The consumer can either consume it in the uncooked or cooked form. Sai Krok Prew is a semi-dry fermented sausage. It is fermented from pork, pork fat, rice, salt, sugar, potassium nitrate and various seasonings. These components are mixed together and stuffed into casings made from the small intestine of pigs and then sun-dried for two to three days. After this period, fermentation will have occurred, giving the desired level of sourness. The sausage is then packed in plastic bags ready for sale. The consumer

has to cook it either by frying or roasting before consumption.

Mum is a large size fermented sausage. It can be classified as a semi-dried fermented sausage. This sausage is formulated from lean beef, liver, spleen, roasted rice and various seasonings. These components are mixed together and stuffed into casings made from beef large intestines. Mum needs to be sun-dried on the first day after production, then air-dried for the rest of the period.

Fermented sausages in Thailand are mainly produced by small scale industries. The production is considered as an art that continues to be passed down from generation to generation. The uniformity of product quality is quite varied from day to day, and even from batch to batch.

Sai Krok Prew was selected for this project because it is the most popular and dominant product for Northeast Thailand. The potential of becoming a national product is high if a few attributes can be improved. The attributes that need to be improved are as follows:

(a) Uniformity of product quality:

The consistency of this sausage, batch to batch and day to day, is very variable. A procedure that can increase the product uniformity is required.

(b) Texture:

The texture of this sausage is poor. The sausage lacks firmness before being cooked. After being cooked, sausage meat crumbles when cut. Texture improvement for this sausage is required.

(c) Shelf life:

The shelf life of this sausage is only three days at room temperature (30-32°C). The cause of this short shelf life is mould growth on the casings. Refrigerated conditions can extend the shelf life. Nevertheless, few distributors have refrigerated storage trucks or display cabinets. The [illegible]

that can extend the shelf life when being stored or transported at ambient temperature. This attribute is considered to be the most important in restricting increased production and national sales.

Therefore, the aim of this project was to study the method that could increase the shelf life of Sai Krok Prew at ambient temperature. Within this aim, there were a number of objectives:

- To produce and evaluate Sai Krok Prew by chemical acidulants instead of bacterial fermentation;

  To do this, the most acceptable pH of Sai Krok Prew was determined. The levels of chemical acidulants (lactic acid solutions and GDL) required to reduce the sausage pH to the desired value were investigated. The uniformity of Sai Krok Prew quality when using GDL was investigated using the rate of pH reduction as a judgement criteria.

- To improve the texture of Sai Krok Prew;

  To study this, the proportions of pork, fat and rice in the formulation were varied, and an optimum formulation determined by sensory testing.

- To produce Sai Krok Prew by using starter cultures;

  To study this, commercial starter cultures were used in the optimum Sai Krok Prew formula. Nitrite levels and types of sugar were varied to study the effects on starter cultures. The uniformity of Sai Krok Prew quality when using bacterial fermentation was investigated in the same manner as Sai Krok Prew production using chemical acidulant.

- To extend the shelf life of Sai Krok Prew up to 14 days at room temperature (30-32°C);

In order to extend the shelf life of this sausage, the experiments were concentrated on the use of potassium sorbate. The minimum effective level of potassium sorbate that could extend the shelf life for both chemically acidified and microbially fermented sausages up to 14 days under the simulated commercial condition was investigated. Acceptability as judged by a taste panel was also evaluated for the sausage produced using potassium sorbate. Experiments with vacuum packing to increase the shelf life of fermented sausage were also carried out.

LITERATURE REVIEW

Acid fermentation in sausages have been studied extensively in U.S.A. and Europe but only a few studies have been done in Thailand. The American and European literature was therefore reviewed to find information that could be applied in a study of fermentation in Thai sausages.

Chemically acidified sausages have been produced mainly by adding Glucono-Delta-Lactone (GDL). The decrease in pH is due to the hydrolysis of GDL yielding gluconic acid. The addition of GDL also has an effect on the microorganisms, texture, flavour and growth of pathogens in the sausages.

Texture is also important in fermented sausage. Nevertheless, there was very little literature that reported on the texture of Thai fermented sausages. Therefore, the overseas literature was reviewed.

Generally, most types of fermented sausages have long shelf lives. But this is not true for Sai Krok Prew due to its traditional processing method and packing method. Similarly to the texture, there was very little literature that reported on the shelf life of Thai fermented sausages. Therefore, the overseas literature concerning fermented sausages' shelf life was reviewed.

2.1 MICROBIOLOGICAL ACIDIFICATION

The action of lactic acid bacteria on added sugars results in a slow production of acid in the sausage. This is a fermentation reaction involving the conversion of sugar to lactic acid, and a resultant reduction in the pH value of the mixture. There are two major methods in microbiological acidification:

2.1.1 FERMENTATION BY NATURALLY OCCURRING MICROORGANISMS

This process is dependent upon chance or random contamination with wild organisms. If the desirable organisms are present to the practical exclusion of the others, the flavour, odour, texture and aroma of the fermented sausage will be ideal. In an attempt to maintain desirable fermentation, the practice of "back slopping" is used extensively. In this case, part of a previously fermented batch is held at 38°C for 24 hours and then added to the meat batch as a starter (Daly, 1973). Nevertheless, there are many disadvantages in this traditional process:

(a) It is a long process, as generally the fermentation is a gradual process lasting over a three to seven day period. This requires not only an excessive amount of handling and labour, but a very appreciable investment in materials and extensive drying room areas.

(b) Because this process is dependent upon the chance or random contamination with wild organisms, the uniformity of the final product quality is unreliable.

(c) Back slopping can also promote the undesirable as well as the desirable organisms. Jensen (1942) stated that this process is never economical.

2.1.2 STARTER CULTURE PROCESS

In the early 1940s, Jensen and Paddock (1940) in their patent observed that several species of the genus Lactobacillus could be utilised as starters. The use of starters in the manufacturing of fermented sausages was introduced in the 1950s (Niinivaara,

1955; Niven *et al.*, 1958). A strain of *Micrococcus auranthiacus*, or other suitable nitrate-reducing micrococcal strains, was suggested for the European types of fermented sausages (Niinivaara, 1955). Diebel (1974) indicated that a starter culture for fermented sausage must possess a rather unique set of physiological characteristics. Among these are:

(a) It must be tolerant of salt and grow vigourously in the presence of at least 6.0% sodium chloride;

(b) It must grow in the presence of at least 100 ppm. nitrite;

(c) It must grow in the range of 27-43°C, preferably with an optimum around 32°C;

(d) It must be homofermentative. Gas production and fermentation products other than lactic acid are not desirable;

(e) It must not be proteolytic or lipolytic;

(f) It must not produce any compounds that are associated with off-flavours such as amines or sulfides;

(g) It must not be harmful to health.

2.1.2.1 LYOPHILIZED STARTER CULTURE

Pediococcus cerevisiae not only met all the requirements for a sausage starter, but it also survived lyophilization (Diebel *et al.*, 1961). Everson *et al.* (1970a) indicated that after refinements in classification

techniques *P. acidilactici* appeared to be the correct nomenclature instead of *P. cerevisiae*. The advantages of lyophilized starter are reducing the production time from around 150 hours to 32-40 hours with final pH and flavour showing only small variations from batch to batch. But at the same time lyophilized starter culture also possesses some disadvantages such as:

- The flavour of sausage soon after removal from the smokehouse is variably described as "harsh, chemical, sharp, unpleasant". However, after two to three days in the cooler, the flavour is found to be the typical tangy flavour of this type of sausage;

- During the rehydration period, the starter culture is not actively growing and yet conditions are ideal for wild contaminants to multiply and influence the ultimate results.

2.1.2.2 FROZEN STARTER CULTURE

Experience from lyophilized starter culture has shown that the rehydration period is the reason for much of the residual product variation. The same culture in a more suitable frozen form is being widely accepted because (Everson *et al.*, 1970a; Everson *et al.*, 1970b; Rothchild and Olsen, 1971).

- It can reduce the production time from around 150 hours to 10-15 hours;

- No rehydration period is required for the culture;

- The uniformity of odour, flavour, texture and pH is reported to be extremely good.

In order to expand the applicability and flexibility of starters over a wider range of sausages, the use of additional lactic acid starter was developed, including *Lactobacillus plantarum, L. plantarum* with *P. cerevisiae,* lactobacilli with micrococci (ratio 1:1), *Streptococcus lactis,* and *Streptococcus diacetilactis* - with *L. plantarum* in the ratio of 1:1 (Hill, 1972; Lactacel, D.S. and Lactacel, M.S.; Niskanen and Nurmi, 1976; Roberts and Smart, 1976).

2.2 NATURE OF THE BIOCHEMICAL ENVIRONMENT AND BIOCHEMICAL CHANGES DURING FERMENTATION

The major biochemical changes are due to the action of bacteria that produce lactic acid (LA). These bacteria utilize carbohydrates to form acids and thus decrease the pH. Most LA bacteria participating in sausage fermentation are homofermentative and ferment added glucose to produce lactic acid as the primary end product of metabolism. Heterofermentative LA bacteria such as Leuconostoc spp. produce lower amounts of LA as well as acetic acid, ethanol, and CO_2. Depending on the type of substrate, other organic acids and high molecular weight compounds (e.g. dextrans and polyalcohols) can also be synthesized by both homo- and heterofermentative LA bacteria (Buchanan and Gibbons, 1974; Bullerman *et al.,* 1969; Carr *et al.,* 1975; Tittsler *et al.,* 1952).

Tandler (1963) stated that the quantity of sugar needed depends on the type of sugar added, the curing substances used, and the ripening process followed. This may be due to the influence of these factors upon microbial activities. Andersen and Ten Cate (1965) reported that

approximately 1% sugar is needed to yield a reduction of approximately 1 pH unit. In methods using starter cultures, recommendations (Anon, 1969, 1972) are for the use of a minimum 0.75% sugar as glucose for proper starter activity.

Carbohydrates other than glucose, primarily sucrose and corn syrups or corn syrup solids, are suggested ingredients in some formulations where starter cultures may or may not be included (Komarik *et al.*, 1974; Kramlich *et al.*, 1973). There are studies which demonstrate that carbohydrates other than the 'simpler' mono- and disaccharides may serve as suitable fermentable substrates for bacterial production of acid in sausage (Tandler, 1963; Coretti and Tandler, 1965; Pyrez and Pezacki, 1974, 1975; Urbaniak and Pezacki, 1975; and Acton *et al.*, 1977).

2.3 NATURE OF THE MICROBIOLOGICAL ENVIRONMENT AND MICROBIAL CHANGES DURING FERMENTATION

Genigeorgis (1976) reported that the major microbial changes that occur during fermentation of sausage is the shifting from gram-negative, catalase-positive, highly aerobic flora to gram-positive, catalase-negative, and mostly microaerophilic flora. He found that the numbers of gram-negative bacteria declined rapidly during fermentation and may not be detected at all after fermentation has progressed for a few days. One of the early changes taking place within the sausage is the decrease in redox potential, which occurs because of muscle enzyme activity, the action of aerobic organisms, and the addition of reducing substances (e.g. ascorbates). Thus, the redox potential soon decreased to the point where aerobic organisms cannot grow, especially in the centre of the sausages, and this paves the way for the growth of microaerophilic LA bacteria. Also as a result of adding sodium chloride and the dehydration associated with

fermentation and aging the brine concentration gradually increases and the water activity (a_W) decreases. Both of these factors act as preferential selective agents for the growth of salt-tolerant LA bacteria over the natural gram-negative flora of fresh meat. Eventually, even the LA bacteria are inhibited by the lowering of a_W.

Tandler (1963) stated that acid production was more rapid in fermentation with monosaccharides as compared to disaccharides. When fermented, a dry starch syrup (undefined), dextrose, or sucrose, were found to yield equal amounts of acid in traditionally ripened dry sausage (Coretti and Tandler, 1965), whereas lactose yielded inferior product results. In testing the fermentative ability of 129 bacterial isolates from ripened sausage, Urbaniak and Pezacki (1975) reported that all isolates fermented dextrose, many fermented sucrose, and a few fermented maltose. Acton *et al.* (1977) found that for sausages containing 1% of carbohydrate, fermentations (24 hr at 38°C) with the starter *Pediococcus acidilactici* showed equivalent pH reduction and lactic acid yields for sucrose or glucose. Maltose yielded 78% of the acidity found for glucose, while lactose and dextrin yielded slight, insignificant levels of acid. When corn syrups were tested in the same manner, Acton *et al.* (1977) found that the amount of acid produced was dependent on the quantity of simpler carbohydrates, glucose and maltose, initially available in the corn syrup preparations, the yields of lactic acid were related to the available 'simple' substrates. Pyrez and Pezacki (1974) found that the peak period of lactic acid production in ripening was correlated with the molecular weight of the carbohydrate. As the molecular weight of the carbohydrate substrate increased, a longer period was required to attain adequate fermentation end products. Pyrez and Pezacki (1974) also stated that the tendency toward homofermentation by the lactic acid bacteria in dry sausage increased as the molecular weight of the carbohydrate increased. From an industrial viewpoint Acton *et al.* (1977) found that

sucrose can be substituted for dextrose and yield a desirable fermentation, when using *Pediococcus acidilactici*. Maltose might also be used in combination with dextrose and sucrose.

Lactic acid bacteria can produce H_2O_2 in certain culture mediums, but whether they do so during fermentation of sausage is questionable (Genigeorgis, 1976). If it is produced in sausage, it is produced by LA bacteria close to the periphery of the sausage, where there is sufficient oxygen. A number of antibiotics and antiobiotic-like substances can be produced by LA bacteria in certain culture mediums and foods, but whether they do so during meat fermentation is still unclear (Hurst, 1972; Hurst, 1973; Mossel, 1975; Niivaara, 1955).

During extended aging of sausages, proteolysis and lipolysis take place. The potential role of such changes on the safety of the products has not been investigated. Extensive proteolysis may shift the pH upward. Whether amines produced from proteins may react with residual nitrite to form nitrosamines during the shelf life of the products is not known.

Micrococci, naturally occuring or added to the sausage mix, can, in addition to their fermentative activities, reduce nitrate to nitrite, thus contributing to the development of odour and flavour as well as to antimicrobial activity (Ingram, 1974). Current trends to shorten fermentation time and the direct addition of $NaNO_2$ may limit the role of micrococci to the early part of the fermentation.

The ability of food poisoning organisms to survive, initiate growth, and (for some) to produce toxins depends on their ability to overcome the inhibitory environment created during fermentation. Important components of this environment are:

(a) Ingoing meat quality, types and numbers of competing organisms including pathogens;

(b) Additives, particularly sodium chloride, nitrite and their changes in concentration;

(c) Changing in chemical and physical conditions, e.g. a_W, pH, redox potential;

(d) External conditions, e.g. relative humidity, temperature;

(e) Casings.

The successful growth of fermenting and spoilage organisms will also be affected by many of the factors affecting pathogens. In addition, competing microorganisms may produce various stimulatory or inhibitory compounds. Inhibition of growth or toxin production of one microbial species can be mediated by another microbial species through the production of acids and lowering of pH, production of H_2O_2 or other inhibitory substances such as antibiotics, volatile compounds and lytic compounds, or through competition for essential nutrients (Daly *et al.*, 1973; Genigeorgis, 1974; Goepfert and Chung, 1970; Haines and Harmon, 1973a & b; Hurst, 1973; Mossel, 1975). On the other hand, stimulation of growth or toxigenesis of one microbial species can be due to the production of nutrients, shifting of pH and redox potential to the optimum and utilization of inhibitory compounds by another microbial species (Hurst, 1973; Seminiano and Frazier, 1966).

2.4 THAI FERMENTED SAUSAGES

There has been little published research work on Thai fermented sausages and what is available is in Thai language. Therefore, some details of these research works are reviewed in detail in this section.

Techapinyawat (1975) investigated the microorganisms during the fermentation of Nam sausage. She found that pork itself carried various types of normal flora, i.e. rods and cocci, both gram-positive and gram-negative. Added potassium nitrate was reduced to nitrite by naturally occuring nitrate-reducing micrococcal strains, thus contributing to the development of colour and flavour as well as to antimicrobial activity. Seasonings were added to improve or modify the flavour and in addition to flavour, seasonings could contribute somewhat to the preservation of the fermented sausage. On the other hand, some spices may carry excessively high bacterial loads that would shorten the shelf life of the sausage. Analyses by a taste panel showed that Nam was most acceptable at 3% salt level. At 2% salt the sausage became unacceptable very quickly and produced poor flavour and texture. At 5% salt, the salt taste was unacceptably high and acid level was too low, suggesting that the salt level inhibited acid production. Techapinyawat recommended from her work that the best consumption period was the fourth day after production. During the first four days, rapid decrease in pH value and increase in acid quantity were found, and this changed only slightly after the fourth day. The decrease in pH value was found to be quicker in formulae that contained less salt than those with high salt (see Appendix 1), but there was not much difference in pH and acid content between high and low salt formulae four days after production. In all cases pH was less than 4.5 and lactic acid content approximately 0.5%. The maximum acid content was obtained on the 35th day at room temperature after production, when 1.25% and 1.44% lactic acid was recorded. Despite the acid production, the pH changed only slightly and never went down below 4.0. This was attributed to the buffer capacity of pork. She also found that there was a wide range of microorganisms

during the initial period after production, but after 24 hours, differential growth rate reduced the numbers of some types of microorganisms. During the 24-72 hour period heterofermentative lactobacilli and homofermentative cocci (Pediococcus spp. including *Pediococcus cerevisiae*) proliferated resulting in a rapid rate of acid production. After 72 hours, homofermentative lactobacilli *(Lactobacillus plantarum)* predominated with some Pediococcus and heterofermentative lactobacilli still growing. The number of bacteria present in the sausage three days after production as determined using MRS agar was $8.08 \times 10^8/g$ of sausage. She considered that on the fourth day with the pH lower than 4.5 and the acid content greater than 0.5% most non-acid producing microorganisms were destroyed, including coliform bacteria, but this situation was suitable for *L. plantarum* to proliferate and resulted in further acid production. *Lactobacillus brevis* (Heterofermentative rod) was also found in large numbers along with *L. plantarum* but growing at a slower rate (other Lactobacilli types were also found). The microorganisms which proliferated during the initial stages, including coliform bacteria, decreased because they could not tolerate this high acid and low pH condition. Seven days after production cell counts on both MRS agar and standard plate count agar decreased drastically and the predominant species were *L. plantarum* and *L. brevis*. Yeasts *(Candida sp.)* were found in Nam, presumably due to the proliferation of this yeast when most of the carbohydrates had been utilised and because of their tolerance of high acid low pH condition. When this type of sausage was kept at room temperature longer than one week, it was found that the sourness increased and chewiness decreased. She recommended that four to five days after production, refrigeration was required to increase the shelf life of this sausage. The addition of 10% starter, from the previous batch five days old (back slopping), shortened the fermentation period to 24-36 hours instead of four days,

and reduced the chance of spoilage.

Rojanasaroj *et al.* (1980) investigated the relationship between the bacterial count and pH in Nam bought from the market. They found that the initial pH was 6.12 and the highest bacterial count on standard plate count agar was found to be 2.70×10^7 cells/g sausage on day one after production. On receipt, the samples were incubated at various temperatures. They found that the most acceptable taste was on the third to fourth day after production, at which time the pH was between 4.45 - 4.72. At each incubation temperature, the most desired taste was associated with the highest viable count. The viable count gradually increased up to the third day and then decreased to reach a minimum on the sixth day after production. They reported that on day seven the viable count increased and might be due to other types of bacteria that could tolerate high acid condition. Details of Rojanasaroj *et al.* is shown in Appendix 2.

Rongvisit (1981) studied Sai Krok Prew. The effects of temperature, rice, salt and sugar contents on the alteration of pH and lactic acid content and some important details are summarised as follows:

(a) The effect of rice:

Rice at 25% level was recommended due to the low cost and high sensory score. When the sausage was fermented at room temperature (30-32°C) the pH of the formula that contained the high level of rice dropped more rapidly during the initial hours after production than the formula that contained a low level of rice. But during the following period the rates of pH reduction were about the same.

(b) Effect of salt:

With salt concentration up to 2% there was no apparent effect on pH change, but at 2.5% salt the rate of pH reduction decreased. The highest sensory score was obtained at 2% salt.

(c) The effect of sugar (sucrose):

At 0.5 and 1.0% sugar the rate of pH reduction was similar. The addition of sugar at levels greater than 0.5% did not increase the acid concentration. Only the 0.5% sugar level was accepted by the panelists.

(d) The effect of incubation temperature:

It was shown clearly that the incubation temperature was the most important factor in the development of acid in fermented sausage. At room temperature (30-32°C) the most desirable sourness level was obtained in 24 hours, i.e. pH range 4.3 - 4.6 (1.3 - 0.9% lactic acid).

(e) Formulation:

The formula that yielded the best flavour was reported as being:

Pork	50.00%
Pork fat	15.00%
Pork skin	5.00%
Cooked rice	25.00%
Peeled garlic	2.50%
Coriander	0.10%
Salt (NaCl)	2.00%
Sugar (Sucrose)	0.50%
Pepper	0.25%
Prague powder	0.05%
($NaNO_3$: $NaNO_2$ = 100 : 1)	

Rongvisit (1980) recommended that the fat level may be increased to improve the texture. The spices may vary, but to obtain the best flavour salt at 2.0% and sugar at 0.5% must be maintained.

2.5 CHEMICAL ACIDIFICATION

The important chemical acidulant that has been used in fermented sausage is Glucono-Delta-Lactone (GDL). GDL has been considered as a suitable agent which lowers the pH value of dry sausage (Sair, 1961, 1963, 1964, 1965; Meester, 1964, 1965). The decrease in pH is due to the hydrolysis of GDL yielding gluconic acid. Approximately 25% of the lactone hydrolyzes in the first ten minutes, and the remainder hydrolyzes more slowly over a three hour period (Sair, 1961). The current practice in many European dry and semi-dry sausage operations is the use of GDL to lower the pH (Schum, 1974). The USDA permits 1% GDL in these products and this usage level will reduce the pH level about 0.5 pH units, which is not low enough to achieve room temperature stability (Acton and Dick, 1977). The product must undergo a subsequent fermentation or be handled under refrigeration (Everson, 1981). A different figure was reported by Klettner and Baumgartner (1980) who reported that 0.5% GDL in the formula will reduce pH by about 0.6 unit. Lactic acid, citric acid and acetic acid have also been used alone or in combination.

2.5.1 THE EFFECT OF GDL ON THE MICROORGANISMS IN FERMENTED SAUSAGE

Both Sair (1963) and Grau (1965) concluded that GDL addition had no effect on the normal bacterial growth in the sausage, but Coretti (1966), Nurmi (1966b) and Genigeorgis (1976) found that GDL had a definite effect on the microbial flora of dry sausage.

Nurmi (1966b) showed that GDL makes conditions favourable for the predominance of Lactobacilli and in addition produced favourable conditions for the rapid development of yeasts.

The addition of lactic acid had a similar effect to that of GDL on the microbial flora.

GDL also has the ability to alter the behaviour of pathogens and their ability to proliferate during fermentation. The effect of chemical acidulation on pathogens was reported by Barber and Diebel (1972), Nderu and Genigeorgis (1977) and Genigeorgis *et al.* (1971).

2.5.2 THE EFFECT OF GDL ON THE TEXTURE OF FERMENTED SAUSAGES

According to Meester (1964, 1965) and Coretti (1966), sausages prepared with a GDL addition are ready for slicing three days after preparation. Nurmi (1966b) found that GDL accelerated the development of the desired consistency but almost similar results were achieved with the addition of lactobacilli together with micrococci. Skulberg (1966) made experiments with the addition of both GDL and lactobacilli. He found that the addition of GDL considerably accelerated the development of the desired consistency but, on the other hand, it did not speed up the ripening process of dry sausages.

2.5.3 THE EFFECT OF GDL ON THE FLAVOUR OF FERMENTED SAUSAGE

Nurmi (1966b) found that a disadvantage in the use of GDL was the occurrence of a peculiar sweetish flavour not characteristic of dry sausage. Everson (1981) reported that the flavour achieved is not a characteristic lactic acid flavour or tang but, apparently, this has come to be accepted

in Europe. Diebel (1974) reported that although some European and American manufacturers have utilized this acidulation procedure, the claim has been made that it is conducive to the production of rancidity as the product ages.

Coretti (1966) found an increase in the peroxide number as compared with the control sausages without GDL. He demonstrated in numerous studies that too great an increase in lactobacilli was the main cause of odour and flavour defects in dry sausage. When a commercial micrococci preparation (Baktofermente) was used together with GDL, the peroxide number was lower (Nurmi, 1966c).

2.6 TEXTURE OF FERMENTED SAUSAGES

Fermented sausages in most cases have a chewy texture (Kramlich, 1971). The use of starter culture can improve the uniformity of texture when materials and process conditions are controlled carefully (Everson *et al.*, 1970a).

At the commencement of the process, the development of firmness is directly influenced by acid development and then later in the process by drying. The direct influence of acid development on firmness is manifest in the region of pH 5.3, approaching the gel point of salted meat protein. The proteins form a gel, with a consequent rapid increase in firmness (Ten Cate, 1960; Klement *et al.*, 1974). Subsequent development of firmness is related to the dehydration of the sausage. Therefore, the influence of the ripening room atmosphere is critical on what will happen to the sausage texture. The relative humidity must be controlled so that the sausage will dry at a controlled rate to prevent case hardening and yet yield a firm dry product (Klettner and Baumgartner, 1980).

Zaika (1978) reported that a certain amount of sodium chloride is necessary for the production of sausage having the proper texture. Sausages containing 2-4% sodium chloride and starter culture had a good firm texture.

In Sai Krok Prew, Rongvisit (1980) proposed the recommended formula (see Section 2.4) that yielded the best sensory testing. She also suggested that her recommended formula could be modified for better texture attributes. Techapinyawat (1975) reported that the use of 10% starter from the previous five day old batch (back slopping) had an adverse effect on the texture of Nam. This back slopping process caused less chewiness (which was undesirable) and she discussed that this might be due to the rapid drop in pH.

Everson (1981) reported that early in the study of the direct acid addition method, the addition of L-lactic acid was tried and the textures obtained were poor and clearly inferior to those obtained with fermentation. Combinations of acetic, lactic, citric and other organic acids were not noticeably successful. In every case, the texture of the sausage was destroyed. The current practice to use GDL to lower the pH gives a better texture because the proper use of GDL permits the gluconic acid to be formed after the sausage has set up and therefore early exuding of liquid from the sausage and loss of structural properties is avoided.

2.7 SHELF LIFE OF FERMENTED SAUSAGE

Fermented sausages have traditionally demonstrated an extended shelf life through a combination of reduced moisture content and pH which control many microorganisms in the sausage. However, surface moulds do grow and for some sausages this is considered desirable as it is thought to provide unique flavour.

2.7.1 EFFECT OF pH AND A_W ON SHELF LIFE OF FERMENTED SAUSAGE

In most instances, the shelf life of a meat product is related to the combined effect of a_W, pH, redox potential, nitrite and the competitive flora (Leistner, 1974). Nevertheless, it is feasible to predict the shelf life of the products using only the a_W, pH and temperature (Leistner and Rödel, 1975). Rödel (1975) studies the a_W of representative German meat products, and their usual shelf life, and related this data to the a_W, pH and temperature requirements reported in the literature for food poisoning and spoilage organisms associated with meats. This study derived a concept for grouping meat products into three categories based on the a_W and pH of the product. Every category demands an appropriate storage temperature. This concept covers bacteria which cause spoilage, as well as food poisoning, but not yeasts or moulds, which usually grow more slowly than bacteria. Table 1 indicates that, according to the concept of Rödel (1975) "easily perishable" meat products have a pH about 5.2 and an a_W above 0.95, and must be stored at or below +5°C. The expected bacteriological shelf life at +5°C is about two weeks, but this is prolonged at lower temperatures. The "perishable" meat products have either a pH of 5.2 - 5.0 (inclusive) or an a_W of 0.95 - 0.91 (inclusive) and must be stored at or below +10°C; the bacteriological shelf life at +10°C lasts about four weeks, but longer at lower temperatures. The "shelf-stable" meat products have a pH at or below 5.2 and an a_W at or below 0.95, or a pH below 5.0 or an a_W below 0.91; these products need no refrigeration and their shelf life is often not limited by bacterial but by chemical or physical spoilage, especially rancidity or discolouration.

* TABLE 1: STORAGE CATEGORIES OF MEAT PRODUCTS BASED ON THE A_W AND pH OF THE PRODUCT, WITH CORRESPONDING STORAGE TEMPERATURES

Category	Criteria	Temperature
Easily perishable	pH 5.2 and a_W 0.95	+ 5°C
Perishable	pH 5.2 - 5.0 (inclusive) or a_W 0.95 - 0.91 (inclusive)	+ 10°C
Shelf-stable	pH 5.2 and a_W 0.95 or only pH 5.0 or only a_W 0.91	No refrigeration required

* From Leistner and Rödel, 1975.

According to this concept, Sai Krok Prew, which has pH lower than 5.0, can be classified as a shelf-stable product. But as mentioned before, this concept covers only spoilage and food poisoning bacteria but not yeasts or moulds. Rongvisit (1980) reported that the shelf life of Sai Krok Prew at room temperature (30-32°C) was only three days, terminated by being spoiled by surface moulds. The shelf life of Sai Krok Prew could be extended up to 14 days by keeping at 4°C. If the casing was soaked in chlorine solution before use and the sausage was kept at 4°C the shelf life could be extended up to 21 days. Techapinyawat (1975) briefly mentioned the extension of Nam's shelf life. She recommended that four to five days after production, refrigeration was required to extend the shelf life of this sausage.

2.7.2 SURFACE MOULDS

Surface mould growth is considered as an undesirable attribute for Thai fermented sausages but, mould growth is encouraged in certain aged fermented sausage, e.g. Italian and Hungarian style salamis. Usually these moulds are penicillium spp. growing on the surface and not producing any toxin principle. Ayres *et al*. (1967) found that the moulds, mainly Penicillium species plus a strain each of Aspergillus and Saccharomyces, were typical of the types found on the surface of dry-cured salami. Undesirable Aspergillus spp. may take over depending on the relative humidity and temperature during aging (Genigeorgis, 1976). Takács (1964) found that *Aspergillus glaucau* group were the most frequent cause of microbial spoilage of biltong. Holley (1981) reported that although sausages evenly covered with dense white mycelium are considered desirable by some groups, the uncontrolled mould growth seen on most dry cured meat products is usually pigmented green, gray or yellow. Pederson (1979) stated that moisture on the surface of the sausage will permit growth of bacteria, yeasts and moulds. Holley also reported that mould growth on cured meat products can take place not only during curing but also during wholesale distribution and in the distribution chain.

At each stage in the distribution chain, pigmented mould can be removed by washing to improve product appearance (Holley, 1981; Pederson, 1979), and the product becomes more costly. But the danger inherent in the practice of mould removal by washing is that the procedure does nothing to remove toxic and allergenic mould metabolites, which may have diffused into the meat as a result of the original mould growth.

The retardation or inhibition of surface mould growth may be carried out by reduction of surface water activity, use of vacuum packaging or by chemical preservatives.

2.7.2.1 REDUCTION OF SURFACE WATER ACTIVITY

The moisture on the surface will permit growth of bacteria, yeasts and moulds. In Italian dry salami, which has a water activity ranging between 0.671 to 0.920, a moisture content of 24.3% to 43.4%, and a pH of approximately 4.3 to 7.1 (Genigeorgis, 1976; Lee *et al.*, 1977; Pullen and Genigeorgis, 1977; Terrell *et al.*, 1978; Vicini and Raeznski, 1977) under conditions of relative humidity (R.H.) greater than 70%, mould will readily form on the salami surface (Holley, 1981). The concept of a_W was applied to the development of new types of meat products, known as intermediate moisture meats (Leistner and Rödel, 1974). Many intermediate moisture foods fall into the 0.80 to 0.85 a_W category and still subject to mould spoilage (Jackson, 1975). Jackson (1975) also stated that ready-to-eat products rarely have a_W less than 0.80. The undesirable Aspergillus mould has its minimal a_W for multiplication associated with meat and meat products at 0.65 (Leistner and Rödel, 1975). Thus, the attempt to prevent mould growth by solely decreasing water activity can adversely affect the eating quality of fermented sausage.

2.7.2.2 USE OF VACUUM PACKAGING

Vacuum packaging of the fully mature cured product

is also used. Ingram and Simonsen (1980) reported that if salamis are vacuum-packaged, surface growth of moulds present before packaging will be inhibited or restricted.

2.7.2.3 CHEMICAL PRESERVATIVES, ANTIBIOTICS

Both potassum sorbate (Baldock *et al.*, 1979; Kemp *et al.*, 1979; Leistner *et al.*, 1975; Wallhäusser and Lück, 1978), and pimaricin, also known as natamycin (Cattaneo *et al.*, 1978; Hechelman and Leistner, 1969; Moerman, 1972) have been found effective as antifungal treatments on cured meat under specific conditions.

Holley (1981) found that dipping the salamis in 2,000 ppm pimaricin for 30 seconds was ineffective in preventing surface mould growth. Pimaricin spray 2,000 ppm was found to be effective but mould growth was replaced by a dusting of yeast colonies that covered the surface to a degree that varied directly with the length of ripening. Moerman (1972) also found that the immersion treatment with pimaricin was not very satisfactory unless salami were stuffed in natural casings. But success in using pimaricin to prevent mould growth on salami was also reported by Cattaneo *et al.* (1978), Hechelman and Leistner (1969), and Moerman (1972).

Sorbate has been shown to inhibit growth of yeasts, moulds and many bacteria. Its activity against bacteria, however, is not as comprehensive as that against yeasts and moulds. The inhibitory effects of sorbic acid

have been reported by numerous researchers including Emard and Vaughn (1952), Ferguson and Powrie (1957), Geminder (1959), Pederson *et al.* (1961) and Huang and Armstrong (1970). In meat products at the present time, the only approved use of sorbate in meats in the United States is that of dipping the casings of stuffed dry sausages in a 2.5% potassium sorbate solution to prevent mould growth on the surface of the product during the drying period.

Holley (1981) found that 2.5% potassium sorbate solution was ineffective and in fact higher viable numbers of fungi were present on 2.5% potassium sorbate-treated than on control casings. Holley (1981) speculated that as sorbate penetrated from the surface, sorbate was diluted to a certain degree depending on the salami diameter which caused a loss of inhibitory ability at the surface. Holley also found that at 10% sorbate there was significant visual mould inhibition and fewer viable fungi. Treatment of salamis with 20% potassium sorbate solution caused the most complete inhibition of surface mould growth. Leistner *et al.* (1975) reported that light smoke during the ripening phase and the dipping of the sausages into a 10 to 20% potassium sorbate solution before storage or shipping proved effective. The acceptable daily intake of sorbic acid in Germany amounts to 25 mg /kg body weight. The residual level of fermented sausage, which has been dipped into a 20% potassium sorbate solution is minimal, about 3% of the acceptable daily intake of sorbic

acid, and thus not harmful. Casings that were treated with acetic acid 2% (vol /vol) for three minutes before being treated with potassium sorbate had improved surface mould growth prevention. Holley (1981) found that the best combination was 20% sorbate, with an acetic acid pretreatment.

Sofos and Busta (1980) reported that sorbate was classified as relatively non-toxic. It was reported that sorbate can be metabolized by the organism in a way similar to the naturally occurring fatty acids.

2.8 DISCUSSION AND CONCLUSION

Pure starter culture has been shown to become a necessary factor in achieving a consistent quality in fermented sausages and avoiding the presence of significant numbers of pathogens. Frozen starter culture seems to be superior to lyophilized starter culture because of its quicker initial response. Nevertheless, when the availability and storage facilities required for frozen starter culture are considered, the superiority of frozen starter over lyophilized cultures may be questionable for sausage production in Thailand.

Sausage fermentation, especially ingredients such as the fermentable sugar and salt, have important effects on fermentation. The amount and type of fermentable sugars influence the fermentation and final product quality.

There is no report of using either starter culture or chemical acidulants in Thai fermented sausages. The products are only produced by natural fermentation. Therefore, the application of pure starter culture and chemical acidulant with Sai Krok Prew should be investigated. From the present knowledge of the control of curing, straight nitrite should replace the use of nitrate and nitrate/nitrite

mixtures in the traditional formulation. Rongvisit's work shows that the texture of Sai Krok Prew could be improved. The present use in some countries of sorbate to inhibit surface mould growth leads to the possibility of applying this to Sai Krok Prew.

CHAPTER 3

MATERIALS AND METHODS

This chapter describes the materials and methods used for processing the Sai Krok Prew. The basic formula, mixing and stuffing, laboratory incubation and the cooking method are described. The chemical, sensory and microbiological analyses used in testing the sausages are also described.

3.1 METHOD OF PROCESSING SAI KROK PREW

The method used was developed from the work of Rongvisit (1980) on Sai Krok Prew. The basic formula recommended by Rongvisit has been modified and a bowl chopper was used instead of a mincer.

3.1.1 RAW MATERIALS

3.1.1.1 MEAT AND FAT

After slaughtering and overnight chilling of the pig carcasses, 60 kg of lean post-rigor meat from several carcasses of the same day's production was selected for the experiments. The lean pork was held overnight at 2-3°C and then coarsely ground through a 20 mm plate and mixed thoroughly for three-four minutes. Samples of 750 g of ground pork were placed in P.V.D.C. bags. Air in the bags was evacuated before closing them with a cryovac clip. The bags were then immersed in 90°C water for one-two seconds, which caused them to shrink into close contact with the meat. This procedure was carried out at the Kiwi Bacon Co Ltd,

Longburn, and the resulting meat samples were transferred to Massey University and kept in refrigerated storage (-20°C). After the meat samples were completely frozen, they were taken out of the freezer, placed in polystyrene boxes, sealed with adhesive tape and returned to the freezer.

Pork backfat was used in this study. It was prepared using the same procedures as for the pork meat, except that each sample contained only 300 g of backfat.

3.1.1.2 FILLER

Australian long grain rice was cooked for 20 mins. in water at the ratio of 1:1.5 (wt/wt , rice/water), using a steam jacketed kettle. Cooked rice was allowed to cool down by leaving it at room temperature overnight. It was then packed in plastic bags in 375 g lots. The pouches were evacuated and heat sealed, and stored in the freezer (-20°C). After the rice was completely frozen, the rice packets were taken out of the freezer and put in polystyrene boxes. These boxes were sealed with adhesive tape and returned to the freezer.

3.1.1.3 SEASONINGS

Garlic:

In order to get fresh good quality garlic, only small amounts were bought at a time from retail sellers.

Pepper and coriander:

Ground black pepper and coriander was bought in a bulk lot from wholesalers and weighed amounts from the same batch used for all experiments.

3.1.1.4 SUGAR

Sucrose and pure dextrose.

3.1.1.5 CURE

Iodized salt and food grade sodium nitrite.

3.1.1.6 CHEMICAL ACIDULANTS

Lactic acid solutions.

Two concentrations of 1.0 and 2.0 Normal lactic acid solutions were prepared by diluting concentrated lactic acid (AR) with distilled water. The concentration of lactic acid solutions was checked by titrating against standard solution of one normal NaOH using phenolphthalein as an indicator.

Food grade Glucono-Delta-Lactone (GDL).

3.1.1.7 STARTER CULTURE

Lactacel MC (Microlife Technics, Sarasota, Florida) supplied by Smith-Biolab Ltd, Auckland, was used for the studies. The starter culture was the frozen starter cultures of *Pediococcus cerevisiae* and *Pediococcus plantarum* in 4 oz cans.

3.1.1.8 PRESERVATIVE

Food grade potassium sorbate - the concentrations of 1, 2, 3, 4, 5, 10 and 15% (wt/wt) were prepared by dissolving the potassium sorbate powder in distilled water.

3.1.1.9 SAUSAGE CASINGS

Commercial salted hog casings prepared from the small intestine of pigs - the casings were first thoroughly washed with tap water to remove salt and then soaked in water for two hours before being used.

3.1.2 SAI KROK PREW PREPARATION

Rongvisit (1980) originally recommended a formula which was slightly modified to suit this study and named "Basic Formula". The components of the Basic Formula are as shown in Table 2:

Table 2: Basic formula for Sai Krok Prew

Ingredients	Quantity (%)
Pork	50.00
Fat	20.00
Cooked rice	25.00
Coriander	0.10
Salt (NaCl)	2.00
Sugar (Sucrose)	0.50
Pepper	0.25
Sodium nitrite	0.05
Peeled garlic	2.50

3.1.2.1 MIXING AND SIZE REDUCTION TECHNIQUE

A mixing technique had to be developed to ensure a proper distribution of sausage ingredients and the desired particle size. A proper distribution of sausage ingredients is important because of their influence upon the rate of fermentation which leads to the uniform product and particle size and influences the texture of a sausage.

The mixing procedure was as follows:

- Pork, fat and rice (see 3.1.1.1 and 3.1.1.2) were thawed overnight at room temperature and were allowed to attain a temperature of 15°C before being mixed.

- The proportion of the sausage ingredients in each batch which weighed 1,500 g is shown in Table 2.

- Pork, fat and rice were first mixed for 30 seconds in the bowl chopper (Scharfen Cutter 60302) 45 cm in diameter with two cutting blades and rotated 16 rpm (unloaded speed).

- The other ingredients were then added and mixing continued for another 30 seconds.

This study used two criteria to justify the effectiveness of the mixing technique:

(a) acid distribution, and

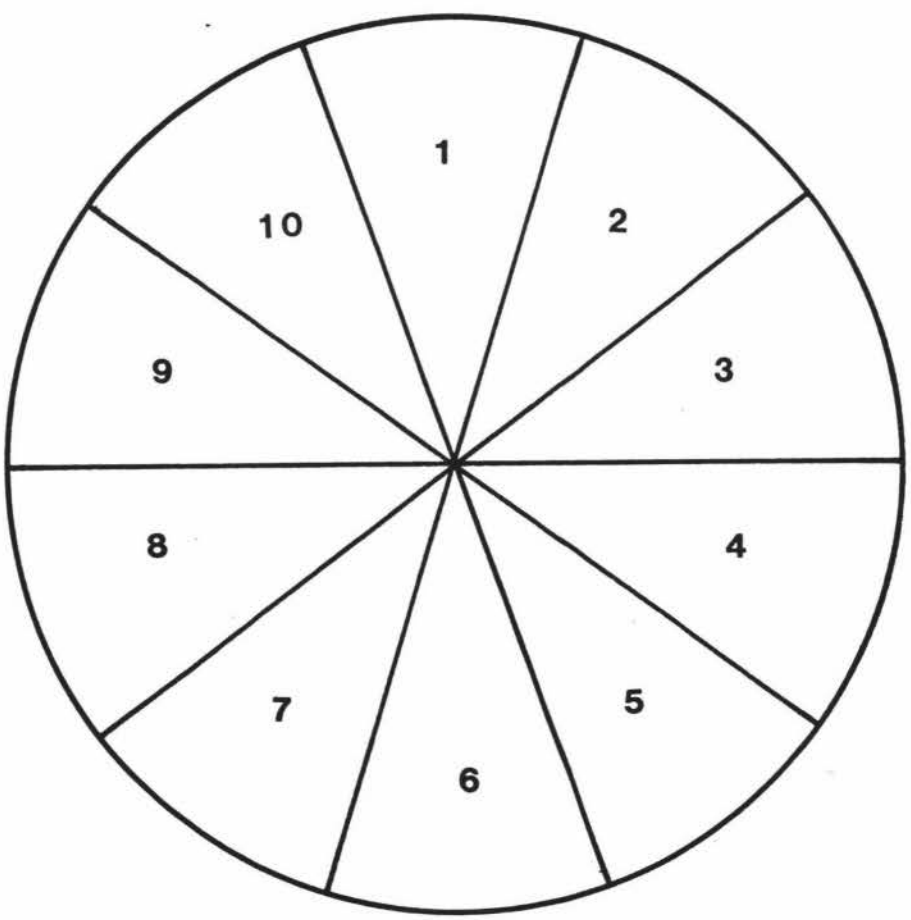
(b) salt distribution.

The procedure used to determine the best mixing technique was as follows:

- 10 ml of 1 N Lactic acid (see Section 3.1.1.6) mixed with the rest of the ingredients were added after the first 30 second mix, and the mixing continued for another 30 seconds.

- The sausage meat was sampled from ten different sites in the bowl chopper (see Figure 1).

Figure 1 Sampling sites in the bowl-chopper (top view)



- pH of the samples was measured and used to indicate the pattern of lactic acid distribution (see Section 3.2.2). Salt (NaCl) content (see Section 3.2.3) was also determined.

- The results were analysed for standard deviation (see Appendix 3).

The acceptability of sausage particle size was checked by sensory evaluation using taste panelists familiar with this type of sausage.

After sampling for pH and salt, the remaining sausage meat was stuffed (see Section 3.1.2.2), cooked, (see Section 3.1.2.4) and presented to five Thai panelists, who commented on the particle size of the sausage in relation to traditional Thai sausages of this type.

The standard deviation of pH and salt concentration was 0.04 pH units and 0.14% respectively, which were very small. This meant that pH and salt concentration differences between site to site in the bowl chopper was very slight. All taste panelists were satisfied with the particle size of the sausage mix. As this mixing technique produced satisfactory results, it was adopted for all experimental batches.

3.1.2.2 SAUSAGE STUFFING

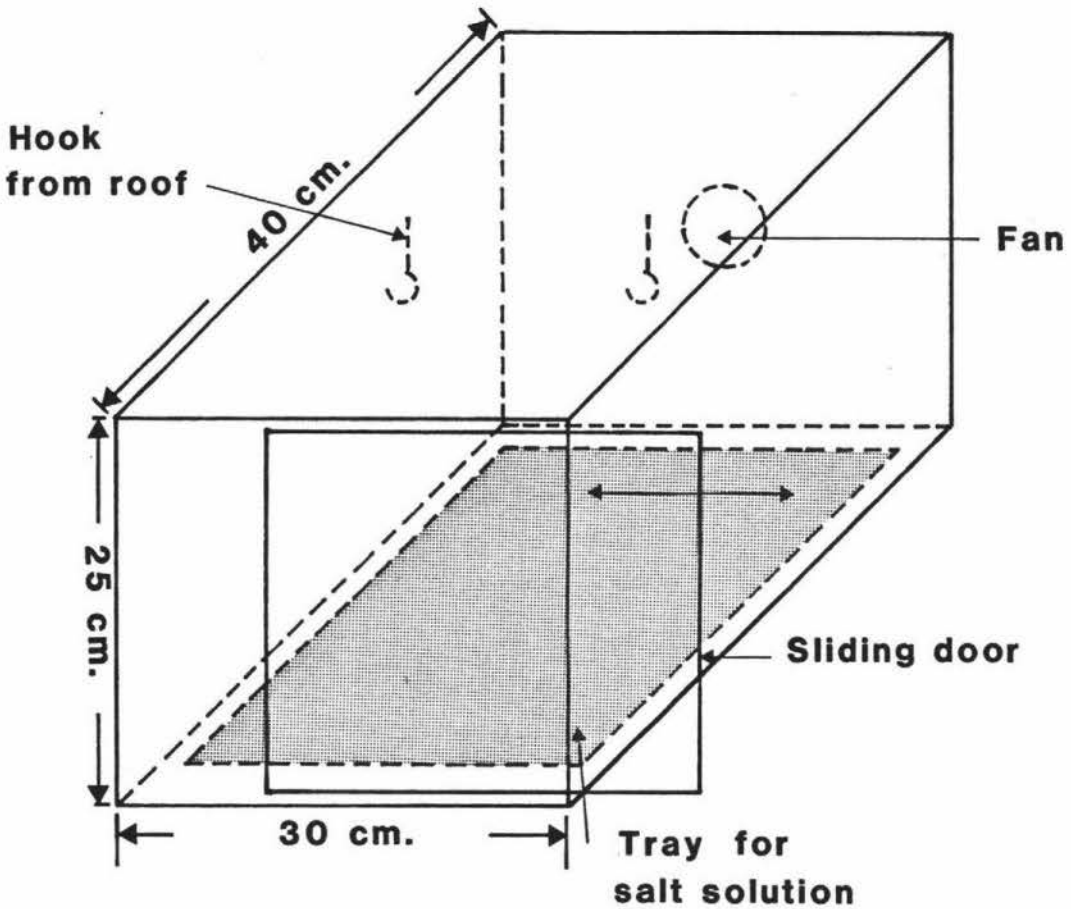
After mixing the sausage mix was stuffed into casings by a horizontal cylindrical shape hand-operated stuffer. The sausages were divided into links, each 8 cm long, by string ties. Ten small holes were made in the skin of each link using a pin, in order to facilitate the removal of excess moisture from inside of the sausage.

3.1.2.3 LABORATORY INCUBATION

Incubator:

Small-scale commercial air-conditioned smokehouses are commonly used to incubate sausages since temperature, humidity and air-flow can be effectively controlled. However, they are generally expensive and not always available (Johnson and Acton, 1975). In this study, simple and relatively inexpensive incubating cabinets which can provide a temperature and humidity-controlled environment were used. The stainless steel cabinet (see Figure 2) measured 30 x 40 x 25 cm in width, length and height respectively. The front panel of the cabinet which was made of hard transparent plastic could slide to open or shut the cabinet. Inside the cabinet, a fan was located on the side opposite to the sliding door. A total of nine cabinets were used during the entire experimental period.

Figure 2
Incubator



Saturated solutions of sodium chloride and potassium sulphate were used to control the relative humidity in the cabinet at 75% and 97% at 30°C respectively (see Appendix 4). The actual humidity was checked with a Thermo-Hygrometer (EP400). (Wallac oy Turkur-Finland).

Incubation process:

Studies of fermented meat in the laboratory have generally involved the use of beakers rather than the conventional sausage casings to contain the meat during the fermentation (Barber and Deibel, 1972; Daly *et al.*, 1973; Deibel *et al.*, 1961). Although this "beaker sausage" technique, first described by Diebel *et al.* (1961) is economical, there are some disadvantages as pointed out by Johnson and Acton (1975). First, the ratio of air to meat surface in the beaker is restricted and considerably different from that found with casings. Second, the fermented material is commonly smoked, cooked and dried for further experiments, but this cannot be carried out conveniently if beakers are used. Therefore, casings were used in this study.

The incubation process was carried out in two stages:

(i) at a temperature of 30-32°C, 75% R.H. to simulate drying conditions in Thailand;

(ii) at a temperature of 30-32°C, 97% R.H. to simulate conditions inside the packaging during distribution and selling in Thailand.

Incubator sterilization:

The incubators were sterilized before and after each experiment. This was done to ensure there were no microorganisms on the inside surfaces of the incubators before starting the experiment. Formaldehyde was used as a sterilizing agent. It required 600 ml of concentrated formalin and 200 g of $KMnO_4$ per 1,000 sq ft chamber (Maddox, 1981) to form the desired formaldehyde fume. The incubators were fumigated for 24 hours.

3.1.2.4 SAUSAGE COOKING

Sausages were deep fried in soybean oil at varying cooking times and temperatures in order to obtain the optimum quality product. Cooking temperatures were measured by a pocket probe pyrometer, model MP (Electronic Development Laboratories Inc., Plainview, N.Y. 11803). A stopwatch was used to control the cooking time. After predetermined cooking times and temperatures, they were placed on a strainer for three minutes so that excess oil was allowed to drain. The sausages were at this stage ready to be presented to the panelists or used in other experiments.

From trial and error, it was found that the optimum cooking time and temperature for this type of sausage was seven minutes at 130°C.

3.2 TESTING METHODS

In this section the general chemical and sensory testing methods which were used throughout the studies are described.

3.2.1 PROXIMATE ANALYSIS

Moisture content (%) of the sausage mix was measured using the AOAC (1980) method - 24.003 (a).

Protein content (%) was determined by the Kjeldahl method, using the conversion factor : x 6.25 (Bradstreet, 1965; Dawson , 1969).

Fat content (%) of the sausage mix was determined using the Soxhlet Extraction method (Massey University, 1978). The sample was prepared by drying about 2 to 5g samples in the same manner as for the determination of moisture content. The extractant used was 125 ml of petroleum ether (B.P. 40-60°C). When the extraction was completed, the flask containing a mixture of solvent and fat was removed and the solvent evaporated off using a rotary film evaporator. Fat residues in the flask were finally dried in a 100°C oven for 30 min , cooled at room temperature (not in a desiccator) and weighed. The percentage of fat content was calculated using the formula:

$$\% \text{ Fat} = \frac{(\text{wt of flask + fat}) - (\text{wt of flask})}{\text{sample wt}} \times 100$$

Ash content (%) was determined as follows:

- weighed 3 to 5g (± 0.01g of well-mixed sample into ashing dish which had been ignited, cooled in a desiccator and weighed soon after attaining room temperature;

- placed in muffle furnace at 550°C. Incinerated until light grey ash was obtained;
- cooled in open for a short time, then finished in desiccator and weighed soon after room temperature was attained.

3.2.2 pH MEASUREMENT

The sausage mix was removed from the casing (AOAC, 1980). Ten grams of sausage mix were blended for 60 seconds with 100 ml distilled water in the Waring blender. pH value of the homogenate was measured using a pH-meter (Ockerman, 1974; Acton *et al.*, 1972).

3.2.3 DETERMINATION OF SALT

Salt concentration (%) of the sausage mix was determined using the standard AOAC (1980) method - 24.010.

3.2.4 DETERMINATION OF SORBATE

The method of analysis for sorbate residuals was developed from the method of Bokus (1968); Wilamowski (1974) and Baldock *et al.* (1979).

The sausage casing was peeled out from the meat by a razor blade, then the casing weighed and transferred into a Waring blender jar containing 100 ml of an alcoholic solution of metaphosphoric acid (5g of HPO_3 in 250 ml of H_2O and diluted to 1 litre with alcohol). The mixture was blended for one minute and allowed to stand for 10 minutes. The contents of the blender jar was then vacuum-filtered through Whatman No. 3 filter paper.

5 ml of the filtrate was transferred to a 250 ml separating funnel and 100 ml of 1:1 petroleum-ethyl ether mixture was added.

The mixture was shaken for one minute, after which the ether layer was recovered and dried with 5 g of anhydrous sodium sulphate. Absorbance of the ether layer was determined spectrophotometrically at 250 nm using the extracts from an untreated sausage casing sample as a blank.

Preparation of the standard curve:

The standard curve was prepared from a stock solution containing 0.134 g of potassium sorbate (equivalent to 0.1 g of sorbic acid) in 100 ml of deionized water.

Aliquots of 1 to 6 ml were made up to 100 ml with the alcoholic metaphosphoric acid solution.

5 ml from each flask were then shaken for one minute with 100 ml of 1:1 petroleum ether-ethyl ether. The ether layer was recovered and dried with 5 g of anhydrous sodium sulfate.

Absorbance was determined at 250 nm against a blank prepared with 5 ml of metaphosphoric acid solution and was plotted against mg of sorbic acid/100 ml of ether. The standard curve of sorbic acid is shown in Appendix 5.

Calculation:

Concentrations of sorbic acid on sausage casings was calculated by the following formula:

$$\frac{\text{mg sorbic acid/100 ml ether}}{\text{wt of sausage casing}} \times 20{,}000 = \text{ppm sorbic acid per g sausage casing}$$

In order to convert the concentration of sorbic acid on the sausage casing to the concentration of sorbic acid per square area of sausage casing and of the sausage mix, ten links of sausage were randomly sampled. Each sausage link was individually weighed, and the casing peeled off by a razor blade. The sausage casings were then weighed and spread over graph paper to determine their areas.

Calculation:

ppm sorbic acid per square cm. of sausage casings = ppm sorbic acid per g sausage casing x 0.0182

ppm sorbic acid per g sausage mix = ppm sorbic acid per g sausage casing x 0.0141

3.3 SENSORY TESTING METHOD

The main sensory attribute that was studied in these experiments was texture. Texture in food is an extremely complex property, comprised of a spectrum of parameters. There are two methods of sausage texture measurement and evaluation; subjective (Taste panel) and objective method (Instrumental method). The subjective method involves taste panelists' selection, taste panel brainstorming and recording. The objective method involves the selection of the appropriate apparatus and interpretation of the result. Nicklin (1981) tried to find the correlation between sensory and instrumental textural measurements in a commercial emulsion-type sausage. She used the extrusion test, multiple compression test and Warner Bratzler Shear test for the objective method and correlated between sensory properties and these instrumental measurements. She found that the relationship between sensory properties and these instrumental measurements was dependent

on the level of addition of protein additives and not all the properties considered to be important in commercial sausages could be related to instrumental measurements. Therefore, only the subjective method was used for the present study. Because of the complex texture characteristic, it was decided to use profile testing.
The taste panelists identified the texture vocabulary of Sai Krok Prew and then the scaling system was developed based on this vocabulary.

The panelists were also asked to comment on the flavour and acceptability; in particular the acceptability of Sai Krok Prew at different acid levels.

The subjective method to measure and evaluate the sausage texture was carried out in the sensory evaluation room in the Food Technology Department at Massey University. Testing sessions were held at 5.00 p.m. as all panelists were available at that time of the day.

3.3.1 TASTE PANEL SELECTION

Five Thai students at Massey University were invited to be the panelists (after the first session one more Thai student was invited to join the following sessions). These panelists were all familiar with this type of fermented sausage.
The number of panelists was limited by the number of people who would be available consistently over the two month training and testing period.

3.3.2 SETTING UP THE TEXTURE PROFILE

The technique used was as follows:

- appointed a moderator;
- the panelists tasted the sausage sample, looking for the dominant "texture notes";
- the moderator collated the terms for the texture notes;
- the moderator reduced the number of texture notes to the six most important;
- during this period the terminology required to describe the texture was developed, standardised and agreed among the panelists.

The sausage used as a model for setting the texture profile was the same as Batch 2 in Section 4.1.1.

The sausages were cooked (see Section 3.1.2.4) immediately after stuffing and served hot to the panel. Sausage samples were presented to the panelists on a plate marked into four sections and the panelists were able to assess the properties of the sausage independently. Water was provided for the panelists to rinse their mouths.

3.3.3 SCALING METHOD DEVELOPMENT

The refinement of the questionnaire obtained from the brainstorming session (see Appendix 6.1) was the application of scale into the questionnaire. The choice of scale was influenced by Baten (1946) who showed that a scale-word anchored at either end of a line yielded a greater product

difference than the typical category scale. The interval scale used consisted of a horizontal line 80 mm long with anchor points 10 mm from each end. The horizontal lines were superimposed on a grid dividing the lines into nine units (1-9) and assigned a number between 1 and 9 to each rating. Both ends of the horizontal line were labelled with a word or expression (the vocabularies obtained from the brainstorming session). Each rating scale between 1 and 9 was labelled with a word or expression to reflect the strength of perception of that property on top of the questionnaire. This questionnaire is shown in Appendix 6.2.

The texture attributes were firmness, rubberiness, juiciness, oiliness, smoothness and stickiness. They were asked to judge the overall texture acceptability from 1 that was the lowest score, to the highest score at 9.

The panelists were asked to scale the samples and also the ideal product on the line by making a vertical mark across the horizontal line.

3.3.4 TASTE PANEL TRAINING

Four training sessions were organised to train the panelists. These training sessions were held one each week. During the first training session the panelists were presented with four types of Sai Krok Prew that were prepared, mixed and stuffed similar to those in Section 4.1.2. For the following three training sessions, the panelists were presented with the samples prepared from Section 4.3.1.

They were asked to evaluate the texture of various sausage treatments using the questionnaire (see Appendix 6.2). Group discussions were held following these sessions to ensure the proper understanding of the terms and scale used in the questionnaire.

3.3.5 TASTE PANEL PROCEDURE

The panelists were presented with three and four samples alternately at two day intervals. They were presented with one whole cooked sausage for each sample. The sample was put on a marked plate and coded with a random number. They were asked to record their perceptions of the various texture attributes on the scales. They were allowed to wash their mouths out with cold water. Discussions were held at the end of each session.

Each judge recorded his/her evaluation by making a vertical line across the horizontal line at the point that best reflected his or her perception of the magnitude of that property, including the ideal point. The values obtained from the judges were measured and tabulated.

The ratio of the sensory score to the ideal for every texture attribute was calculated for two-way analysis of variance to see the differences between the judges and samples.

3.4 MICROBIOLOGICAL ASSAY

In this project, all microbiological assays were carried out immediately after sampling. The entire sample was macerated by blending for two minutes in a sterile blender cup.

3.4.1 METHOD OF SAMPLING

The whole sausage chubs were randomly sampled for the microbiological assay. The string at the end of the sausage chub was removed by the sterile razor blade. The sausage was then placed in the sterile petri plate. The plate was labelled with the sample number and any other desired information prior to preparing the dilutions.

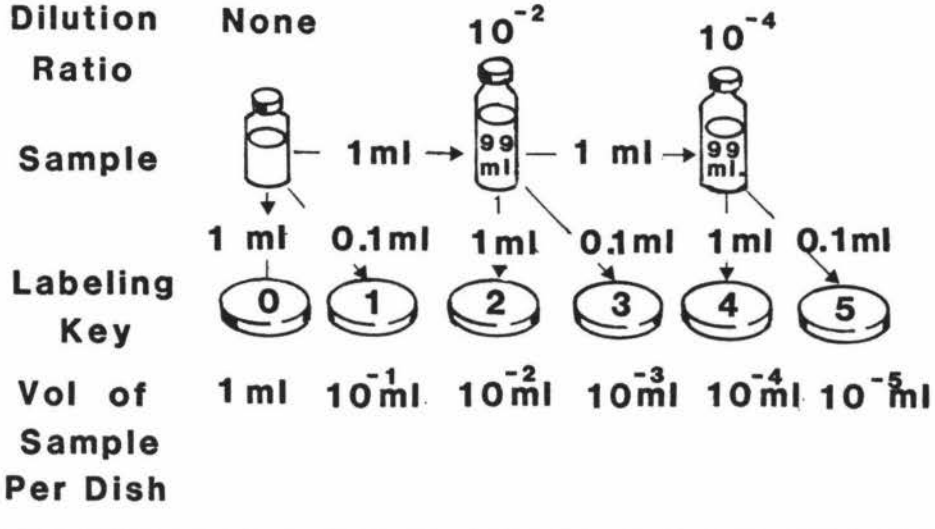
3.4.2 DILUTING SAMPLES

For the most accurate colony count, the dilution(s) should be selected so that the total number of colonies in a plate will be between 30 and 300 (Breed and Dotterrer, 1916). If the count is expected to be in the range of 3.0×10^3 to 3.0×10^5 per ml or g , prepare plates containing 1:100 and 1:1000 dilutions. Figure 3 shows a schematic of examples for preparing dilutions.

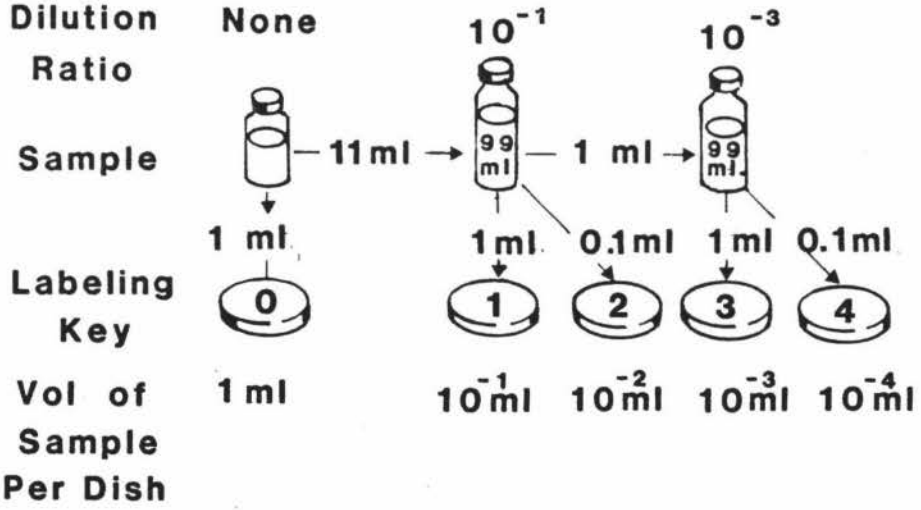
Figure 3

Method of preparation of dilutions

1. Method of employing 1 ml of sample



2. Method of employing 11 ml of sample



3.4.3 TOTAL VIABLE MICROORGANISMS

In order to determine the total viable microorganisms in Sai Krok Prew, 10 g samples of sausage mix were blended for two minutes in a sterile blender cup with 90 ml quantities of 0.85% saline. The subsequent decimal dilutions were prepared with the same diluent. At each step in the dilution series, each dilution from the initial dilution were mixed by the vortex mixer for five seconds before being transferred into the plates. Plate count agar and the pour plate technique were used to determine the total viable microorganisms. Plates were incubated at 30°C for 48 to 72 hours before counting.

3.4.4 LACTIC ACID PRODUCING BACTERIA

In order to determine lactic acid producing bacteria, 10 g samples of sausage mix were blended for two minutes in a sterile blender cup with 90 ml quantities of M.R.S. broth and subsequent decimal dilutions were prepared with the same diluent. The vortex mixer was used to mix the dilutions as before, which were transferred onto the plates

in a similar manner to Section 3.4.2. Molten M.R.S. agar (45°C) was poured into the plates and mixed thoroughly. When the medium had set, another layer of uninonculated M.R.S. agar was poured over the surface to produce a layer-plate. Plates were incubated at 37°C for three days before counting.

3.4.5 YEASTS AND MOULDS ON SAI KROK PREW'S CASING

The procedure to determine yeasts and moulds on Sai Krok Prew's casing was as follows:

- Sausage skins were slitted longitudinally and peeled aseptically by a sterile razor blade.
- The sausage skin surface area was measured against the sterilised graph paper.
- The sausage skin was transferred into a sterilised stomacher bag, 100 ml of 0.1 (wt /vol) peptone was added and stomached for one minute.
- The casing was removed and the subsequent decimal dilutions were prepared with the same diluent.

The vortex mixer was used to mix the dilutions before being transferred onto the plates similar to Section 3.4.2.

- Potato Dextrose Agar and the pour plate technique were used to determine the viable counts of yeasts and moulds.
- Plates were incubated at 22°C for three to five days before counting.

CHAPTER 4

EXPERIMENTAL

In the first experiments, added chemical acidulants were used without fermentation to determine the relationships between the amount and effects of acid and pH reduction. Fresh chemically acidified Sai Krok Prew tended to burst during the cooking process. Therefore, cooking stability of the chemically acidified Sai Krok Prew at various times during the drying stage was investigated.

The attempt to improve the texture of Sai Krok Prew was carried out using a Mixture Design with pork, fat and rice as the variable factors, using the empirical equation relating the sensory testing result to the mixture composition. The Lagrange multiplier technique was used to determine the mixture with the optimum texture.

The formula from the optimization process was used to study pH reduction of Sai Krok Prew, using a starter culture.

Lactacel MC was used as the starter culture. Variations in the content of sodium nitrite, sucrose and dextrose were studied to find their effect on pH reduction.

The effect of potassium sorbate on the shelf life and acceptability of chemically acidified and microbially acidified Sai Krok Prew was studied. The effect of vacuum packaging on shelf life was also investigated.

4.1 pH REDUCTION USING CHEMICAL ACIDULANTS

European "fermented" sausage commonly has a pH value of 4.8 - 5.0. Rongvisit (1980) found that for Sai Krok Prew, the most acceptable pH range was between 4.3 - 4.5. In the first study lactic acid solution was used as acidulant and in the following studies GDL powder.

4.1.1 pH REDUCTION USING LACTIC ACID

From the knowledge of dry and semi-dry fermented sausage, the addition of extra moisture into the system should be avoided, or kept at a minimum, which makes uniform addition of lactic acid difficult. The reports of Nurmi (1966a) and Everson (1981) do not mention the strength of lactic acid used. Therefore, a preliminary experiment to determine the relationship between the strength and amount of acid and the reduction in pH was necessary.

To investigate the relationships between the amount of added lactic acid (1 N) and pH of sausage mix, the following experiment was carried out. Pork, fat and rice (see Section 3.1.1.1 and Section 3.1.1.2) were thawed overnight at room temperature and were allowed to attain a temperature of 15°C before being mixed.

- The proportion of the sausage ingredients in each batch which weighed 1,500 g is shown in Table 2.

- The ingredients were mixed in a bowl chopper for 60 seconds.

The sausage mix was divided into six portions of 200 g each and to each portion different amounts of lactic acid and distilled water were added as shown in Table 3.

Table 3: The amounts of 1N lactic acid and distilled water

Portion no.	ml of 1 N lactic acid	+	ml of distilled water *	% added lactic acid in meat (wt/wt)
1	0		25	0.00
2	5		20	0.23
3	10		15	0.45
4	15		10	0.68
5	20		5	0.90
6	25		0	1.13

* Distilled water was added to standardise the amount of liquid added.

- The addition was carried out dropwise from a 25 ml burette and the mixture was manually stirred with a glass rod during the addition.

After the addition of acid and water, and thorough mixing, the pH value of the mixture was measured using a pH meter.

The relationships between the amount of added lactic (1 N) and texture of the sausage mix from the preliminary experiment showed that 1N lactic acid was too weak for the addition. In order to reduce the sausage meat pH down to the most desirable pH, 4.4, (Rongvisit, 1980) without deteriorating the sausage texture, an increased strength of 2 N was therefore used in the next experiment.

The procedure used was similar to the basic method in Section 3.1.2.1. The lactic acid was added after 30 seconds mixing

in the bowl chopper and the mixing continued for further 30 seconds. The amounts of 2 N lactic acid and distilled water added are shown in Table 4.

Table 4: The amounts of 2N lactic acid and distilled water

Batch no.	ml of 2 N Lactic acid	+	ml of distilled water *	% added lactic acid in meat (wt/wt)
1	0		90	0.00
2	30		60	0.36
3	60		30	0.72
4	90		0	1.08

* Distilled water was added to standardise the amount of liquid added.

The sausage mix after addition of acid and mixing was stuffed into casings (see Section 3.1.2.2). The pH value of the mixture was measured using a pH meter.

4.1.2 pH REDUCTION USING GLUCONO-DELTA-LACTONE (GDL)

The objective of this experiment was to study the extent of pH reduction as a result of the addition of different levels of GDL.

With the exception of the substitution of GDL for lactic acid, the sausage ingredients were prepared, mixed and stuffed into casings in the same manner using the same proportion as described in Section 4.1.1. There were four levels of GDL added during mixing, 0.0, 1.0, 1.5 and 2.0 g GDL/100 g sausage mix. The sausages were then held under the drying stage conditions (see Section 3.1.2.3) during which three random samples were taken from each treatment at

times 0, 1, 2, 3, 4, 6, 8 and 24 hrs. for the determination of pH.

4.2 BURSTING OF SAUSAGES DURING THE COOKING PROCESS

From the experiments in Section 4.1 using lactic acid and GDL as an acidulant to reduce the pH of sausage mix, when the sausages were cooked soon after the ultimate pH was attained, there was found to be a high incidence of bursting of sausages during the subsequent cooking process, especially for sausages with lactic acid addition. This incidence might have been due to the rapid reduction in pH of the sausage mix which decreased the water holding capacity (WHC) of the pork, i.e. increased the free moisture in the sausage mix. This free moisture could vapourise when cooked, resulting in burst sausage skins. Some part of this free moisture can be removed during the holding period (which will indirectly affect the fat content) by means of a low humidity atmosphere (see Section 3.1.2.3). Therefore, it was decided to vary the holding periods and observe the change in pH, moisture and fat content with respect to the bursting of sausages during the cooking process. In this study GDL, which was in the form of a powder, was used so that the differences in moisture would be attributed solely to the inherent moisture content of the sausage mix. The level of GDL was 2.0 g /100 g sausage mix found from the previous experiment to give a pH of 4.4. The procedure for preparing, mixing and stuffing the sausages was the same as in the GDL experiments in Section 4.1.2. Sausages were held under the drying stage conditions and six randomly selected sausages were taken at each of the times 0, 1, 3, 5, 7, 24, 48 and 72 hrs. Four sausages were analysed for pH, moisture content and fat content. The remaining two samples were cooked (see Section 3.1.2.4) and then analysed for moisture content and fat content.

4.3 MIXTURE DESIGN

In order to improve the sausage texture, levels of pork, fat and rice were varied.

From the experiments in Section 4.1 it was found that GDL yielded much better texture than lactic acid. Therefore, GDL was selected to be used in mixture design.

In mixture experimentation, it is impossible to vary one ingredient or component while holding all others constant. As soon as the proportion of one component is altered, so is that of at least one other component, since the sum of all components is always 1.0. For this reason, conventional experimental designs such as those discussed by Cochran and Cox (1957) and Davies (1956) cannot be applied. Instead, a new set of experimental plans simply called 'Mixture Designs' has been developed. If properly applied, these designs can lead to an optimum product. Frequently in mixture experimentation, it is impossible to vary the proportion of each component from 0 to 1.0. Each component is constrained within certain limits. Mathematically,

$$0 \leqslant a_i \leqslant x_i \leqslant b_i \leqslant 1.0, \quad i = 1, 2, \ldots, q$$

Among the solutions to the problem of selecting an experimental design in this situation are the extreme vertices designs of McLean and Anderson (1966). They recommended taking all possible combinations of the bounds a_i and b_i for the components taken q-1 components at a time. Subtraction from 1.0 will yield the level of the remaining component provided it is within its bounds. Otherwise, that vertex is deleted from the experiment. The maximum number of design points for a q component design will be $q.2^{q-1}$.

In this sausage formulation, there were nine components. With these nine components, an extreme vertex design could have as many as 2,048 vertices (9.2^{9-1}). Some of these vertices can be eliminated. The methods of elimination of some of the design points were discussed by McLean and Anderson (1966), Scheffé (1965) and Hare (1974).

In this study, sausage components could be divided into two groups:

(a) Main component: This was composed of pork, fat and rice. These three elements weighed 0.95 (95%) of the formulation and were varied in the mixture design.

(b) Minor component: This was composed of garlic, coriander, salt, sucrose, pepper and sodium nitrite. These minor elements were 0.05 (5%) of the formulation and were fixed at constant levels.

4.3.1 PRELIMINARY MIXTURE DESIGN

The Sai Krok Prew formulae obtained from communication with two producers (Lee and Rut, 1980) and Rongvisit's Thesis (1980) provided the figures for the constraints of pork, fat and rice in the formula. In this preliminary experiment, pork, fat and rice were varied subject to the constraints:

$$0.50 \leqslant x_1 \leqslant 0.70$$
$$0.15 \leqslant x_2 \leqslant 0.40$$
$$0.05 \leqslant x_3 \leqslant 0.30$$

where x_1 = pork ratio

x_2 = fat ratio

x_3 = rice ratio

The total number of points considered were equal to $q.2^{q-1}$, where q was the number of variable factors. Therefore, the total number of points = 3.2^{3-1} = 12. These points are listed in Table 5.

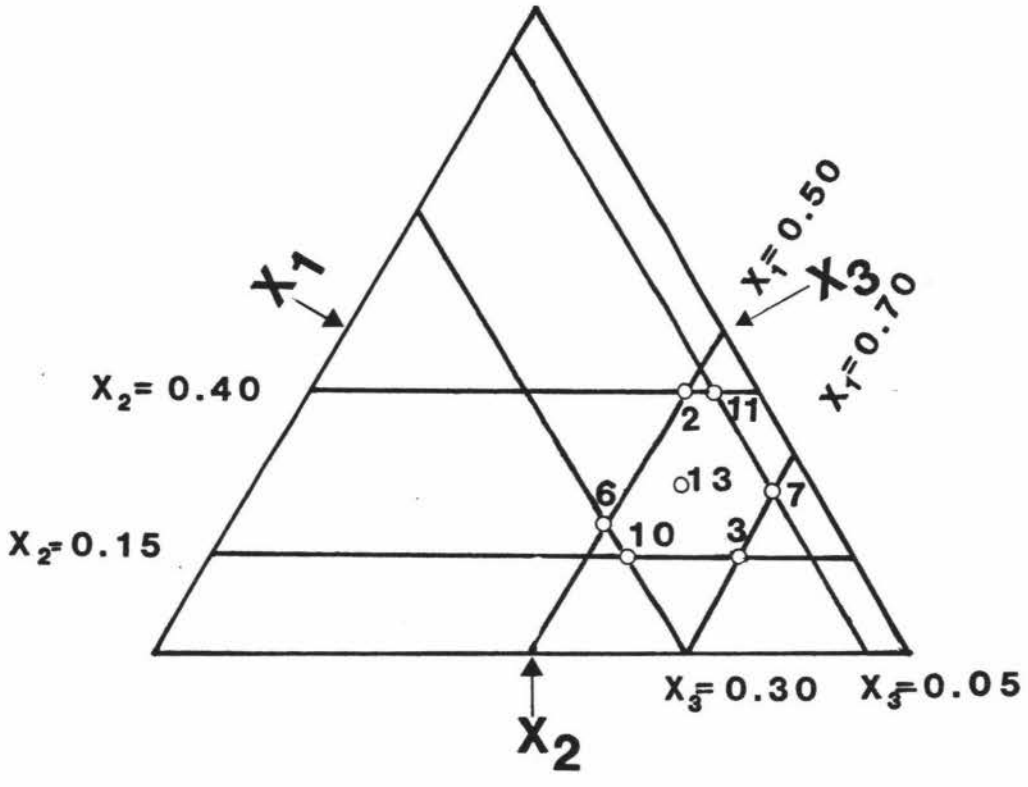
Six of these points 1, 4, 5, 8, 9 and 12, were eliminated because the remaining component was beyond its bounds. The remaining six points along with a centre point (no. 13) were run. The coordinates of the centre point (0.58, 0.26, 0.16) were calculated by averaging the coordinates of the six vertices. The resulting experimental design is illustrated graphically in Figure 4.

Table 5: Preliminary Extreme Vertex Design

Vertex	X_1	X_2	X_3
1	0.50	0.15	0.35
2 *	0.50	0.40	0.10
3 *	0.70	0.15	0.15
4	0.70	0.40	-
5	0.50	0.45	0.05
6 *	0.50	0.20	0.30
7 *	0.70	0.25	0.05
8	0.70	-	0.30
9	0.80	0.15	0.05
10 *	0.55	0.15	0.30
11 *	0.55	0.40	0.05
12	-	0.70	0.40
Centre (13) *	0.58	0.26	0.16

* Points in design

Figure 4 Preliminary Extreme Vertex Design



Therefore, at a batch size of 1,500 g , the amounts of pork, fat and rice which weighed 95% of the formula are as shown in Table 6.

Table 6: The amounts of pork, fat and rice in the preliminary mixture design experiment

Vertex	Pork (g)	Fat (g)	Rice (g)
2	712.50	570.00	142.50
3	997.50	213.75	213.75
6	712.50	285.00	427.50
7	997.50	356.25	71.25
10	783.75	213.75	427.50
11	783.75	570 00	71.25
Centre (13)	226.50	370.50	228.00

For each run the minor component was fixed at 0.05 (5%) level.

The amount of GDL was added to each run at the concentration of 2 g GDL/100 g sausage mix. Therefore, in each run the main component in Table 6 was mixed with:

- peeled garlic 37.50 g
- coriander 1.50 g
- salt 30.00 g
- sucrose 7.50 g
- pepper 3.75 g
- $NaNO_2$ 0.75 g

GDL was added 30 g to 1,500 g of sausage mix , i.e. 2 g / 100 g sausage mix.

- Sausage ingredients were prepared, mixed and stuffed into casings similar to Section 4.1.2.

- The sausages were then held under the drying stage conditions for 48 hours.

- The sausages were cooked. The remaining samples (unburst) were used to train the panelists. The questionnaire (see Appendix 6.2) was employed during the training sessions.

From this experiment it was found that the samples that contained a high level of fat (40%), i.e. vertex number 2 and 11, were very oily before cooking and burst along their full length during the cooking process.

4.3.2 FINAL MIXTURE DESIGN

From the preliminary experiment (see Section 4.3.1) it was found that the upper limit of fat level at 40% was too high, i.e. the constraint of the fat content had to be reduced.

In this experiment, the constraints of pork and rice were the same as in the preliminary study but the upper limit of fat content was reduced to 30%.

The experiment was carried out as follows:

- pork, fat and rice had components subject to the constraints

$$0.50 \leqslant X_1 \leqslant 0.70$$

$$0.15 \leqslant X_2 \leqslant 0.30$$

$$0.05 \leqslant X_3 \leqslant 0.30$$

where X_1 = pork ratio

X_2 = fat ratio

X_3 = rice ratio

- The total number of points considered were similar to the preliminary experiment and these points are listed in Table 7.

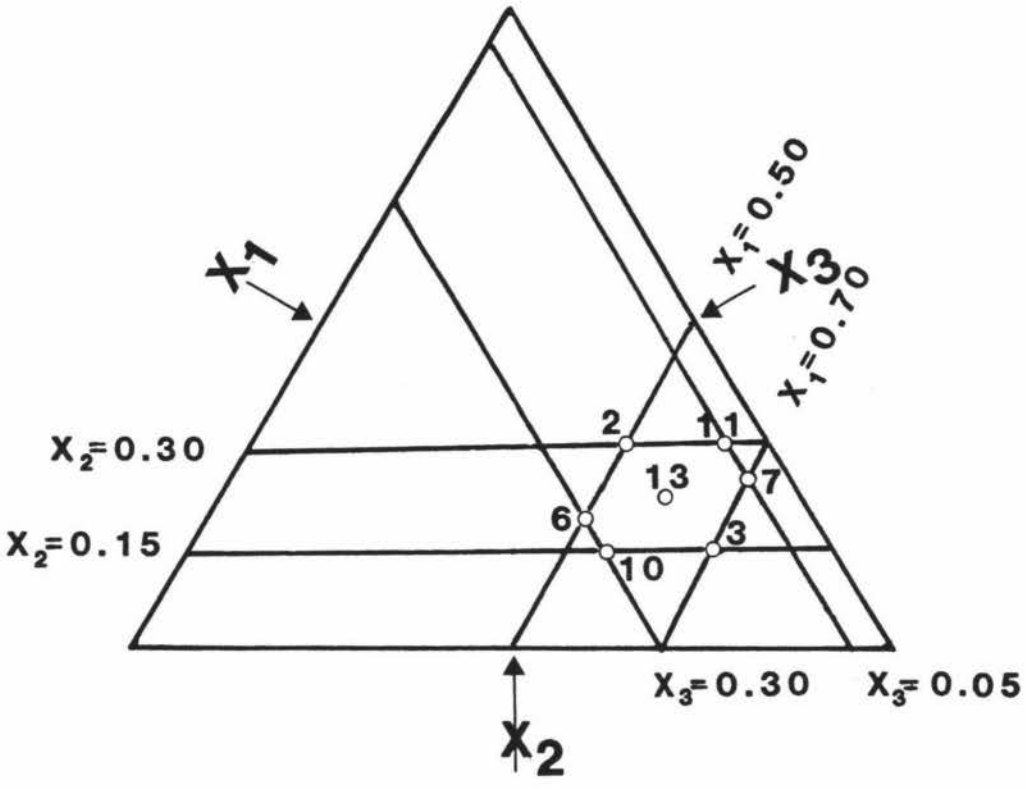
- Similarly to Section 4.3.1, six of these points 1, 4, 5, 8, 9 and 12 were eliminated because the remaining component was beyond its bounds. The remaining six points along with a centre point were run. The coordinates of the centre point (0.60, 0.22 , 0.18) were calculated by averaging the coordinates of the six vertices. The resulting experimental design is illustrated graphically in Figure 5.

Table 7: Final Extreme Vertex Design

Vertex	X_1	X_2	X_3
1	0.50	0.15	0.35
2 *	0.50	0.30	0.20
3 *	0.70	0.15	0.15
4	0.70	0.30	0.00
5	0.50	0.45	0.05
6 *	0.50	0.20	0.30
7 *	0.70	0.25	0.05
8	0.80	0.15	0.05
9	0.80	0.15	0.05
10 *	0.55	0.15	0.30
11 *	0.65	0.30	0.05
12	-	0.30	0.70
Centre (13)*	0.60	0.22	0.18

* Points in design

Figure 5 Final Extreme Vertex Design



- The amounts of pork, fat and rice were calculated similarly to the preliminary study and shown in Table 8.

Table 8: The amounts of pork, fat and rice in the final mixture design experiment

Vertex	Pork (g)	Fat (g)	Rice (g)
2	712.50	427.50	285.00
3	997.50	213.75	213.75
6	712.50	285.00	427.50
7	997.50	356.25	71.25
10	783.75	213.75	427.50
11	926.25	427.50	71.25
Centre (13)	855.00	313.50	256.50

- To each mixture was added 5% of minor component and 30 g GDL similar to the preliminary study.

- Random numbers were used to designate the order of runs. The runs were split into two sets of 48 hr. intervals to suit the limited amount of incubators and reduce the fatigue of the panelists that might occur if too many samples were tested at a time.

- The first set were the vertices numbers 10, 7 and 2. Sausage ingredients of each run were prepared, mixed and stuffed into casings (see Section 4.1.2).

- Four sausages from each run were randomly sampled. Two sausages were analysed for the initial pH and the remaining two were analysed for initial moisture and fat content (see Section 3.2.1).

- The sausages were then held under the drying stage conditions for 48 hours.

- Four sausages from each run were randomly sampled, two sausages were analysed for the "before-cooked" pH, and the remaining two were analysed for "before-cooked" moisture and fat content.

- The sausages were cooked.

- Two sausages from each run were randomly sampled and analysed for "after-cooked" moisture and fat content.

- The cooked samples were presented to the panelists and tested as described in Section 3.3. The mean score for every texture attribute was used to determine the correlation with the moisture and fat content of cooked sausage.

The compositions of the extreme vertices and the centre point, with the average overall texture acceptability, were employed to determine the equation for predicting the relationship between pork, fat and rice, and its effect on the sensory score. The multiple regression predicting equation containing no constant term (Hare, 1974) was determined by using Standard Computer Package called 'Minitab' (Ryan et al., 1976).

4.3.3 TEXTURAL OPTIMIZATION

The multiple regression model for predicting the overall texture acceptability score (y) was used to optimize the texture acceptability.

In most engineering problems - especially real-world formulations - the object is to optimize (maximize or minimize, in this case maximize) a criterion (or objective function) subject to several constraints. When a few nonlinear constraints are imposed on the problem there are very few known solution techniques available. Certainly there are relatively few efficient solution techniques. The mathematical technique of Lagrange multipliers has been developed to convert constrained optimization problems into unconstrained optimization problems, (Krajewski and Thompson, 1981).

Calculation:

From the sensory scores a quadratic model fitted to the data produces an adjusted R^2 value of 0.9981. The resulting equation for predicting sensory scores (y) is:

$$y = 6.13X_1 - 107X_2 - 25.8X_3 + 196X_1X_2 + 49.2X_1X_3 + 184X_2X_3 \quad (1)$$

where X_1 = pork ratio

X_2 = fat ratio

X_3 = rice ratio

The prediction equation was maximized subject to the constraint that the sum of the components is equal to 1.0. Lagrange multipliers to incorporate the constraint into the

prediction equation were employed. Partial derivatives of the augmented equation with respect to all of the variables produce a set of simultaneous equation which when solved yields either a maximum or a minimum. The set of equation was:

$$-\lambda + 196X_2 + 49.2X_3 = 6.13 \quad \text{(2)}$$

$$-\lambda + 196X_1 + 184X_3 = 107 \quad \text{(3)}$$

$$-\lambda + 49.2X_1 + 184X_2 = 25.8 \quad \text{(4)}$$

$$-X_1 + X_2 + X_3 = 1.00 \quad \text{(5)}$$

where λ = Lagrange multiplier

y will be maximized at the mixture of

$X_1 = 0.6179 = 61.79\%$

$X_2 = 0.2169 = 21.69\%$

$X_3 = 0.1652 = 16.52\%$

yields $y_{max} = 6.6$

4.3.4 TESTING THE PREDICTION AND OPTIMIZATION

In order to test the goodness of the prediction model in Section 4.3.2 and otpmization (maximize) from Section 4.3.3 the same group of panelists were invited to judge the sample once again. The final mixture from the optimization was processed into Sai Krok Prew. The amount of pork, fat and rice from the calculation (see Section 4.3.3) was changing from 100% to 95% of the formula (because the formula contained 95% main component). The remaining 5% was the minor component. GDL was added at the concentration of 2 g / 100 g sausage mix. Therefore, in 1,500 g batch the amounts of pork,

fat and rice were:

$$\text{pork} = (0.6179 \times \frac{95}{100}) \times 1{,}500 = 881 \text{ g}$$

$$\text{fat} = (0.2169 \times \frac{95}{100}) \times 1{,}500 = 309 \text{ g}$$

$$\text{rice} = (0.1652 \times \frac{95}{100}) \times 1{,}500 = 235 \text{ g}$$

- Weighed the minor component and GDL.

- The amounts of pork, fat and rice from the calculation were mixed with the minor component and GDL, stuffed into casings, held in the drying cabinet and cooked in the same manner as Section 4.3.2.

- Each panelist was presented with two cooked sausages, but the sausages were assigned with different random numbers. The overall texture acceptability scores from all panelists were averaged and compared with the scores from Section 4.3.3.

- Chemical analysis of the sample was carried out as in Section 4.3.2.

4.4 STARTER CULTURE FOR ACID FERMENTATION

Starter culture used for the studies was Lactacel MC (Microlife Technics, Sarasota, Florida) supplied by Smith-Biolab Ltd., Auckland. Lactacel MC was a frozen starter culture of *Pediococcus cerevisiae* and *Lactobacillus plantarum* in 4 oz. cans.

Different types of media were used to investigate the activity of lactic acid bacteria by Honoré (1979), Techapinyawat (1975), Rongvisit (1980), Acton *et al.* (1972) and Daly *et al.* (1973). The media used by these research workers were checked with the Oxiod

Manual (1979). It was speculated that MRS medium would be the suitable medium for the studies on Lactacel MC where *P. cerevisiae* + *L. plantarum* were examined together. A preliminary study was done to confirm the suitability of MRS medium.

It was found that both Gram positive rod (Lactobacillus) and Gram negative cocci (Pediococcus) were able to grow on MRS agar. The visual appearance for both types of colonies were very similar, therefore MRS agar could be used for the total count of lactic acid bacteria but not the differential count between Lactobacillus and Pediococcus.

4.4.1 CELLS CONCENTRATION OF LACTACEL MC

The objective of this experiment was to determine the cells concentration of Lactacel MC starter cultures.

Lactacel MC 0.1 g was diluted through a dilution series (see Section 3.4.2) using Ringer's solution ¼ strength as a diluent. MRS agar and layer-plate technique (see Section 3.4.4) were used in this study.

4.4.2 GROWTH RATE OF LACTACEL MC ON MRS BROTH

The objective of this experiment was to determine the growth rate of Lactacel MC on MRS broth, which was an ideal substrate. The procedure was as follows:

- Duplicated media of MRS broth 500 ml in 1 litre flask.
- Inoculated MRS broth with 0.6250 g of Lactacel MC (≃ 0.125% wt/col inoculum size).

- Checked sample at two hour intervals.

- Determined the viable cells count using the dilution series technique (see Section 3.4.2).
  MRS agar and layer-plate technique (see Section 3.4.4) were used in this study.

- Incubated the plates at 37°C for three days before counting.

4.4.3 METHODS OF USING THE STARTER CULTURE (LACTACEL MC) IN SAI KROK PREW

Lactacel MC was kept frozen until just prior to its being used. Frozen Lactacel MC was used at the level of 0.125% (wt/wt) of the sausage formula. Therefore, for batch size of 1,500 g sausage mix, 1.875 g of Lactacel MC was dissolved with 20 ml of Ringer's solution ¼ strength before being added into the sausage mix. The procedure used was similar to the basic method in Section 3.1.2.1. The slurry of Lactacel MC was added after 30 seconds mixing in the bowl chopper and the mixing continued for a further 30 seconds.

4.4.4 MICROBIAL GROWTH DURING FERMENTATION

The objective of this study was to determine the viable cells of both total viable microorganisms and lactic acid producing bacteria during the fermentation. The procedure was carried out as follows:

- Sausage ingredients of equal batch size and preparation were similar to the chemical acidified sausage in Section 4.3.4 except that sodium nitrite was reduced to

150 ppm level and GDL was replaced by the starter culture (see Section 4.4.3).

- Sausage mix was stuffed and incubated under the drying stage conditions.

- After each time interval (0, 5, 10, 15 and 72 hours) after the addition of starter culture, duplicate samples of sausages were prepared for total viable microorganisms and lactic acid producing bacteria (see Sections 3.4.3 and 3.4.4).

4.5 THE EFFECTS OF NITRITE LEVELS AND DEXTROSE ON pH REDUCTION

The supplier of starter culture (Microlife Technics) stated that Lactacel MC grows well in formulations with 100-156 ppm sodium nitrite and produces acid from glucose, fructose, maltose, lactose, glycogen, starch, sucrose and corn syrup solids (Anon., 1981).

Nitrite level is important because of its ability to inhibit the growth of both desirable and undesirable microorganisms. In methods using starter cultures, recommendations (Anon.,1969, 1972) are for the use of a minimum 0.75% sugar as glucose for proper starter activity.

4.5.1 EFFECT OF NITRITE ON RATE OF pH REDUCTION

The procedure was as follows:

- Sausage ingredients and preparation were similar to Section 4.4.4 except that the nitrite levels in the formulation were varied at 75, 150 and 500 ppm.

- Sausage mix was stuffed and incubated under the drying stage conditions.

- Samples were taken at 2 hour intervals up to the 26th hour. Duplicated samples of sausages were analysed for pH.

- The results were analysed for analysis of variance to find if there were differences in rate of pH reduction between the three treatments.

4.5.2 EFFECT OF SUCROSE AND DEXTROSE ON RATE OF pH REDUCTION

The objective of this experiment was to test for suitability of sucrose and the necessity of dextrose as fermentable substrates in Sai Krok Prew inoculated with Lactacel MC.

Sausage ingredients and preparation were similar to Section 4.4.4, with 150 ppm sodium nitrite, and for the first treatment sucrose, and second treatment dextrose. In the second treatment the formulation was similar to the first treatment but sucrose was replaced by dextrose. The samples were incubated and sampled similarly to Section 4.5.1.

The results were analysed for analysis of variance to find if there was a difference in rate of pH reduction between the different sugars.

From the experiments in Sections 4.5.1 and 4.5.2, nitrite at 150 ppm and sucrose were selected for further studies. In an attempt to investigate the consistency of Sai Krok Prew production when using Lactacel MC, three batches of Sai Krok Prew were produced at a two-day intervals. These three batches were prepared with the same ingredients (nitrite

150 ppm and 0.5% sucrose in the formulation), mixed, stuffed, dried and sampled similar to the experiments in Sections 4.5.1 and 4.5.2.

4.6 EFFECT OF POTASSIUM SORBATE ON SHELF LIFE AND ACCEPTABILITY OF SAI KROK PREW

In order to investigate the effect of dipping the sausage in potassium sorbate solution on shelf life of Sai Krok Prew, firstly the shelf life of both GDL acidulated and microbial fermented Sai Krok Prew without potassium sorbate were determined. Then GDL acidulated Sai Krok Prew was used to determine the minimum effective level of potassium sorbate required to extend the shelf life to 14 days. The knowledge of minimum potassium sorbate solution required was then applied to microbial fermented Sai Krok Prew kept under simulated commercial conditions. The effect of dipping the sausage in potassium sorbate on rate of fermentation was also investigated.

4.6.1 SHELF LIFE OF GDL ACIDULATED SAUSAGE AND FERMENTED SAI KROK PREW

The objective of this experiment was to investigate the shelf life of both GDL acidulated sausage and bacterial fermented Sai Krok Prew. The experiment was carried out as follows:

- For GDL acidulated sausage, sausage ingredients were prepared, mixed and stuffed similar to those described in Section 4.3.4.

- For bacterial fermented sausage, sausage ingredients were prepared, mixed and stuffed similar to those described in Section 4.4.4.

- Incubated both types of sausages under the drying stage conditions.

- The sausages were inspected for the surface mould growth by visual inspection

4.6.2 EFFECT OF POTASSIUM SORBATE ON GDL ACIDULATED SAI KROK PREW SHELF LIFE

The objectives of this experiment were to investigate the effective level of potassium sorbate in preventing the surface mould and its effect upon the acceptability of the sausages. The experiment was carried out as follows:

- Prepared and mixed sausage ingredients similar to those described in Section 4.3.4.

- Stuffed the sausage mix but did not puncture the holes at this stage.

- Dipped the sausages in aqueous solution of 0 (control), 5, 10 and 15% (wt/vol) of potassium sorbate for 60 seconds.

- Punctured 10 holes in each sausage link similar to Section 3.1.2.2.

Sausages from each treatment were sampled. Two sausages were analysed for the initial potassium sorbate residue (see Section 3.2.4). The other two were analysed for the initial viable surface yeasts and moulds (see Section 3.4.5).

- Diferent treatments were incubated separately under the drying stage conditions.

- During the incubation period surface yeasts and moulds were enumerated when they could be observed by visual inspection.

- Every four days, six sausages from each treatment that were not showing surface mould were cooked, each treatment with separate cooking oil, and presented to the same group of panelists. Each panelist was presented with one sausage from each treatment and asked for overall acceptance or non-acceptance of the samples. The test was carried out up to 14 days after production.

The preliminary study showed that as low as 5% sorbate solution could extend the shelf life at least up to 14 days. However, it was also found that holding the sausage under the drying stage conditions up to 12 days yielded an overdried product and the product was too hard to be accepted. Commercially the sausage is held in two stages:

(i) Drying stage:

Normally, this drying stage is accomplished by sun-drying for two days (average 30-32°C, 75% R.H.).

(ii) Packaging stage:

After sun-drying stage, the sausage is packed in a plastic bag and kept at room temperature until it is purchased (30-32°C, 97-99% R.H.).

Hence, in order to prevent the excessive drying the commercial conditions were simulated for the next studies.

The investigation of the minimum effective level of potassium sorbate that could extend the sausage shelf life up to 14 days under the simulated commercial conditions was carried out as follows:

- Sausages were prepared, mixed, stuffed, dipped in potassium sorbate solution and the casings punctured similar to the preliminary study. The potassium sorbate concentrations were 0, 1, 2, 3 and 4%.

- The different treatments were incubated separately under drying stage conditions for two days and transferred to an incubator at the 30-32°C and 97-99% R.H. (packaging conditions).

- The sausages were inspected for surface mould growth by visual inspection.

- The treatments that gave a shelf life up to 14 days were analysed for residual potassium sorbate (see Section 3.2.4), cooked and presented to the panelists as in the preliminary study.

4.6.3 EFFECT OF POTASSIUM SORBATE ON FERMENTED ACIDULATED SAI KROK PREW

It was found from Section 4.6.1 that dipping the GDL acidulated sausage in 4% (wt/vol) solution of potassium sorbate for 60 seconds could extend the shelf life up to 14 days under the simulated commercial conditions. In this experiment, Lactacel MC was employed to convert sugar in the sausage mix into lactic acid.

The effect of potassium sorbate 4% solution on microbial acidulated Sai Krok Prew shelf life and acceptability were carried out as follows:

- Sausage ingredients similar to those described in Section 4.4.4 with 150 ppm sodium nitrite level were used. The ingredients were prepared, mixed, stuffed, treated with 4% (wt/vol) potassium sorbate and incubated under the simulated commercial conditions similar to Section 4.6.2.

- The sausages were observed for surface mould.

- After holding for 14 days, the sausages were cooked and presented to the panelists in the same manner as in Section 4.6.2.

To determine the effect of dipping fermented Sai Krok Prew in potassium sorbate on rate of pH reduction:

- Prepared and mixed sausage ingredients similar to those described in Section 4.4.4 with 150 ppm sodium nitrite.

- Divided the sausages into two halves. The first half was stuffed and incubated under the drying stage conditions. The second half was stuffed, treated with 4% potassium sorbate in the same manner as in Section 4.6.2 and incubated under the same conditions similarly to the untreated half.

- At each time interval duplicated samples of sausages from each treatment were measured for pH.

- The results were analysed for analysis of variance to observe if there was a difference in the rate of pH reduction between the treated and untreated sausages.

4.7 THE EFFECT OF VACUUM PACKAGING ON SHELF LIFE OF MICROBIAL FERMENTED SAI KROK PREW

The objective of this experiment was to investigate the effect of packaging upon the shelf life of microbial fermented sausage. The experiment was carried out as follows:

- Sausages were prepared similar to those described in Section 4.4.4 with 150 ppm sodium nitrite.

- After stuffing, sausages were packed in gas impermeable pouches with two links of sausages in each pouch.

- The pouches were evacuated by the vacuum pump for one minute and heat sealed, then incubated at 30-32°C.

- Every two days one pouch was randomly sampled for any off-odour. Surface mould was also observed by visual inspection.

4.8 CHEMICAL COMPOSITION OF FERMENTED SAI KROK PREW

Fermented Sai Krok Prew was analysed for moisture, protein, fat and ash content after being incubated for 14 days under the simulated commercial conditions.

CHAPTER 5

RESULTS

5.1 pH REDUCTION USING CHEMICAL ACIDULANTS

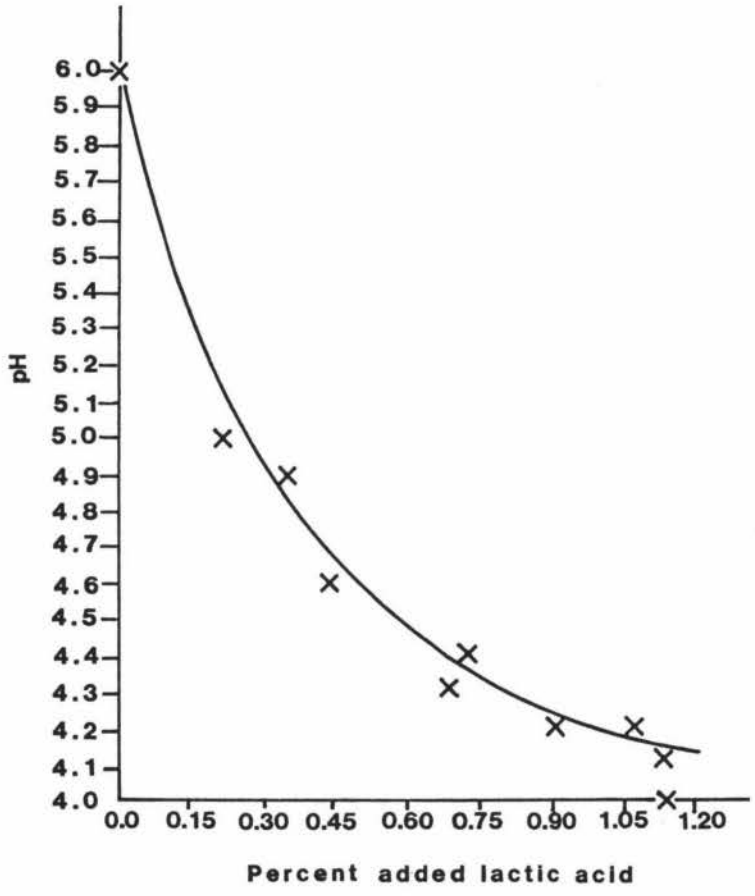
5.1.1 LACTIC ACID

The relationship between added lactic acid and reduction of sausage mix pH is shown in Figure 6 (detailed results in Appendix 7). It was found that the relationship between the amount of added acid and pH reduction was nonlinear. Rapid pH reduction occurred between pH 6.0 - 5.0 and very slow reduction below pH 4.4, i.e. more acid was needed to accomplish the same reduction of pH below 4.4.

The panelists were presented with four different levels of chemically acidified sausages (pH 6.0, 4.9, 4.4 and 4.2) and asked for the most desirable level of sourness. It was found that 60% of the panelists preferred the pH 4.4 sample, and the other 40% the pH 4.2 sample. Therefore, to achieve the most desirable level of sourness, added lactic acid at the range of 0.72 - 1.08% (wt/wt) was required.

It was also found from this experiment that the addition of lactic acid solution gave poor texture sausage. Sausage mix was watery and there was a lot of free fluid oozed out during the stuffing process, especially with the samples which had pH lower than pH 4.6.

Figure 6 Relationship between added lactic acid and pH reduction in sausage mix



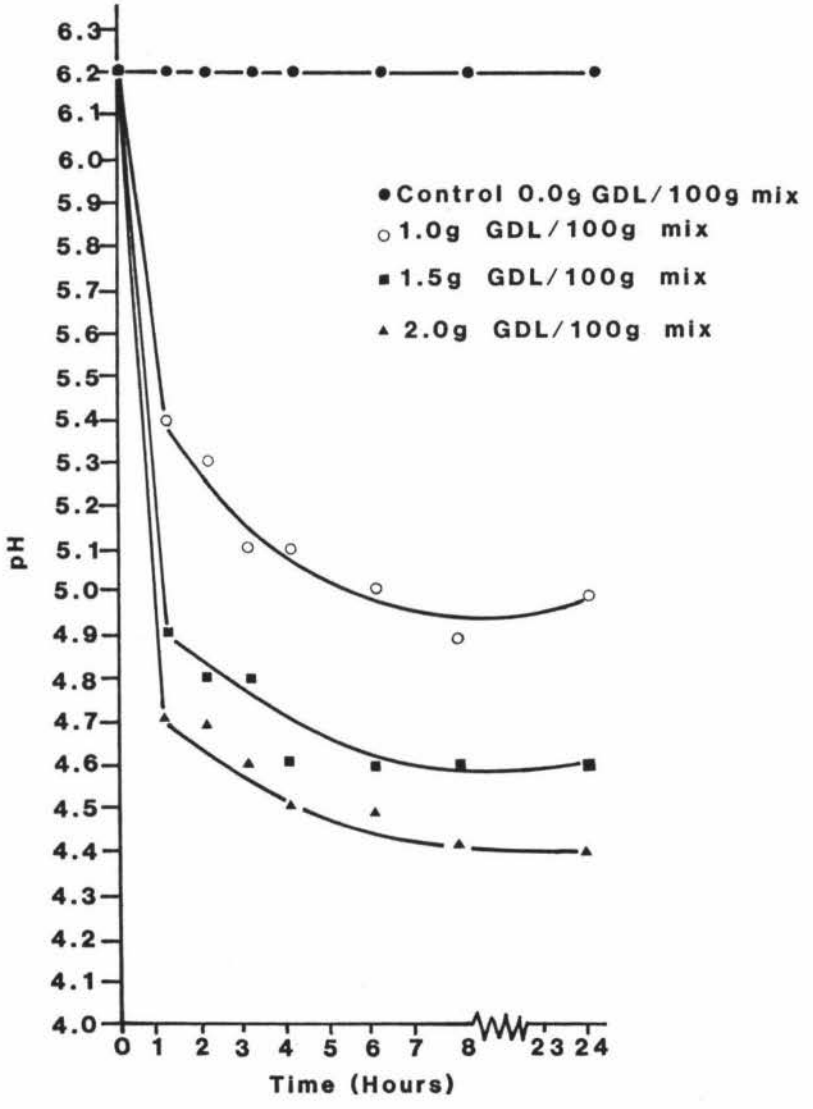
5.1.2 GLUCONO-DELTA-LACTONE (GDL)

Four levels of GDL were added to the sausage mix. The change in pH of the sausages with time in the incubator is shown in Figure 7 (detailed results in Appendix 8).

It was found that the pH of the control (0 g GDL/100 g mix) did not decrease and maintained above 6.0 throughout the experiment. The pH of the other three treatments decreased rapidly during the first three hours and continued to decrease very slightly up to 24 hours. The relationships between pH reduction and GDL concentration was nonlinear with 0. 1.0, 1.5 and 2.0 g GDL/100 g sausage mix decreasing the sausage mix by 0, 1.2, 1.6 and 1.8 pH units respectively.

At the concentration of GDL 2.0 g/100 g sausage mix was not exactly on the ideal of pH 4.2 - 4.4 but by this time the sausage had a very harsh flavour. Therefore, decreasing the sausage pH lower than pH 4.4 by increasing the concentration of GDL was undesirable. It was also found that the texture of the sausages from all treatments was good.

Figure 7
pH change with time in the incubator (30-32 °C,75%R.H.)
for sausages formulated with different levels of GDL



5.2 COOKING STABILITY

Sai Krok Prew was acidified with 2 g GDL/100 g sausage mix and held at various times under the drying stage conditions. Chemical compositions of Sai Krok Prew and cooking stabilities at different stages are shown in Table 9.

Table 9: Sai Krok Prew cooking stability at various holding times under the drying stage conditions

Holding time (hour)	pH	Uncooked sample		Cooked sample		
		moisture content (%)	fat content (% dry wt)	moisture content (%)	fat content (% dry wt)	cooking stability
0	6.1	54.9	41.0	47.8	30.0	Slightly burst
1	5.0	53.1	44.3	47.4	32.0	Slightly burst
3	4.5	52.9	46.0	45.6	32.8	Burst
5	4.4	52.2	46.3	44.9	33.8	Burst
7	4.4	51.0	50.3	46.7	34.9	Burst
24	4.4	47.1	52.6	43.3	35.1	Burst
48	4.4	43.6	60.0	42.1	37.3	Not burst
72	4.4	42.0	60.0	42.1	37.7	Not burst

With the use of 2 g GDL/100 g sausage mix, the cooking stability was improved after holding the sausage 48 hours under the drying stage conditions, i.e. the bursting of the sausages decreased on drying. Moisture content of the sausage decreased from 54.9% to 43.6% and pH decreased from 6.1 to 4.4 during this period. Fat content (% dry wt) increased from 41.0 to 60.0% but not proportionally to the decrease in moisture content.

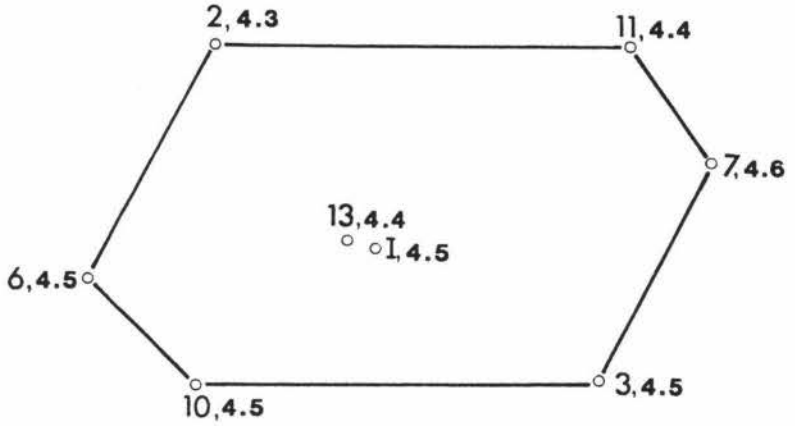
5.3 MIXTURE DESIGN

5.3.1 CHEMICAL COMPOSITIONS IN MIXTURE DESIGN

Chemical compositions of the design points immediately after the addition of GDL, before cooking and after cooking, are shown in Table 10. pH before cooking, moisture and fat content after cooking, of each vertex are also demonstrated in Figures 8 and 9 respectively.

Figure 8

pH values at the design vertices of mixture before cooking



I = Optimized vertex

Figure 9

Moisture and fat content (dry basis) of mixture design vertices after cooking

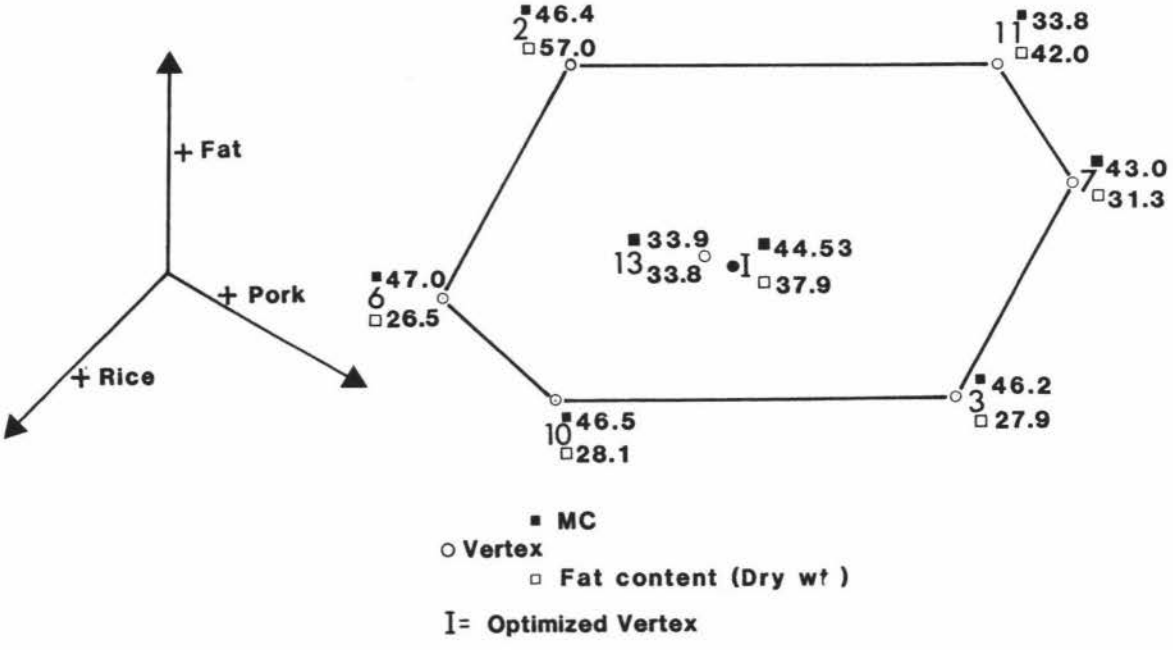


Table 10: Chemical compositions of Sai Krok Prew immediately after the addition of GDL, before cooking and after cooking

Vertex no.	Immediately after the addition of GDL			Before cooking			After cooking	
	pH	Moisture content (%)	Fat content (% dry basis)	pH	Moisture content (%)	Fat content (% dry basis)	Moisture content (%)	Fat content (% dry basis)
2	6.1	51.9	45.7	4.3	46.5	51.6	46.4	57.0
3	6.1	59.6	39.4	4.5	52.5	42.0	46.2	27.9
6	6.1	54.6	45.5	4.5	50.4	41.6	47.0	26.5
7	6.1	56.3	50.4	4.6	52.4	50.1	43.0	31.3
10	6.1	56.7	58.5	4.5	52.5	56.7	46.5	28.1
11	6.1	50.2	63.7	4.4	48.4	54.8	33.8	42.0
13	6.1	55.5	48.2	4.4	49.7	47.5	33.9	33.8

From the chemical composition it was found that the moisture content was reduced partly on drying and further on cooking. Some vertices lost a greater amount of moisture than the others. In particular, vertices nos. 11 and 13. The average moisture loss was 4.6% during the drying stage and a further 7.9% was lost during the cooking process. The pattern of change in fat content was not obvious during the drying stage but the fat content changed obviously during the cooking process. The average fat content (dry basis) decreased 1% during the drying stage and a further 14% during the cooking process. The pH differences between vertices both immediately after the addition of GDL and before cooking were very slight for all vertices.

From Figure 9 it can be seen that after cooking, vertices no. 11 (which contained a low level of rice and a high level of fat) and no. 13 (mid point of all component percentages) was found to contain low moisture 33-34%, and the remaining vertices had fairly high moisture contents 43.0 - 47.0%. Vertices nos. 3, 6 and 10 which had low pork fat in the formulae were found to contain low fat content (26.5 - 28.1%) after cooking. Vertices nos. 2 and 11 which had high pork fat in the formulae were found to contain high fat content after cooking, 57% and 42% respectively. The centroid (13) was found after cooking to have a low moisture content and fairly low fat content, 33.9% and 33.8% respectively.

5.3.2 SENSORY SCORES IN MIXTURE DESIGN

The mean scores for all judges were determined for each texture attribute and the overall texture acceptability. The ratios of the texture score and the ideal scores for each judge were determined and then calculated for the mean ratio for every texture attribute (except the overall texture acceptability because the ideal point was always 9). The mean score, mean ratio and the mean of overall texture acceptability for each mixture design vertices are shown in Table 11.

Table 11: Mean score and mean ratio of mixture design vertices

Vertex No.	Firmness		Rubberiness		Juiciness		Oiliness		Smoothness		Stickiness		Mean of Overall Texture Acceptability
	Mean Score	Mean Ratio	Mean Score	Mean Ratio	Mean Score	Mean Ratio	Mean Score	Mean Ratio	Mean Score	Mean Ratio	Mean Score	Mean Ratio	
2	4.0	0.7	3.7	0.7	6.0	1.0	5.6	1.1	7.4	1.2	4.8	0.8	5.2
3	7.0	1.3	5.7	1.1	4.2	0.7	4.3	0.9	5.1	0.8	5.7	0.9	5.5
6	4.7	0.8	6.0	1.1	5.6	0.9	5.0	1.1	6.0	0.9	6.1	1.0	5.5
7	6.5	1.3	5.3	1.0	4.6	1.0	4.4	0.9	4.3	0.7	5.5	0.9	6.3
10	5.2	1.0	4.7	0.9	5.5	1.0	5.2	1.1	6.4	1.0	5.4	0.9	5.7
11	6.1	1.2	4.5	0.8	4.2	0.7	4.5	1.0	5.6	0.9	3.9	0.6	4.9
13	6.0	1.0	5.4	1.0	4.4	0.7	4.5	0.9	4.5	0.7	5.6	0.9	7.1
Average		1.0		1.0		0.9		0.9		0.9		1.0	

The ranges of mean ratio for firmness, rubberiness, juiciness, oiliness, smoothness and stickiness were 0.7 - 1.3, 0.7 - 1.1, 0.7 - 1.0, 0.9 - 1.1, 0.7 - 1.2 and 0.6 - 1.0 respectively. The smaller the range indicated the products were closer to the ideal product texture. The oiliness of all samples was very close to ideal and firmness showed the widest variation from the ideal but no factor varied a great deal from the ideal. The differences in the mean ratios was not due to any single level of pork, fat and rice but rather due to the interaction of these three factors.

5.3.3 PREDICTION EQUATION

The matrix to determine the equation for predicting the relationship between pork, fat and rice and its effect on the overall texture acceptability score is shown in Table 12.

Table 12: Extreme vertices and average overall texture acceptability scores

Vertex	Proportion of Pork	Proportion of Fat	Proportion of Rice	Acceptability Sensory Score
2	0.50	0.30	0.20	5.2
3	0.70	0.15	0.15	5.5
6	0.50	0.20	0.30	5.5
7	0.70	0.25	0.05	6.3
10	0.55	0.15	0.30	5.7
11	0.65	0.30	0.05	4.9
Centre (13)	0.60	0.22	0.18	6.6

The prediction equation containing no constant term was

$$y = -6.13X_1 - 107X_2 - 25.8X_3 + 196X_1X_2 + 49.2X_1X_3 + 184X_2X_3$$

where X_1 = pork ratio

X_2 = fat fatio X_3 = rice ratio

5.3.4 TESTING THE PREDICTION AND OPTIMIZATION

From the calculation in Section 4.3.3, it was predicted that the overall texture acceptability score (y) would be maximized at 6.6 when pork, fat and rice ratio equal to 0.6179, 0.2169 and 0.1652 respectively. The predicted proportions and acceptability sensory score were almost identical to the centre point of the designed vertices. The experimentation with the predicted proportions was carried out (see Section 4.3.4). The result for average overall texture acceptability from the panelists was found to be 7.1.

The difference between the predicted and actual score was slight. Therefore, the result from this optimization could be adopted. The chemical composition and sensory score of all texture notes were also presented in Appendix 9.

5.4 STARTER CULTURE FOR ACID FERMENTATION

5.4.1 CELL CONCENTRATION OF LACTACEL MC

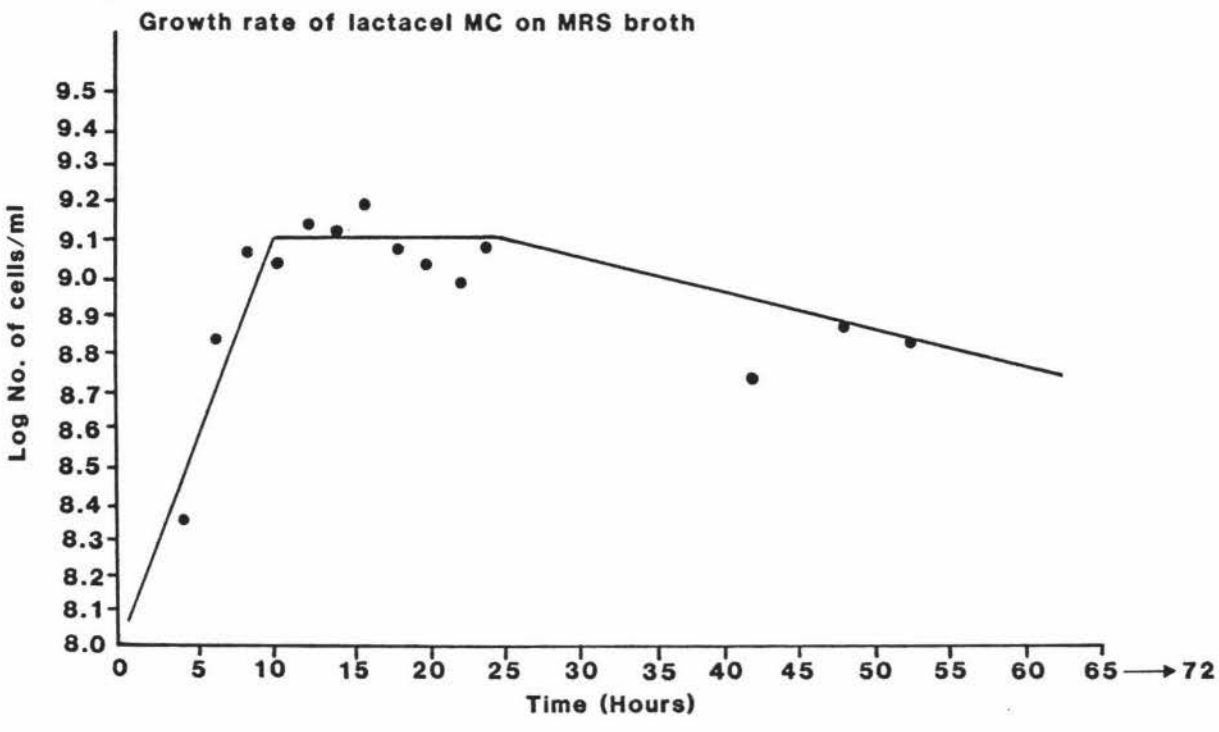
Cell concentration of Lactacel MC was determined using MRS agar and layer-plate technique. It was found that the concentration of Lactacel MC was 2.95×10^{10} cells/g.

The recommended inoculation rate (use rate) for Lactacel MC is 0.125% (wt/wt) of the sausage formula. Therefore, this recommended usage rate will provide 3.69×10^{7} cells of viable *P. cerevisiae* and *L. plantarum* organisms per gram of sausage mix.

5.4.2 GROWTH RATE OF LACTACEL MC ON MRS BROTH

The growth rate of Lactacel MC on MRS broth was determined using MRS agar and layer-plate technique. The result is shown graphically in Figure 10.

Figure 10



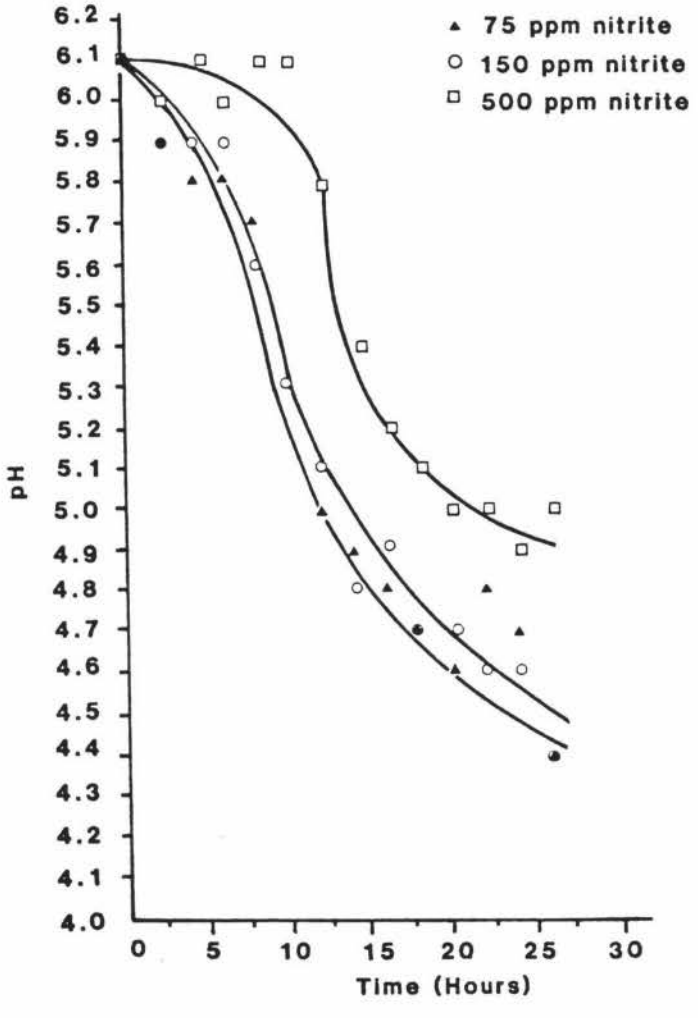
It took nine hours for Lactacel MC to reach the stationary phase. After incubation for 25 hours the growth curve started to level off and went into the decline phase.

5.5 THE EFFECTS OF NITRITE AND DEXTROSE ON THE RATE OF pH REDUCTION

5.5.1 EFFECT OF NITRITE ON THE RATE OF pH REDUCTION

The effect of nitrite levels at 75, 150 and 500 ppm in the formula on rate of pH reduction is shown in Figure 11 (detailed results in Appendix 10).

Figure 11
Effect of nitrite on the rate of pH reduction



After the sausages were fermented 26 hours the pH of 75 and 150 ppm nitrite treatments were 4.4 while the 500 ppm nitrite still remained at 5.0. The analysis of variance to determine if there was a difference between the mean reduction of pHs found that there was a significant difference in rate of pH reduction among the treatments. By Scheffé's method (Scheffé, 1959) it was found that the rate of pH reduction of

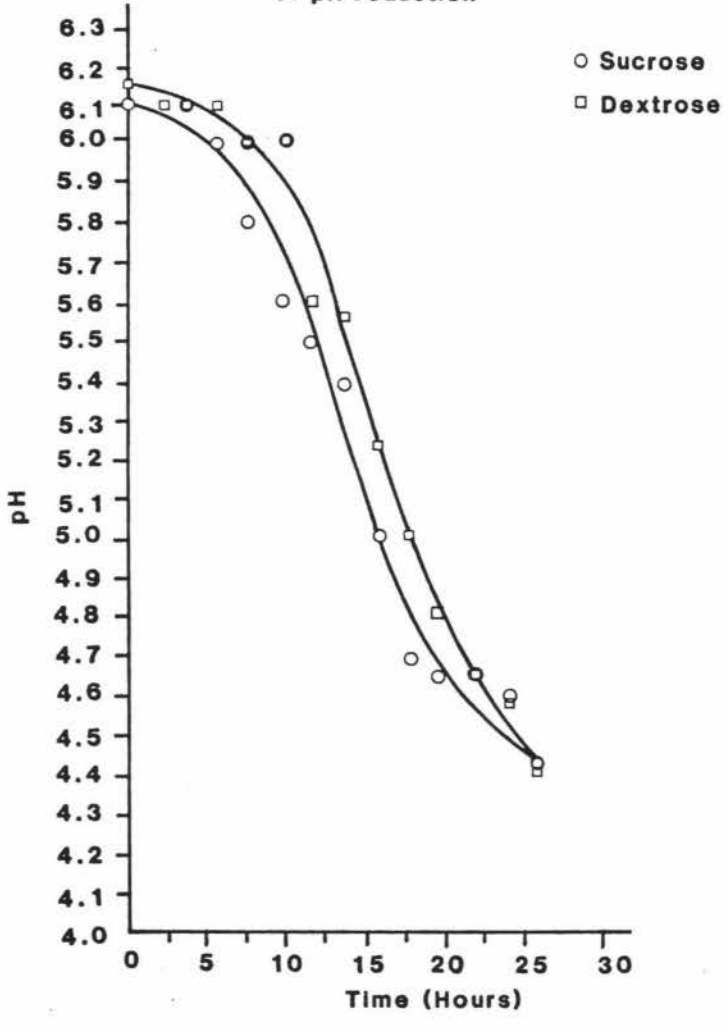
of 500 ppm nitrite treatment was significantly slower than 75 and 150 ppm treatments (at 25% level), but there was no significant difference between 75 and 150 ppm nitrite treatments.

5.5.2 EFFECT OF SUCROSE AND DEXTROSE ON RATE OF pH REDUCTION

The effect of sucrose and dextrose on the rate of pH reduction is shown in Figure 12 (detailed results in Appendix 11).

Figure 12

Effect of sucrose and dextrose on the rate of pH reduction

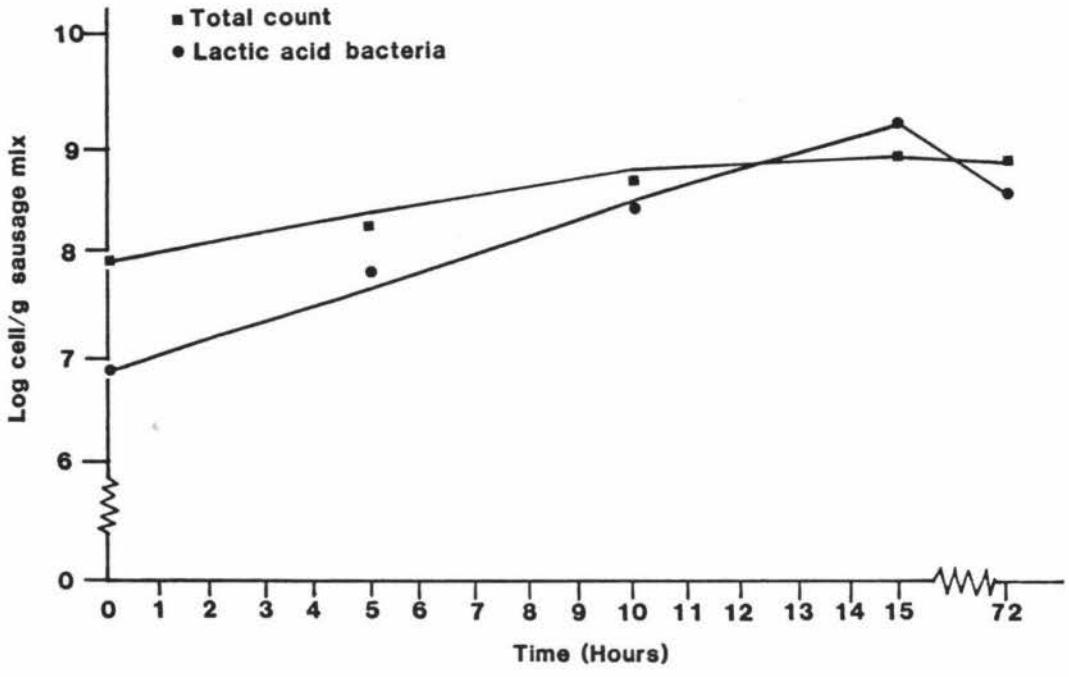


After the sausages were fermented for 26 hours, the pHs of both treatments was 4.4. The analysis of variance to determine if there was a difference between the mean reduction of pHs found that there was no significant difference between the treatments.

5.5.3 MICROBIAL GROWTH DURING FERMENTATION

Total viable microorganisms and lactic acid producing bacteria during the fermentation is shown graphically in Figure 13.

Figure 13
Changes in microbial growth during fermentation

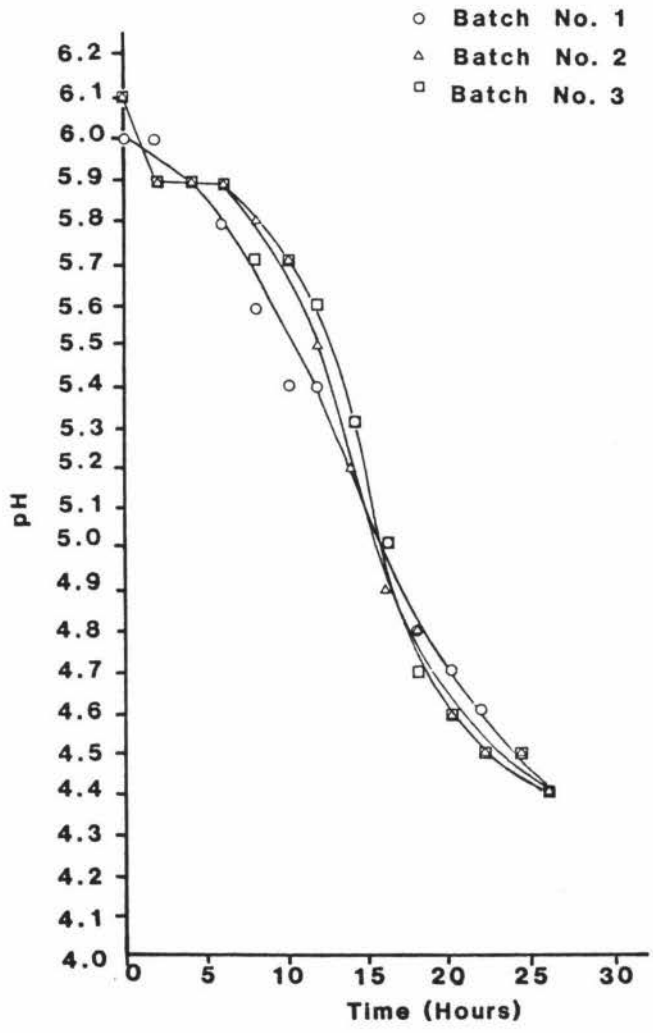


The difference between the number of total viable microorganisms and lactic acid producing bacteria was slight. The numbers of both types reached a maximum after being incubated for 15 hours.

5.5.4 THE CONSISTENCY OF pH REDUCTION USING LACTACEL MC

Three batches of Sai Krok Prew were produced at two-day intervals. Each batch contained 150 ppm nitrite and 0.5% sucrose. The results are shown in Figure 14 (detailed results in Appendix 12).

Figure 14
Reproducibility of Lactacel MC to reduce pH with different batches of Sai Krok Prew



The differences in rate of pH reduction between batches were very slight. After the sausages were fermented for 26 hours, the pH of all batches were reduced to pH 4.4. The analysis of variance to determine if there was a difference between the mean reduction of pHs found that there was no significant difference between batches.

5.6 EFFECT OF POTASSIUM SORBATE ON SHELF LIFE AND ACCEPTABILITY SAI KROK PREW

5.6.1 SHELF LIFE OF GDL ACIDULATED AND FERMENTED SAI KROK PREW

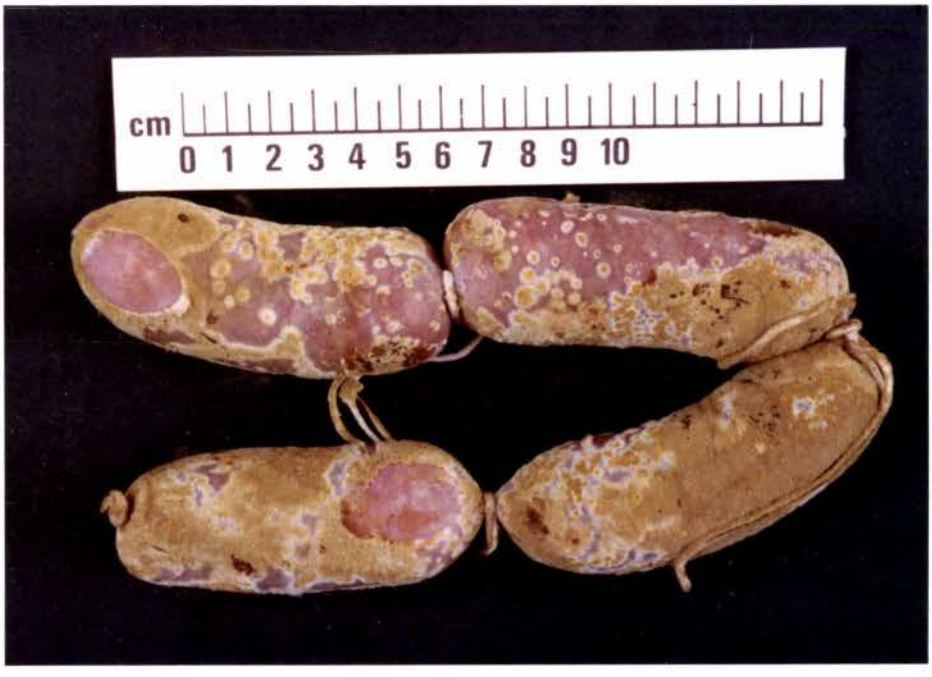
Shelf life of GDL acidulated sausage and bacterial fermented sausage were investigated. It was found that the shelf life for both types of sausages was three days. The white and yellow mycelia were found to be the dominant moulds that caused spoilage (see Figure 15).

5.6.2 EFFECT OF POTASSIUM SORBATE ON SHELF LIFE OF SAI KROK PREW

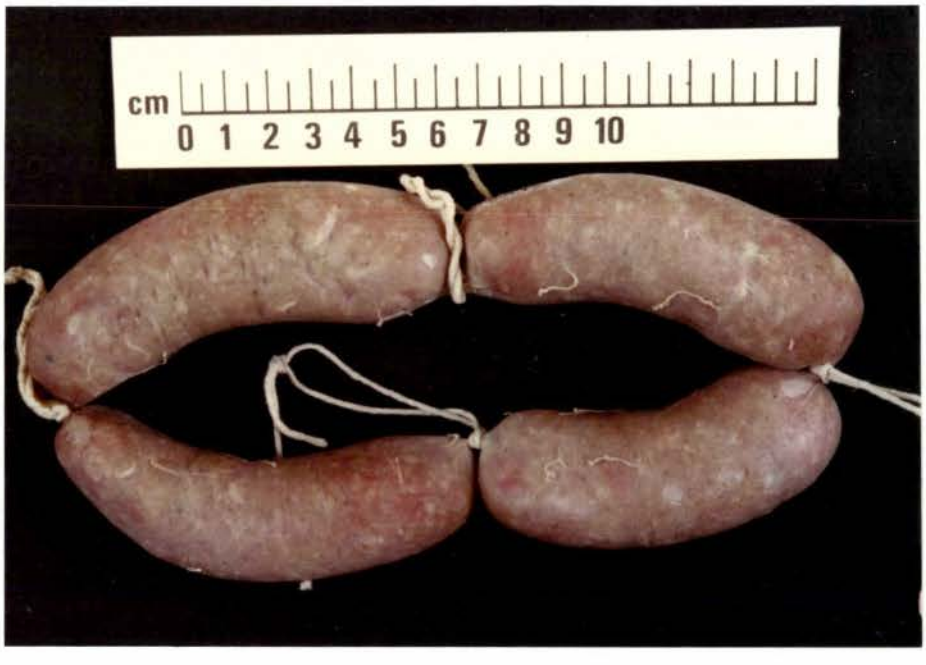
Treatment with 5, 10 and 15% potassium sorbate increased the shelf life from three days to longer than 14 days. But the taste panelists started to reject these 5, 10 and 15% potassium sorbate treated sausages after being held 12 days under the drying stage conditions because of the overdryness. The sausage texture became too hard to be accepted. The initial potassium sorbate residue, moulds and yeasts on the casings are shown in Table 13.

Figure 15

Sai Krok Prew three days after processing



Control



Treated with 4% potassium sorbate solution

Table 13: Initial (day 0) potassium sorbate residue[a/], mould and yeast on the sausage casing

Dipped in potassium sorbate solution %	Potassium sorbate residue			Mould	Yeast
	ppm per g sausage casing	ppm per sq cm sausage casing	ppm per g sausage mix	per sq cm (estimated)[b/]	per sq cm (estimated)[b/]
0	0	0	0	170	700
5	2964	54	42	<100	627
10	4598	84	65	<100	181
15	13741	250	194	<100	320

a/ All values expressed as ppm sorbic acid

b/ Speck (1976)

From the result above, it was found that sorbate residues on the casing were not proportional to the concentration of sorbate solutions, most likely because more of the solution adhered to the skin at the higher concentrations. The initial number of moulds per square centimetre of sausage casings was found to be between 170 (Est.) to less than 100 (Est.). The initial number of yeasts per square centimetre of sausage casings were found to be between 700 to 181 (Est.).

The minimum level of potassium sorbate solution required was investigated by mixing more dilute sorbate solutions, 1-4%. The results are shown in Table 14.

Table 14: Shelf life of GDL acidulated sausage treated with different levels of potassium sorbate

Potassium sorbate solution (%)	Days before surface mould appeared
0	4
1	6
2	9
3	9
4	>14

Only at 4% level could sorbate extend the shelf life up to 14 days. The potassium sorbate residues (expressed as sorbic acid) after processing and 14 days after processing were 2,447 and 98 ppm respectively.

All panelists accepted the samples treated with 4% potassium sorbate solution and had no complaint about the overdryness because the sausages were dried only 48 hours.

5.6.3 EFFECT OF POTASSIUM SORBATE ON SHELF LIFE OF SAI KROK PREW

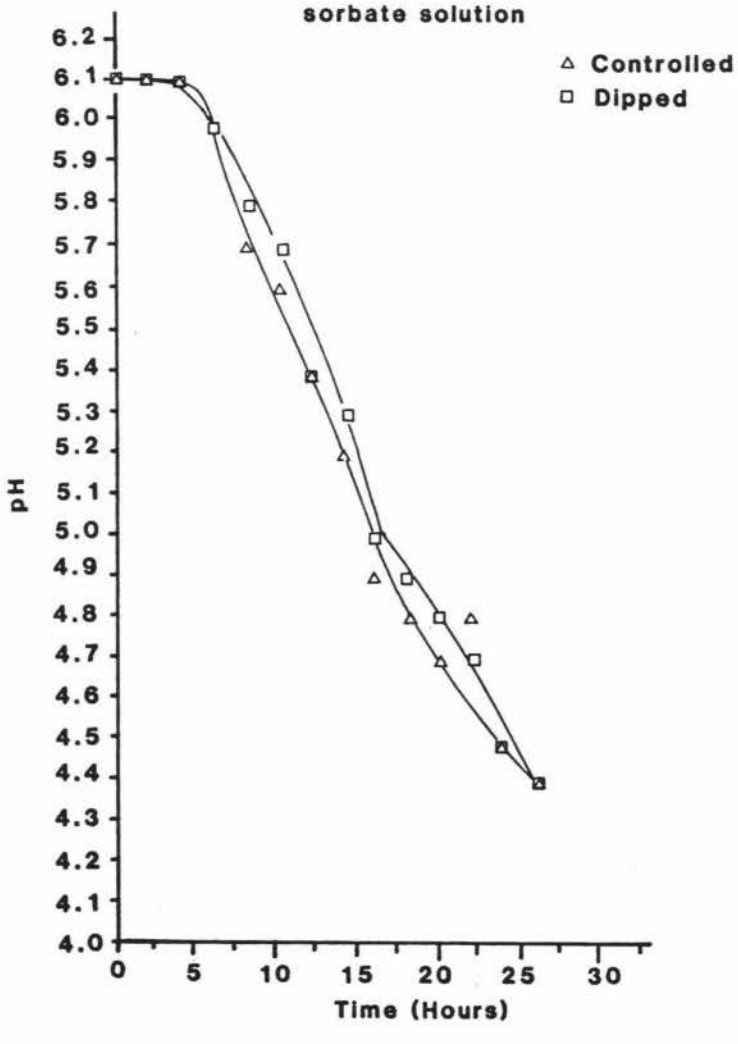
It was found that by dipping the fermented sausage in 4% potassium sorbate solution, this could extend the shelf life up to 14 days. The effectiveness of potassium sorbate was the same for GDL acidulated and bacterial fermented sausages.

The sausage was cooked and presented to the taste panelists after incubation for 12 days under the simulated commercial conditions. All panelists accepted the sample.

The effect of dipping fermented Sai Krok Prew in 4% potassium sorbate solution on rate of pH reduction is shown in Figure 16 (detailed result in Appendix 13).

Figure 16

Effect on rate of pH reduction of dipping fermented Sai Krok Prew in 4% potassium sorbate solution



After the sausages were fermented for 26 hours the pH of the controlled and dipped treatments were found to be the same, at 4.4. The analysis of variance to determine if there was a difference between the mean reduction of pHs found that there was no significant difference between the controlled and dipped treatments.

5.7 THE EFFECT OF PACKAGING IN GAS IMPERMEABLE FILM ON SHELF LIFE OF SAI KROK PREW

It was found that the sausage shelf life could be extended up to 10 days by using vacuum packaging in a gas impermeable pouch, and evacuating air in the pouch before heat sealing. After 10 days in the pouch the surface mould started to appear. There was no evidence of putrid odour during the keeping period.

5.8 CHEMICAL COMPOSITIONS OF FERMENTED SAI KROK PREW

The chemical compositions of fermented Sai Krok Prew 14 days after production were as follows:

- Protein 24.91%
- Fat 28.51%
- Moisture 31.83%
- Ash 4.25%

CHAPTER 6

DISCUSSION

In this study of the processing of Sai Krok Prew, three important areas affecting the final quality of the Sai Krok Prew were selected:

- the type and rate of acid production;
- the proportion of pork, fat and rice;
- surface mould growth affecting storage.

6.1 GENERAL ASPECTS OF ACID PRODUCTION IN SAI KROK PREW

The important aspects that were studied were:

i. the use of adding chemical acidulants - lactic acid solution and Glucono-Delta-Lactone (GDL) powder to produce artificially acidifed Sai Krok Prew;

ii. the use of pure starter culture to produce Sai Krok Prew;

iii. the effect of sugar and nitrite upon the rate of acid fermentation.

6.1.1 PRODUCTION OF CHEMICALLY ACIDIFIED SAI KROK PREW

Lowering the pH of sausage mix with straight lactic acid solution was very rapid; with GDL it was slower but still very much faster than in the fermentation. The hydrolysis of GDL to gluconic acid, which lowered the pH value was found to be most rapid during the first hour after addition and then continued for a three-four hour period. This result was similar to that obtained by Sair (1961) who stated that

approximately 25% of the lactone hydrolysis occurred in the first ten minutes, and the remainder hydrolysed more slowly over a three hour period. The relation between pH reduction and addition of both lactic acid solution and GDL powder was non-linear, i.e. more acid was required per unit drop of pH as the pH was lowered. This suggested that there must be some buffering substances in the sausage mixture, most likely from the pork. Bate-Smith (1948) indicated that buffering substances in meat such as protein, the phosphate compounds, carnosine, anserine, and even lactic acid itself (at the lower pH ranges) aid in preventing rapid pH change. In this experiment the lowest pH attainable with chemical acidulants was 4.1.

A poor, watery texture was found with lactic acid treatment and there was a lot of free fluid released during the stuffing process. This 'shorting out' was found to be more obvious with the treatment with a pH less than 4.6. The 'shorting out' and poor watery texture was not found with the GDL treatment and the Sai Krok Prew had a better texture. The watery texture of the lactic acid treated Sai Krok Prew can be explained by the very rapid drop in pH. The water holding capacity (WHC) of the pork tissue falls with pH because of an overall reduction of reactive groups available on the proteins for water binding (Forrest, 1975). Minimum water is retained around 5.0 - 5.1 which corresponds approximately to the isoelectric point of the fibrillar proteins in the normal ionic environments (Sair and Cook, 1938; Grau *et al.*, 1953). Where sodium chloride is added to the meat as in Sai Krok Prew, the isoelectric point is moved towards a lower pH (Niivaara and Pohja, 1954).

In this case minimum WHC is in the pH range between 4.0 - 4.5. This corresponds with the result in the present experiment where in the straight lactic acid treatment there was a poor watery texture at pH below 4.6. The 'shorting out' and poor watery texture was not found in the GDL treatment, which gave a very much better texture in Sai Krok Prew than lactic acid. This result confirmed the statement of Everson (1981) that the proper use of GDL permits the gluconic acid to be formed after the sausage has set up and 'shorting out' is avoided. The rapid drop in pH by straight lactic acid solution might cause an excessive protein denaturation which caused the 'shorting out'. Furthermore, the addition of lactic acid solution introduced the undesirable moisture into the sausage mix and enhanced the 'shorting out' incidence.

The rapid decrease in WHC (especially with lactic acid treatment), increasing the amount of unbound water, caused bursting. This unbound water may be vapourised during the cooking process, increasing the pressure inside the casing and so causing the bursting of Sai Krok Prew. However, bursting could also have been caused by high rapid fat release during the cooking process. Sai Krok Prew is not an emulsion sausage, and therefore the fat particles are not coated by solubilized proteins. The high cooking temperature (130°C) melted the fat into oil and this oil could have increased the pressure inside the casing. If the moisture vapour and the hot oil were not released quickly enough, the accumulated pressure could have caused bursting. The bursting was found to be more severe with lactic acid

solution treatment than GDL treatment. The reason for this was most likely that the addition of lactic acid caused a sudden decrease in pH, whereas GDL permitted the gluconic acid to be formed and decrease the sausage pH slower than lactic acid treatment. This was confirmed when the Sai Krok Prew were cooked immediately after stuffing. There was a great deal of bursting with the lactic acid treated Sai Krok Prew, but with the use of GDL, it was found that only a few sausages were slightly burst when cooked. The pH of GDL sausage mix was still relatively high, between 5.0 and 6.0 during the first hour after the addition of GDL. In this pH range, the WHC is reasonably high and would retain most of the water in the sausage mix in the form of bound water. As the pH of GDL treated Sai Krok Prew was reduced further, bursting increased. If kept long enough, i.e. 48 hours there was a significant loss of moisture from the sausages by evaporation and drip and bursting on cooking was reduced. There did not appear to be any loss of fat on storage, the relative fat content increasing. The decrease in moisture content and increase in fat content were not exactly proportional. This might be due to the loss of some water soluble compounds and salt soluble proteins with the drip.

The aroma and flavour of chemically acidifed sausages were found to be much inferior to those of fermented sausages. There was a lack of aroma and the flavour was harsh. However, it was found that the panelists accepted the flavour of GDL treated sausages more than that of the lactic acid treated sausages. This was also reported by Everson (1981). He also stated that the flavour achieved was

not a characteristic lactic acid flavour or tang as in the fermented sausage. Nurmi (1966b) showed the occurrence of a peculiar sweetish flavour in GDL sausages but this was not found in this present experiment.

6.1.2 THE UNIFORMITY OF SAI KROK PREW QUALITY USING A STARTER CULTURE

It was found that the rate of pH reduction was the same for all experimental batches and agreed with the technical bulletin of the starter culture supplier (Anon, 1981). The technical bulletin stated that a pH of 5.0 or less can be expected in less than 24 hours. Once again this proved that Lactacel MC can be applied to the Sai Krok Prew system. This rate of pH reduction was very similar to Rongvisit's work, although she did not use pure starter culture or back-slopping. The reason for this might be the presence of lactic acid bacteria on the equipment, working tables, and storage shelves and rooms used over and over again for sausage making. This could heavily seed the sausages with the bacteria necessary for fermentation. This was shown by comparing the number of lactic acid bacteria and rate of pH reduction during the initial stage, and the late stage of her work. She reported that with the same incubation temperature and formulation, the number of lactic acid bacteria were found during the initial experiments and later experiments to be $5.0 - 7.0 \times 10^5$ and 5.1×10^8 cells/g sausage, 24 hours after processing respectively, i.e. approximately three log cycles higher during the later runs. For the pH, Rongvisit required two-three days to reduce the pH down to 4.5 during the initial runs, but only 24 hours was required during the later runs.

It is most likely that the use of starter culture would not increase greatly the rate of pH reduction in commercial fermentation. However, this present experiment showed that the pure starter culture gave improved microbiological control of other microorganisms than the starter culture. Immediately after inoculation, the total viable count for the sausage was found to be a log cycle higher than the lactic acid bacteria count. During fermentation to pH values of 6.0, 5.3, 4.8 at times 5, 10, 15 hours after inoculation respectively, there was a stepwise increase in lactic acid bacterial counts to the highest level of 1.9×10^9 cells/g sausage. Ten hours after inoculation the number of total viable bacteria were essentially the same as the number of lactic acid bacteria. This shows that Lactacel MC was actively growing shortly after it was inoculated into the sausage mix. The total viable count increased very slightly and this increase could be attributed to the increase of lactic acid bacteria. This means that the rapid action of starter culture inhibited the growth of undesirable bacteria which are usually present in the meat. In most of Rongvisit's work, there was a much greater ratio of total viable count to lactic acid bacteria count during the first 48hours after production. This meant that during the first 48 hours there were a high number of bacteria other than the desirable lactic acid bacteria, which might include pathogens. The reason that the starter culture treatment gave better microbiological control may be due to the level of addition of lactic acid bacteria which was in such numbers as to ensure dominance over the natural flora. The frozen Lactacel MC also included a nutrient medium and a

stabilizer, such as glycerol. This starter culture has a very short lag phase enabling the bacteria to begin growing almost immediately upon being warmed up.

Furthermore, this present experiment found that during the first five hours, the starter culture multiplied rapidly by approximately one log cycle, but did not yet produce acid. After five hours the rate of multiplication became slower but acid started to be produced.

The maximum number of lactic acid bacteria in MRS broth and sausage mix were essentially the same. Nevertheless, the maximum number of lactic acid bacteria in MRS broth was attained five hours faster than in the sausage mix. This presumably was due to the perfect formulation of MRS broth that suits the growth of lactic acid bacteria. Furthermore, the sausage mix contained cured salt which may slow down the multiplication of lactic acid bacteria.

There is a great deal of variation in final product pH in the commercial production of Sai Krok Prew (personal communication with many firms). The formulations, in particular salt and sugar levels, are varied according to the weather. This may introduce seasonal variability in product characteristics, e.g. change in flavour and sometimes cause no fermentation. The use of pure starter culture may reduce the variability. The benefit of using starter culture will be enhanced if the temperature and humidity are controlled during the first 24 hours of fermentation. The individuality and distinctiveness of Sai Krok Prew flavour was not affected by pure starter culture. The panelists could not distinguish any difference in flavour of

Sai Krok Prew produced with the use of pure starter culture from the conventionally made Sai Krok Prew. This is presumably because the product distinctiveness is associated with the spicing (especially the 2.5% fresh garlic), salting, degree of tang, residual sugar (if any) and meat formulation. This was also stated by Diebel (1974) that the removal of product individuality by starter culture is absolutely unfounded and the spices, etc. could overwhelm and essentially mask the differences.

6.1.3 THE EFFECT OF NITRITE ON THE RATE OF FERMENTATION

Nitrite has five distinct roles in sausage. Modification and stabilisation of the colour, production of the characteristic cured meat flavour, inhibition of putrefaction, prevention of botulism and an antioxidant effect. To achieve all these benefits from nitrite, the ingoing level of nitrite should be between 100 - 200 ppm (Ingram, 1974). Nitrite is currently allowed in Thailand for cured meat, up to a maximum ingoing level of 200 ppm.

The present practice in Thailand for Sai Krok Prew is to use up to 500 ppm of nitrate and/or prague powder. Straight nitrite is not used although the rate of the natural fermentation is relatively fast and the role of micrococci to the early part of fermentation may be limited (Krol and Tinbergen, 1974). It is unlikely that there is a significant reduction of the nitrate to nitrite. During storage before retail sale, there may be a slow breakdown of nitrate to nitrite. To obtain better control of the beneficial effect of nitrite, it would be preferable to add nitrite directly. This study showed there was no

significant difference in the rate of pH reduction when 75 and 150 ppm nitrite were added, but it was significantly slower with the addition of 500 ppm nitrite. This confirmed the recommendation of the supplier of the starter culture who recommended a nitrite level at 100 - 150 ppm sodium nitrite. Above this, the starter culture and the naturally occurring microflora may be inhibited.

In this present experiment using nitrite alone, it was found that the colour of Sai Krok Prew was a more pinky colour than the commercial product. The acceptability of this colour would have to be tested on the consumer in Thailand. The effect of nitrite levels on the fermentation by wild organisms would also have to be tested. The main problem in using nitrite is the possible formation of unacceptable levels of carcinogenic nitrosamines by interaction between nitrite and organic constituents. Studies up to date throughout the world have shown very few cases of nitrosamines formation in fermented sausage. Nevertheless, nitrosamines will have to be tested in Sai Krok Prew made with levels of nitrite recommended.

6.1.4 THE EFFECT OF SUGAR ON RATE OF FERMENTATION

The result from the experiment showed that sucrose or dextrose in the formulation were readily fermented at the same rate. This result confirmed Rongvisit's work and Acton *et al.* Rongvisit (1980) found that sucrose could be metabolised to produce lactic acid. Acton *et al.* (1977) found that *P. acidilactici* readily fermented sucrose

in fermented sausage and sucrose can be substituted for dextrose and yield a desirable fermentation. Acton *et al.* also found that there was no difference in the rate of fermentation between sucrose and dextrose. This is good from the industrial point of view as it can reduce the cost of production.

According to Ten Cate (1960) lactic acid is mainly formed following the stoichiometry of homofermentation. Each mole of glucose yields two moles of lactic acid by the Embden-Meyerhof pathway.

$$C_6H_{12}O_6 \longrightarrow 2CH_3CHOHCOOH$$

Based on this stoichiometric analysis one g of fermentable carbohydrates would yield one g lactic acid. The amount of fermentable carbohydrates in the formulation is important. The fermentable carbohydrates must be at a level to allow lactic acid bacteria in the mix to utilize it and produce sufficient acid to preserve the sausage and to produce the desirable flavour.

It was found that pH 4.4 was the most desirable level of sourness (60% of the panelists preferred the pH 4.4 and the other 40% the pH 4.2). Lactic acid 0.72% was required to decrease the pH down to 4.4. Therefore, it can be assumed for practical purposes that in fermented sausage lactic acid bacteria had to produce the same amount of lactic acid (0.72%) from added carbohydrates to reduce the pH down to this pH 4.4 to achieve the most desirable levels of sourness.

Therefore, from the stoichiometry, 0.72% of fermentable carbohydrates is required to produce 0.72% lactic acid. However, this stoichiometric calculation was based on the assumption of 100% conversion efficiency of lactic acid bacteria to convert fermentable carbohydrates to lactic acid.

However, the conversion efficiency of Lactacel MC to metabolise carbohydrate and produce lactic acid cannot be 100% (Buchanan and Gibbon, 1974; Pederson, 1979; Andersen and Ten Cate, 1965; Pezacki and Szotak, 1962; and De Ketelaere *et al.*, 1974). Nevertheless, the discrepancy between the calculation and the actual amount required should be small. Research at Kulmbach has shown that by using starter cultures, only lactic acid is produced, whereas small amounts of other acids such as acetic, propionic, butyric and pyruvic were recovered from commercial samples which presumably had not used starter cultures (Klettner and Baumgartner, 1980).

From the present experiments, it was found that lactic acid bacteria could produce lactic acid and reduce the sausage pH from 6.1 - 6.2 to 4.4 in 26 hours with either 0.5% sucrose or dextrose. Rongvisit (1980) also found that at the sucrose level of 0.5% the sausage pH was reduced from 5.9 to 4.4 in 24 hours. By the stoichiometric relationship, the pH from 6.0 to 4.4 required at least 0.72% fermentable carbohydrates. Therefore, lactic acid bacteria must have been able to metabolise other substrates in Sai Krok Prew, other than sucrose and dextrose to lactic acid.

The other substrates may be the glycogen in pork and the dextrins and starch in cooked rice. The amount of glycogen in pork should be very little. This means that cooked rice, which mainly contained polysaccharides, some dextrins and a neglible amount of mono and disaccharides (Richards and LeLievre, 1981) could be metabolised by lactic acid bacteria. *P. acidilactici* could develop some lactic acid from dextrin (Acton *et al.*, 1977), and the supplier of the starter culture claimed that Lactacel MC can produce acid from starch. However, Buchanan and Gibbons (1974) stated that Pediococcus cannot produce acid from starch but did not comment on the effect of Lactobacillus on starch. The report by Buchanan and Gibbons is presumably derived from experiments with laboratory enriched media and the activities of microorganisms may be different in sausage mix.

6.2 SAUSAGE FORMULATION

This present experiment confirmed that using the extreme vertices designs has an important contribution for conducting experiments with mixtures when several factors have constraints placed on them. These designs can guide to an optimum product with a small number of experiments.

The statement by Gorman (1966) seemed to agree with the result found in this present experiment - that the choice of constraints on the individual factors is crucial and often in exploratory work, the constraints cannot be set precisely and may have to be estimated experimentally. The preliminary experiment in this study showed that fat at 40% was too

extreme and the result helped to achieve a feasible region of composition. The feasible region in the final extreme vertices design covered the recommended formula given by Rongvisit (1980) and Lee (personal communication, 1980). The constraints in the final extreme vertices design were almost an ideal set of constraints because it was found that the centroid of the experiment yielded the highest overall texture acceptability, and was the closest to the optimum mixture.

Hare (1974) found that Lagrange multipliers was an effective technique to locate a maximum in a constrained system. Similarly, in this present experiment, Lagrange multipliers was shown to be a promising technique.

6.2.1 COMPARISON OF OPTIMUM FORMULATION WITH THAI FORMULATIONS

When compared with Rongvisit's formulation, it was found that the acceptability score could be improved by changing the proportion of pork from 50% to 58.7%, fat from 15% to 20.6% and rice from 25% to 15.7%. This was presumably due to the higher amounts of pork and fat providing a firm, juicy and oily product which was more desirable to the panelists. The decrease in the rice proportion yielded better acceptability, which may be due to the effect of rice causing grittiness to the sausage texture. However, there is one point that should be kept in mind; the size of the panel in this experiment was relatively small (due to the panelists' availability), and to confirm the acceptability a larger group in Thailand would have to taste the Sai Krok Prew.

The present optimum formulation would be more expensive than Rongvisit's formulation because the major cost of Sai Krok Prew is pork (see Appendix 14). The formulation can be reduced to a 55% pork, with the fat content 22% and rice 23%, and only reduce the acceptability by a marginal amount (see Equation (1)). However, if the pork is reduced as low as 50%, then the acceptability is significantly reduced. Obviously, in commercial practice, cost and therefore price would have to be balanced with the degree of acceptability. The protein content of the present optimum formulation was higher than the Thai commercial sausages (see Appendix 15).

6.2.2 SENSORY EVALUATION

It was difficult to obtain a significant difference between the samples when studying the specific attributes of the texture because of the significant differences between the panel members' scores. The firmness and smoothness were found to be significantly different between samples. This may be due to the ability of the panelists who could detect the differences in high and low level pork samples (affecting the firmness) and differences in high and low level of rice samples (affecting the smoothness).

The correlation between the chemical composition of the cooked sausage and the mean scores for every texture attribute was found to be non-significant, except for the relationship between fat content and rubberiness. As fat content decreased, rubberiness increased. The reason for the lack of correlation with the other attributes might be because the texture in sausage is an extremely complex property, comprised of a

spectrum of parameters (mechanical, geometric and others) related to fat and moisture (Szczesniak, 1963). When tested in the mouth, these various sensations were perceived, analysed, integrated and interpreted in the evaluation. But when they were tested for correlation with the chemical composition, only one texture attribute was used at a time, thus resulting in problems of correlation.

This complex property of sausage texture could be seen again with the different levels of pork, fat and rice in the extreme vertices design. There was not any single level of pork, fat and rice that could explain the mean ratio of the actual texture score to ideal score by itself. Instead, the interaction between the levels of pork, fat and rice seemed to be more appropriate to explain the mean ratios.

6.3 SHELF LIFE

6.3.1 USE OF POTASSIUM SORBATE

The shelf life of both GDL acidulated and bacterial fermented Sai Krok Prew was only three days at 30-32°C and 95% R.H. The shelf life could be extended to at least 14 days under simulated commercial conditions in Thailand by dipping the sausage in 4% potassium sorbate solution for one minute.

When considering the residual of potassium sorbate (calculated as sorbic acid) on the sausage, it was found that the length of storage influenced residual concentration.

With 4% potassium sorbate treatment, the sorbic acid concentration decreased approximately 96% after stroage for 14 days (2247 to 98 ppm). Decreased sorbic acid concentration during storage was also found by Baldock *et al.*, (1979). They found that the sorbic acid residues decreased about 91% (from 2100 to 142 - 228 ppm) after 30 days storage at 21.1 $\pm$ 0.5°C and 70 $\pm$ 5% R.H. The greater decrease in sorbic acid concentration during storage found in this present study may be due to the higher storage temperature and relative humidity than in Baldock *et al.'s* work.

The minimum required potassium sorbate solution to extend the shelf life agreed with the results of Baldock *et al.*, and Holley. Baldock *et al.*, (1979) found that 5% potassium sorbate treatment is about the lowest concentration for fungal inhibition on storage of whole country ham up to 30 days at 21.1 $\pm$ 0.5°C and 70 $\pm$ 5% R.H. Holley (1981) found that 2.5% potassium sorbate treatment was ineffective and 10-20% potassium sorbate was required to prevent surface mould growth on Genoa salami.

Although the initial concentrations of sorbic acid residual seemed fairly high, these values were from the exterior surface of Sai Krok Prew (ppm of casing weight) and did not reflect the concentration of sorbic acid residues in the overall whole sausage. The residues per gram of overall sausage were fairly low.

The initial level of sorbic acid residues when converted to ppm for the whole sausage was only 35 ppm and decreased to only 1 ppm after 14 days storage. Sorbate was classified as relatively non-toxic (Deuel *et al.*,]954; and Deuel *et al.*, 1954b), and Food and Drug regulations in Thailand allow

the use of sorbic acid or potassium sorbate up to 1,000 ppm. Therefore, the use of 4% potassium sorbate to treat Sai Krok Prew is within and much lower than the maximum allowance. Furthermore, Sai Krok Prew must be cooked before being consumed. A very small amount of potassium sorbate would be left over after cooking, and therefore it is safe for consumption.

The application of potassium sorbate to Sai Krok Prew had an effect on levels of moulds and yeasts, as shown by the reduction in moulds from 170 to less than 100 colonies/sq cm (checked on PDA). This inhibitive effect, especially on moulds, was found beneficial for extending Sai Krok Prew's shelf life as shown by the lack of mould growth after 14 days. This was also found by Baldock *et al.* (1979), who reported a slight reduction in numbers of moulds on day zero in country hams sprayed for one minute with potassium sorbate (2.5, 5 or 10%). Treatment with potassium sorbate gave no off-flavours on any of the experimental Sai Krok Prew. This result agreed with the work by Kemp *et al.* (1979) who found no off-flavours or off-colours on ham treated with potassium sorbate.

There was no difference in rate of pH reduction between the potassium sorbate treated sausages, and non-potassium sorbate treated sausages. This may be due to the very small amount of potassium sorbate that could diffuse into the sausage meat inside the casing and, also, the activity of sorbate against bacteria is not as comprehensive as that against yeasts and moulds (Sofos and Busta, 1980).

6.3.2 VACUUM PACKAGING

Vacuum packaging without sorbate dipping could extend the shelf life of Sai Krok Prew from three days up to ten days at 30-32°C. This was similar to what was found by Techapinyawat (1975) who found that Nam (bought from the market) which was wrapped in the microaerophile condition spoiled on the day seven. The reason for this phenomenon may be due to the strictly aerobic nature of moulds. Furthermore, the surface of Nam and Sai Krok Prew (in vacuum packaging) were soaked in the liquid that dripped out from the sausage mix which reduced the chance for mould on the casing to contact with the oxygen residual in the package.

CHAPTER 7

CONCLUSION

1. Addition of straight lactic acid cannot be used to produce Sai Krok Prew.

2. GDL can be used alone to produce a low quality Sai Krok Prew with poor flavour but acceptable texture.

3. GDL might be used at a low level for rapidly reducing the initial pH for Sai Krok Prew that is also fermented. Further work would have to be done to prove this.

4. Pure starter culture can be used to produce high quality Sai Krok Prew. The rate of pH reduction with pure starter culture was similar to the natural fermentation, but it obviously provided a better control of the microbiological activities in the sausage mixture.

5. The control of fermentation is improved by the use of starter culture so that the fermentation is very similar from batch to batch.

6. Nitrite in the range between 75 - 150 ppm can be used to replace prague powder in the production of Sai Krok Prew.

7. There is no need for glucose as a medium in the sausages for the growth of lactic acid bacteria. Sucrose alone is adequate.

8. The texture is improved by increasing the pork and fat level and decreasing the rice level.

9. Shelf life can be increased by dipping the sausage in 4% potassium sorbate solution and also by vacuum packaging.

Further studies that are recommended before the full scale commercial production can be carried out are:

1. The possibility of producing starter culture in Thailand.

2. The mutual benefit of using low quantity of GDL and starter culture.

3. Comparison of the cost and acceptability of Sai Krok Prew made with pork varying from 61% to 50% and the use of low grade pork.

4. The acceptability of the colour of Sai Krok Prew made with straight nitrite.

Other possibilities to be studied in Thailand would be:

5. The acceptability of low quality Sai Krok Prew using only GDL.

6. The effect of using straight nitrite in natural fermentation.

The study is a basis for a modern technological process of making Sai Krok Prew which will have a storage shelf life up to 14 days at 30 - 32°C as compared with the present three days shelf life. The basis for the formulation would be pork, fat, rice, sugar, garlic, nitrite and spices. The mixture would be mixed in the bowl chopper and starter culture slowly added. The mix would be stuffed into pig's casings immediately after mixing. The sausages would be dipped in 4% potassium sorbate solution for one minute and then hung for 24 - 26 hours at room temperature. After testing to ensure that the pH value was correct, the sausages would be vacuum packed in plastic bags and distributed at ambient temperature.

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APPENDIX 1

PH AND ACID CONTENT AT DIFFERENT STAGES OF NAM - 3%, 4% SALT LEVEL AND FROM THE MARKET:

Time	pH 3% NaCl	pH 4% NaCl	pH from market	% acid 3% NaCl	% acid 4% NaCl	% acid from market
0 hr	5.64	5.90	6.33 (3 hr)	0.22	0.22	0.10 (3 hr)
12 hr	5.16	5.68	5.45	0.22	0.21	0.16
24 hr	5.27	5.50	4.98	0.23	0.21	0.26
36 hr	5.01	5.12	4.77	0.26	0.25	0.32
48 hr	4.77	4.90	4.65	0.32	0.27	0.41
60 hr	4.64	4.74	4.56	0.35	0.30	0.45
72 hr	4.63	4.66	4.60	0.40	0.34	0.61
4 days	4.45	4.45	4.55	0.50	0.47	0.56
5 days	4.35	4.36	4.60	0.62	0.58	0.68
6 days	4.35	4.36	4.60	0.68	0.63	0.71
7 days	4.32	4.33	4.46	0.85	0.69	0.75
14 days	4.27	4.36	4.70	0.97	0.96	0.69
21 days	4.15	4.33	5.57	1.25	1.19	0.53
28 days	4.13	4.30	5.63	1.38	1.27	0.52
35 days	4.17	4.39	6.10	1.45	1.28	0.52
42 days	4.25	4.49	6.25	1.42	1.28	0.43

APPENDIX 2

pH AND BACTERIAL COUNT AT DIFFERENT TEMPERATURES AND STAGES:

Day	pH	Room temp. 27.4 - 29.1°C	30°C	32°C	37°C	40°C
1	6.12	270×10^5	264×10^5	260×10^5	240×10^5	158×10^5
2	4.91	129×10^6	145×10^6	107×10^6	94×10^6	290×10^5
3	4.72	148×10^6	135×10^6	132×10^6	165×10^6	73×10^6
4	4.55	119×10^6	133×10^6	109×10^6	127×10^6	36×10^6
5	4.48	62×10^6	61×10^6	76×10^6	75×10^6	33×10^6
6	4.44	32×10^6	42×10^6	53×10^6	40×10^6	280×10^6
7	4.41	131×10^6	119×10^6	122×10^6	109×10^6	43×10^6

APPENDIX 3

PH AND SALT CONCENTRATION OF SAUSAGE MIX FROM TEN DIFFERENT SITES IN THE BOWL CHOPPER:

Site	pH	Salt concentration (%)
1	5.9	2.70
2	5.8	2.56
3	5.9	2.60
4	5.8	2.66
5	5.8	2.74
6	5.8	2.84
7	5.8	2.56
8	5.8	2.57
9	5.8	2.30
10	5.8	2.56
	S.D. = 0.04	S.D. = 0.14

APPENDIX 4

SUITABLE SALTS TOGETHER WITH THEIR CORRESPONDING RELATIVE HUMIDITIES IN THE 5° - 40° C RANGE (ROCKLAND, 1960):

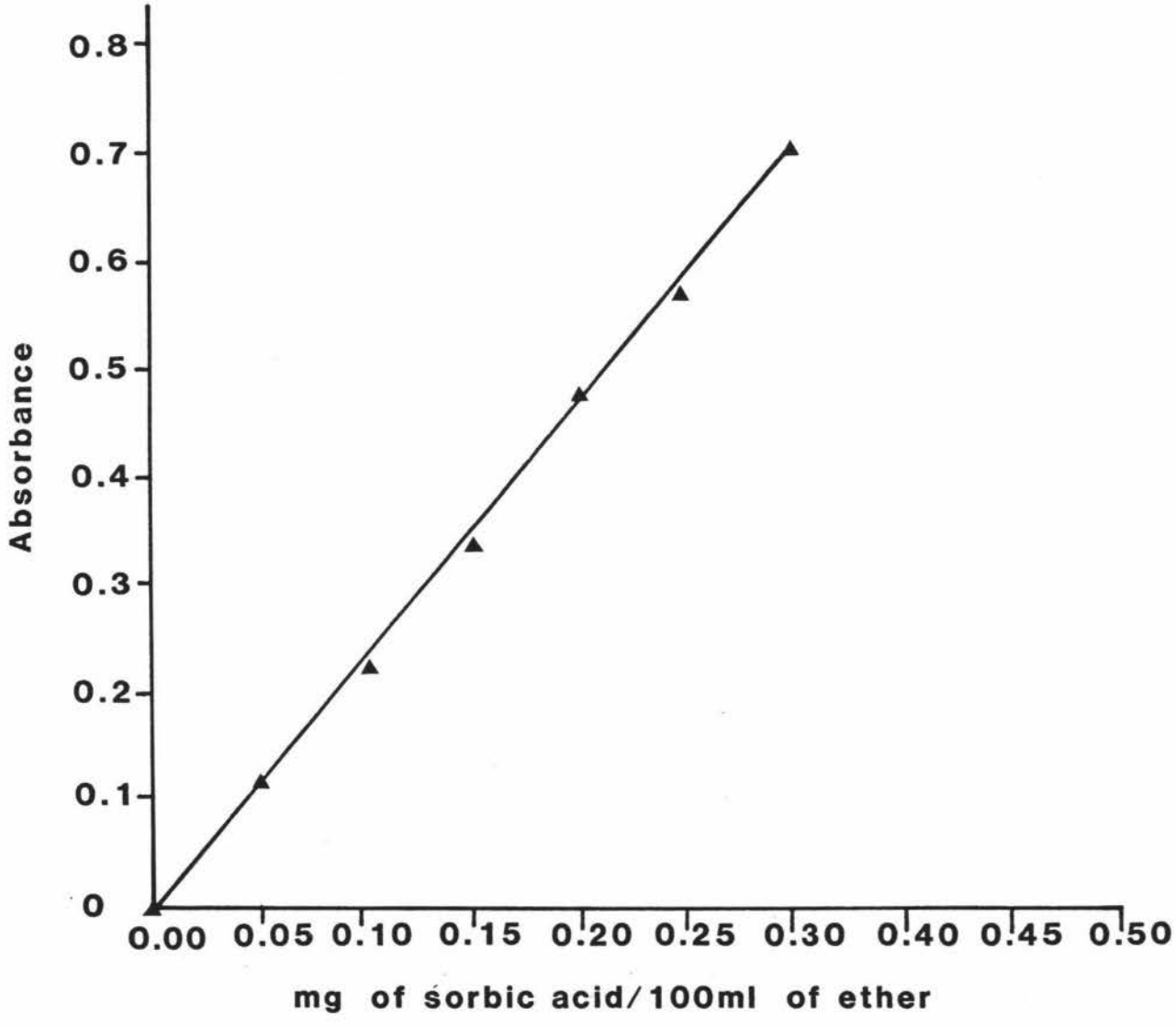
Salt	Relative Humidity % at deg. C							
	5	10	15	20	25	30	35	40
Lithium chloride	16	14	13	12	11	11	11	11
Potassium acetate	25	24	24	23	23	23	23	23
Magnesium bromide	32	31	31	31	31	30	30	30
Magnesium chloride	33	33	33	33	33	32	32	31
Potassium carbonate	-	47	45	44	43	42	41	40
Magnesium nitrate	54	53	53	52	52	52	51	51
Sodium bromide	59	58	58	57	57	57	57	57
Cupric chloride	65	68	68	68	67	67	67	67
Lithium acetate	72	72	71	70	68	66	65	64
Strontium chloride	77	77	75	73	71	69	68	68
Sodium chloride	76	75	75	75	75	75	75	75
Ammonium sulphate	81	80	79	79	79	79	79	79
Cadmium chloride	83	83	83	82	82	82	79	75
Potassium bromide	-	86	85	84	83	82	81	80
Lithium sulphate	84	84	84	85	85	85	85	81
Potassium chloride	88	87	87	86	86	84	84	83
Potassium chromate	89	89	88	88	87	86	84	82
Sodium benzoate	88	88	88	88	88	88	86	83
Barium chloride	93	93	92	91	90	89	88	87
Potassium nitrate	96	95	95	94	93	92	91	89
Potassium sulphate	98	97	97	97	97	97	96	95
Disodium phosphate	98	98	98	98	97	96	93	91[b]
Lead nitrate	99	99	98	98	97	96	96	95
Group B								
Zinc nitrate	43	43	41	38	31	24	21	19
Lithium nitrate	61	59	55	49	41	31	19	11
Calcium nitrate	-	66	60	56	54	51	48	46
Cobalt chloride	-	-	73[a]	67	64	62	59	57
Zinc sulphate	95	93	92	90	88	86	85	84

[a] 18°C

[b] 38°C

Appendix 5

Standard curve for sorbic acid determination



APPENDIX 6

APPENDIX 6.1:

THE QUESTIONNAIRE RESULTING FROM THE BRAINSTORMING SESSION:

TEXTURAL EVALUATION OF FERMENTED SAUSAGES

Name: .....

Date: .....

You have been presented with ..... samples of fermented sausages coded ..... I would like you to score each property - and your ideal product - by making a line on the scale.

Very soft — Very firm

Crumbliness — Rubbery

Dry — Juicy

Not oily — Oily

Gritty — Smooth

Non-sticky — Sticky

Overall texture unaccepted — Overall texture accepted

APPENDIX 6.2:

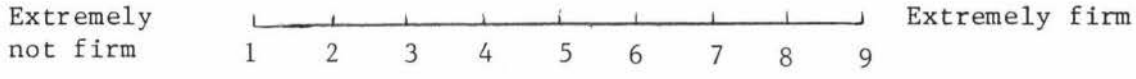
THE MODIFIED QUESTIONNAIRE:

TEXTURAL EVALUATION OF FERMENTED SAUSAGES

Name:.....

Date:.....

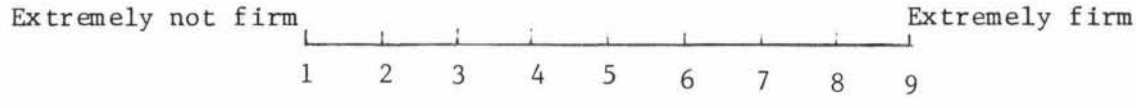
You have been presented with ..... samples of fermented sausages coded ..... I would like you to score each property and your ideal product by making a line on the scale. The structured scale is shown below as an example.



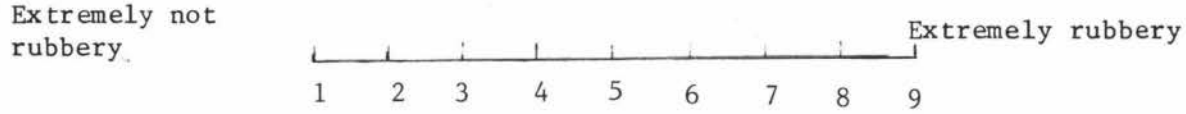
9 Extremely firm
8 Very much firm
7 Moderately firm
6 Slightly firm
5 Neither firm, nor not firm
4 Slightly not firm
3 Moderately not firm
2 Very much not firm
1 Extremely not firm

****

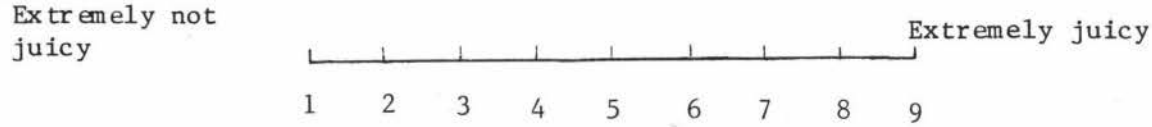
1. Firmness (softness):



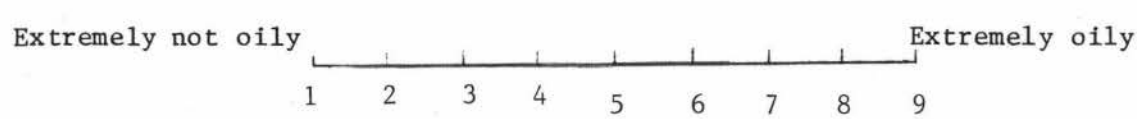
2. Rubbery (crumbliness):



3. Juiciness (dryness):



4. Oiliness (not oiliness):



5. Smoothness (grittiness):

Extremely not smooth — Extremely smooth

1 2 3 4 5 6 7 8 9

6. Stickiness (non sticky):

Extremely not sticky — Extremely sticky

1 2 3 4 5 6 7 8 9

7. Overall texture acceptability:

Extremely not accepted — Extremely accepted

1 2 3 4 5 6 7 8 9

APPENDIX 7

RELATIONSHIP BETWEEN ADDED LACTIC ACID AND REDUCTION OF SAUSAGE MIX pH:

% added lactic acid (wt/wt)	Sausage meat pH
0.00	6.0
0.23	5.0
0.36	4.9
0.45	4.6
0.68	4.5
0.72	4.4
0.90	4.2
1.08	4.2
1.13	4.1

APPENDIX 8

pH CHANGE WITH TIME IN THE INCUBATOR (30-32°C, 75% R.H.) FOR SAUSAGES FORMULATED WITH DIFFERENT LEVELS OF GDL:

Time after processing (hours)	g GDL/100g sausage mix			
	0.0	1.0	1.5	2.0
	pH			
0	6.2	6.2	6.2	6.2
1	6.2	5.4	4.9	4.7
2	6.2	5.3	4.8	4.7
3	6.2	5.1	4.8	4.6
4	6.2	5.1	4.6	4.5
6	6.2	5.0	4.6	4.5
8	6.2	4.9	4.6	4.4
24	6.2	5.0	4.6	4.4

APPENDIX 9

THE OPTIMIZED VERTEX:

APPENDIX 9.1:

CHEMICAL COMPOSITION:

Initial pH:		6.1
"	Moisture content (%)	55.70
"	Fat content (% dry basis)	46.95
Before cooked pH:		4.5
" "	Moisture content (%)	42.73
" "	Fat content (% dry basis)	39.81
After cooked pH:		Not determined
" "	Moisture content (%)	44.53
" "	Fat content (% dry basis)	37.86

APPENDIX 9.2

SENSORY SCORE:

Judges	Firmness	Rubbery	Juiciness	Oiliness	Smoothness	Stickiness	Overall Texture Acceptability
1	7.1	6.2	6.7	6.5	6.7	5.2	7.2
2	4.5	3.9	6.0	3.4	6.0	3.3	6.8
3	7.9	7.7	2.4	2.4	5.4	7.8	7.9
4	6.9	5.3	5.9	5.9	7.8	6.8	7.7
5	5.7	4.5	4.4	3.7	6.6	7.7	6.5
6	6.1	5.9	6.3	6.3	6.3	6.3	6.4
							$\bar{x}$ = 7.1

APPENDIX 10

RATE OF pH REDUCTION USING LACTACEL MC AT DIFFERENT LEVELS OF SODIUM NITRITE:

Incubation period (hr)	pH		
	75 ppm nitrite	150 ppm nitrite	500 ppm nitrite*
0	6.1	6.1	6.1
2	5.9	5.9	6.0
4	5.8	5.9	6.1
6	5.8	5.9	6.0
8	5.7	5.6	6.1
10	5.3	5.3	6.1
12	5.0	5.1	5.8
14	4.9	4.8	5.4
16	4.8	4.9	5.2
18	4.7	4.7	5.1
20	4.6	4.7	5.0
22	4.8	4.6	5.0
24	4.7	4.6	4.9
26	4.4	4.4	5.0

* Significant at 25% level

APPENDIX 11

RATE OF pH REDUCTION USING SUCROSE AND DEXTROSE ON THE FERMENTABLE SUGARS:

Incubation period (hr)	pH	
	Sucrose	Dextrose
0	6.1	6.2
2	6.1	6.1
4	6.1	6.1
6	6.0	6.1
8	5.8	6.1
10	5.6	6.1
12	5.5	5.6
14	5.4	5.6
16	5.0	5.2
18	4.7	5.0
20	4.7	4.8
22	4.7	4.7
24	4.6	4.6
26	4.4	4.4

APPENDIX 12

THE CONSISTENCY OF pH REDUCTION OF FERMENTED SAI KROK PREW BETWEEN BATCHES:

Incubation period (hr)	pH		
	Batch no. 1	Batch no. 2	Batch no. 3
0	6.0	6.1	6.1
2	6.0	5.9	5.9
4	5.9	5.9	5.9
6	5.8	5.9	5.9
8	5.6	5.8	5.7
10	5.4	5.7	5.7
12	5.4	5.5	5.6
14	5.3	5.2	5.3
16	5.0	4.9	5.0
18	4.8	4.8	4.7
20	4.7	4.6	4.6
22	4.6	4.5	4.5
24	4.5	4.5	4.5
26	4.4	4.4	4.4

APPENDIX 13

RATE OF pH REDUCTION BETWEEN THE CONTROLLED AND DIPPED IN 4% POTASSIUM SORBATE SOLUTION SAI KROK PREW:

Incubation Period (hr)	pH	
	Controlled	Dipped
0	6.1	6.1
2	6.1	6.1
4	6.1	6.1
6	6.0	6.0
8	5.7	5.8
10	5.6	5.7
12	5.4	5.4
14	5.2	5.3
16	4.9	5.0
18	4.8	4.9
20	4.7	4.8
22	4.8	4.7
24	4.5	4.5
26	4.4	4.4

APPENDIX 14

COST OF RAW MATERIALS: [1]

Raw material	Cost/kg (Baht)[2]	Cost/kg sausage (Baht)[2]
Pork	45.00	26.42
Fat	20.00	4.12
Rice	8.50	1.33
Garlic	35.00	0.88
Coriander	120.00	0.12
Sodium chloride	2.00	0.05
Sucrose	12.00	0.06
Pepper	100.00	0.25
Sodium nitrite	210.00	0.03
Pigs' intestine	170.00	3.40

1 Based on the current price in Thailand local market

2 17.2 Baht = NZ$1.00

APPENDIX 15

CHEMICAL COMPOSITIONS OF SAI KROK PREW FROM SOME NORTHEASTERN PROVINCES OF THAILAND[1] AND IN THIS PRESENT STUDY:

Source	Protein (%)	Fat (%)	Moisture (%)
Nakorn Ratchasema	11.99	28.45	58.00
Mahasarakarm	7.10	20.20	54.51
Roi Ed	6.96	28.27	74.06
Kalasin	9.26	19.13	58.34
Khon Kaen	8.82	28.81	49.03
Present study * (after storage for 14 days)	24.91	28.51	31.83

1 From Rongvisit, 1980

* Ash content = 4.25%