THE SYSTEMATIC DEVELOPMENT OF A CONTROLLED FERMENTATION PROCESS USING MIXED BACTERIAL STARTER CULTURES FOR NHAM, A THAI SEMI-DRY SAUSAGE

A thesis presented in partial fulfilment of
the requirements for the degree of
Doctor of Philosophy in
Product Development in Food Fermentation
at Massey University

Pairote Wiriyacharee
1990

ABSTRACT

The aim of this thesis was to improve the quality of Nham, a traditional Thai fermented sausage, using systematic product development techniques. The traditional Nham fermentation depends upon a random bacterial flora. This study identified the starter cultures which could be used and developed an industrial production method using mixed starter cultures.

Systematic experimental designs were used to guide the development of the Nham fermentation; identification of the important processing factors using a Plackett and Burman experimental design, formulation and process development using full factorial designs in sequence of 2^4, 2^3 and 2^2, and then a storage test of the product, testing of formulation and process in Thailand and finally a production trial of the new process in a factory in Thailand. Chemical, physical, microbiological, and sensory evaluation were used during the systematic product development. The study ended with consumer testing of the prototype product in the target market in Chiang Mai city and two villages - Ban Don Chai and Ban Ma-Kran.

The important factors affecting Nham quality were the mixed starter cultures and the carbon sources used in the Nham formulation. When the Nham base was inoculated with Lactobacillus plantarum 10^3 cfu.g^{-1}, Pediococcus cerevisiae 10^6 cfu.g^{-1} and Micrococcus varians 10^3 cfu.g^{-1} acid production, firmness and colour development were optimum, the product was microbiologically safe and the sensory properties were acceptable to consumers. The addition of carbon sources increased acid development. 0.5% glucose and 6% cooked rice were optimum levels in the Nham formulation.

Temperature and relative humidity also affected the Nham fermentation. Temperature increased the rate of pH reduction, the firmness and colour development. High relative humidity decreased the weight loss. The Nham fermented at 30°C and 97% relative humidity had optimum acid production and sensory properties.

Nham is sold in Thailand between 20°C and 30°C, and at relative humidity as high as 97%. Experimental samples stored under similar conditions had a shelf life of 11 days and 9 days respectively. When the product was chilled at 10°C and 1°C, the shelf life was extended to 63 days and 103 days respectively. Enterobacteriaceae and Staphylococcus aureus counts fell during storage and no yeasts or moulds were observed. Off-flavour development controlled the shelf life.

Product profiles were determined for the Nham by Thai consumers and the ideal ratio method was used during the sensory product testing. A profile test using linear scaling with fixed ideal points was used for the trained sensory panels. The floating ideal point was used with consumer panels when the prototype product was close to ideal profile. Category scaling was used in the consumer testing of the final product. Sensory evaluation by one hundred and twelve families in Chiang Mai province indicated that appearance, texture, and flavour of Nham made with the mixed starter cultures were good.

The Nham successfully developed by using the systematic product development had a high quality in terms of consistency, microbiological safety and long shelf life and was also accepted by the target Thai consumers. The product could be produced in a simple plant with the existing equipment in fermented meat product factories in Thailand but there would need to be an increase in technology of culture preparation and controlled fermentation. The product could be sent from the cottage industry in the North to all provinces in Thailand, particularly to Bangkok, and also had a potential to be exported to overseas countries if chilled conditions were used.

ACKNOWLEDGEMENTS

This research study was financially supported by the New Zealand Bilateral Aid Programme administered by the Ministry of Foreign Affairs; I would like to take this opportunity of thanking this assistance.

I shall always be grateful to my chief supervisor, Dr Mary D. Earle; Director, Product Development Sub-Department, Massey University, for her constant inspiration, valuable guidance, patience and encouragement throughout this research, and also for giving me the opportunity of working on this topic.

To my co-supervisors; Dr J. D. Brooks, Food Technology Department, Massey University; Dr G. Page, Director, NZ Dairy Research Institute; and Mrs L. Rujanakraikarn, Food Science and Technology Department, Chiang Mai University; I wish to express my appreciation for their many useful suggestions and criticisms.

I would also like to express my appreciation to many people, both in New Zealand and Thailand, who assisted this research in many ways. In particular I would like to take this opportunity of thanking the following:

* Prof. R. L. Earle and the staff of the Faculty of Technology, Massey University for the use of facilities and for their support throughout.
* Prof. P. A. Munro and the staff of the Department of Food Technology and the Sub-Department of Product Development, Massey University especially Mrs Joan Brookes for providing facilities and willing assistance and encouragement.
* Prof. E. L. Richards and Dr I. Maddox for their advice on techniques of chemical analysis.
* Dr I. F. Boag, Production Technology Department, Massey University, for his valuable suggestion in the experimental design and analysis.
* Dr A. M. Anderson, Product Development Sub-Department, Massey University, for his valuable discussion.
* Dr Hugo Varela-Alvarez, Computer Centre, Massey University, for his useful suggestions in computer analysis and help in programming.
* Mr S. L. Oldfield and Dr B. H. P. Wilkinson for their valuable advice and comments on some parts of this research.
* Mrs J. Cleland for her willing assistance in preparation of microbiological materials and equipment.

* Mrs M. Bewley for her willing assistance in preparation of chemical materials and equipment.
* Asso. Prof. N. Na-Lumpang, Dean of Agriculture Faculty, Chiang Mai University for his permission to do some parts of this research in Thailand.
* The staff of Chiang Mai Livestock Breeding and Research Centre, Ministry of Agriculture and Cooperatives, Chiang Mai, for their help in the production trial.
* The Thai students at Massey University and Chiang Mai University who were members of the taste panels.
* The people in the King's Project, Chiang Mai, who participated in this research.
* The consumers in Chiang Mai city; Ban Don Chai, Yangkram district; Ban Ma-Kran, Shongkual district, who tested the final product.
* The staff of the Library and the Registry, Massey University for willing assistance in locating reference materials, and for advice on Ph.D. study programme, respectively.
* Mr Wichien Chatupote for his help in computer programming in particular chart programme.
* Miss V. J. Davis for her work in typing and correcting the thesis.

Finally, I would like to thank my mother and brother, also his family, for their support mentally during the course of my study.

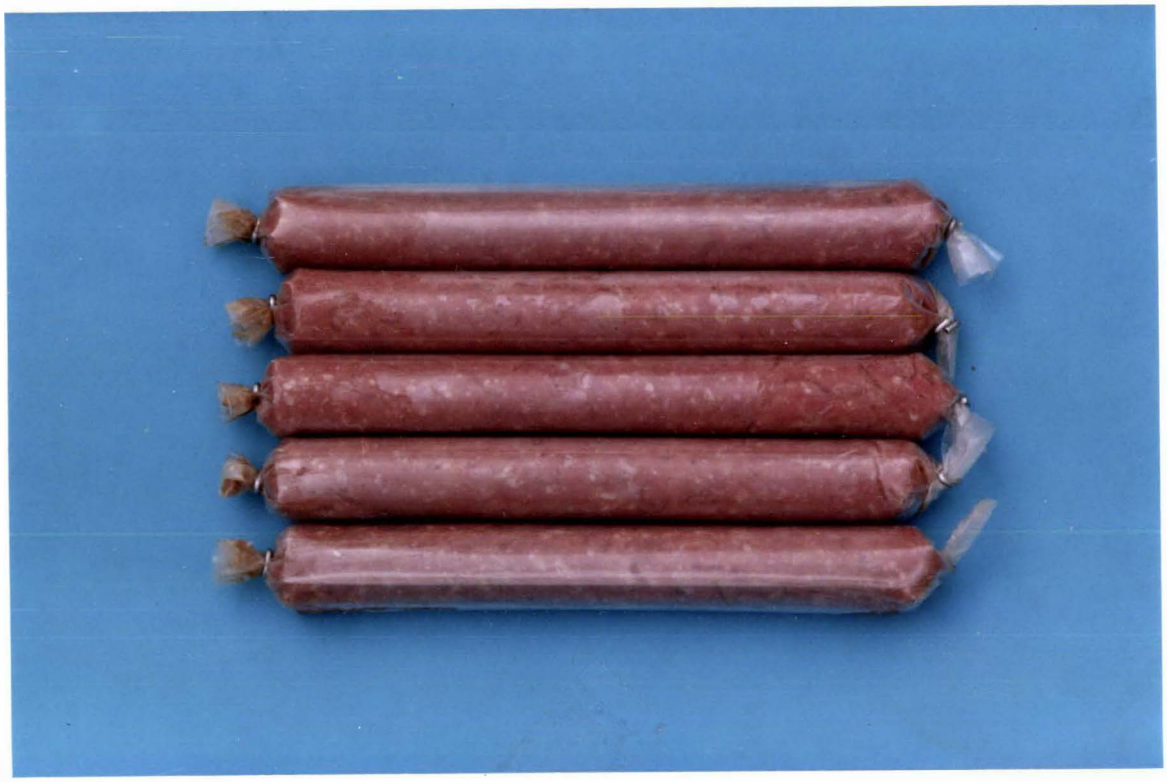
TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

EXPERIMENTAL NHAM, THAI SEMI-DRY SAUSAGE



CHAPTER 1

IMPROVEMENT OF THAI FERMENTED MEAT PRODUCTS

1.1 INTRODUCTION

Fermentation is one of the oldest food preservation methods and remains one of the most economical methods of producing and preserving food highly acceptable to man. Foods have been prepared for thousands of years in Southeast Asia, including Thailand, by the traditional methods of drying, salting, pickling, fermentation or a combination of these methods. These indigenous fermented foods are very important in that they are socioculturally bound in the village traditions; but with the movement of people from the villages to the cities, they are now eaten in both rural and urban households.

Some of these fermented processes and products were superseded in the last seventy years by the physical methods of food preservation - canning, chilling and freezing. In recent years, there has been an increased interest in fermentation because of two factors, rising energy costs and the current interest in "natural" food preservation (Pederson, 1979; Peters, 1980). Increasing quantities of fermented foods were exported from Thailand, mostly to Asian countries but also to markets in Europe and America (Wongkhalaung and Boonyaratanakornkit, 1986).

Important fermented products are meat products; in Thailand fermented meat sausages are marketed internally and some exported. There is an urgent need to set up standards for these fermented meat products to ensure wholesomeness and acceptability. However, before these standards are introduced, improvements are necessary in terms of hygienic practice, better eating quality, and longer shelf-life of fermented products. There is a need to introduce modern knowledge and technology into the historic fermentation methods.

1.2 FERMENTED MEAT SAUSAGES IN THAILAND

Salting and curing of fresh meat has proved an effective means to control the fresh meat microflora. The curing salts, sodium chloride, sodium nitrate or nitrite, create a different microenvironment in the meat mixture which favours the growth of specific types of gram positive microorganisms which inhibit the fresh meat microflora. This microbial inversion occurring during the salting and curing process, provides the accidental origin of all fermented meat sausages.

In Thailand it was found that the traditional fermentation process for meat could be accomplished much faster when the meat was cut into small pieces, mixed with salt and spices, and then stuffed into flexible containers which served to hold the meat tightly and to provide convenient units in which to store the products. These containers were initially either the animals' intestines or leaves, but today many types of sausage casings are available. These traditional sausages became an effective preservation method for meat. The ancient Thai people quickly learned that the important factors in meat fermentation were proper hygiene in the cutting and addition of salt in the formulation, coupled with control of temperature by leaving the sausages in the sun. However, this finally resulted in growth of fungi. Therefore, the sausages had to be consumed soon after fermentation was completed.

Fermented meat sausages are deliberately incubated, or aged, during their processing cycle to allow for sufficient microbial activity to alter the product characteristics. Although microorganisms generally are considered the adversary in the processing of meat, fermented sausages rely upon controlled microbial activity of specific types of bacteria. Traditionally, good manufacturing practices and proper environmental conditions were relied upon to ensure the desired type of microbial development and the extent of the microbial activity. Some companies making the sausages had guilds for their protection; most formulation and processing secrets were passed from one generation to another, with an intentional lack of written documentation.

Generally, there are three types of fermented meat sausages in Thailand. These are Nham, a semi-dry fermented sausage produced from pork with pork skin and usually not smoked (Adams, 1986), Sai-krok-Prew a semi-dry fermented sausage mainly made from cooked rice and pork (Vichiensanth, 1982), and Mum a semi-dry fermented sausage made from beef (Wongkhalaung and Boonyaratanakornkit, 1986). Nham is the most popular Thai fermented sausage. Nham manufacture is a traditional household industry, and originally a dominant product in Northern Thailand particularly Chiang Mai, Chiang Rai, Lampang, and Lamphun provinces. The Nham is produced today mainly by small scale industries. The production is considered as an art that continues to be passed down from generation to generation. Nowadays, the eating of Nham is not only concentrated in Northern Thailand but has spread to all parts of Thailand; therefore, the potential market for it is still growing. Additionally, Nham seems to be a good product for exporting.

1.3 NHAM PRODUCTION

Nham traditionally is made from fresh lean pork that is trimmed, minced, mixed thoroughly with salt, potassium nitrate, cooked rice and seasonings, and packed either in banana leaves (Adams, 1986) or in cylindrical plastic bags (Pakrachpan, 1981). Nham production in Thailand is dependent on chance contamination with wild organisms. It is a long process, generally the fermentation lasts three to five days. This requires not only an excessive amount of handling and labour, but also a very appreciable investment in materials. Because the natural fermentation depends upon the native lactic acid bacteria present on the raw materials, equipment and processing conditions, large variations in the nature and type of bacterial occur. This may cause poor quality or a quality which is not consistent. An intensive investigation of the microbiology, chemistry, product and process improvement of Nham fermentation is needed to enable the traditional empirical methods of manufature to be changed successfully to the large scale, low cost industrial production. It is especially important to develop short ripening times and a highly standardised product.

1.4 MARKETING ASPECTS OF NHAM

Nham is a most popular traditional fermented meat sausage which is sold uncooked at the retail level. It may be cooked before being eaten.

There are a wide variety of Thai sausages in the market place, mostly made by hand on a trial and error basis in the home or on a small scale. Each community has developed its own sausage favourites, and they vary a great deal owing to the variations in spices used and the variations in ambient temperatures in each area. This results in different flavours and nonuniformity. The products lack standardization, which causes low market share. Therefore principles of modern food processing technology must be applied to improve product uniformity, safety and storage stability, so that it is easy to store, distribute and market. The reduction of costs in all areas of production and marketing is important, as there are many competitors in the market place.

Consumers normally prefer to buy the Nham from the market and keep it in a kitchen cabinet (or in a refrigerator if they want to store the product for later consumption). Usually, the companies label the dates for eating on the package of Nham but do not state the expiry date. Therefore, the consumers do not know the shelf-life of the product and eat it as quickly as possible.

Nham originally occurred in Northern Thailand. It was distributed through many parts of Thailand as immigrants started streaming to other parts to find a job, particularly into the central city, Bangkok. They often settled in a close community and usually prepared the traditional foods for consumption. Therefore, Nham is very well known as a native food of Thais. The companies' salesmen sell both directly to fermented food shops and delicatessens in many parts, or to jobbers and wholesalers, who service the market. Usually, the market for this product is associated with other foods which are produced on the same production line e.g. Northern style sausage (Sai-Auo), cylindrical pork product (Mu-Yo), fried pork (Mu-Ping), fried pork skin (Kep-Mu). Sometimes, the producers sell the product themselves or distribute to the supermarkets, local markets or service stations, and perhaps make to order. As supermarkets came on the scene in Thailand, they perceived a need for a wide selection of self-service items that was not only Western style meat and meat products, but also Nham and other native foods.

One of the market channels for Nham is the catering market, such as restaurants. Nham is eaten as a snack with whisky or other alcoholic beverages, sometimes served with fried rice and eggs, and used to prepare some delicious hot soups. There is a very great opportunity for Nham in the fast food area, in the form of take-out sandwiches utilizing various sausage items including Nham. Another example will be in the Pizza area. This market may use Nham, as the number of people who find pizza a completely acceptable new food is growing every year.

There are some problems with Nham, in terms of marketing - short shelf-life in the market, and the possibility of food poisoning. It is also high-priced, semi-perishable, and labour intensive. It has high energy costs if kept under refrigeration in the market place. Additionally, the manufacturers have a heavy exposure to risk of losing a large stock through a process failure. Pork meat is quite expensive and the raw material cost is increasing more quickly than increase in selling price. In addition, large scale production of Nham has the problem of the short storage life. A longer shelf-life is required so that the Nham can be distributed to the market place. Therefore, the Nham market needs the product to have consistency of quality, safety and longer shelf-life. The Nham should stay fresh and not turn rancid or develop an off-flavour, or change in colour when it is in the market place.

On the other hand, Nham production depends upon the natural fermentation; the product quality therefore varies from batch to batch. The shelf-life of the product is quite short - approximately a week at Thai ambient temperatures. Chilled conditions can extend the shelf-life, but normally the product is stored at ambient temperatures. The sanitation of

the processing is also poor because of the lack of knowledge and technology. Thus, the Nham process needs to be studied to improve the product quality, to give a more uniform standard quality and to develop the technology for application of the process on the industrial scale before launching extensively in the Thai and export markets.

1.5 NHAM FERMENTATION

As Nham is packed into cylindrical plastic bags, which exclude air, and is held in the bags during fermentation, a microenvironment is selected for those microorganisms that are not only salt tolerant, but which can also grow in the absence of air. In these gram-positive, fermentative types of microorganisms, lactic acid bacteria are predominant (Comenuanta, 1966; Techapinyawat, 1975). During the initial period of fermentation of traditional Nham, most of the microorganisms are rods and cocci, both gram positive and negative, which produce acid (Techapinyawat, 1975). Fermentable carbohydrates are used by heterofermentative lactobacilli (Lactobacillus brevis), homofermentative lactobacilli (Lactobacillus plantarum) and homofermentative cocci which are Pediococcus cerevisiae (Comenuanta, 1966; Technipinyawat, 1975; Srisomwong, 1985), Pediococcus pentosaceus and Pediococcus acidilactici (Tanasupawat and Daengsubha, 1983). The fermentable carbohydrates are used by those organisms to produce organic acids, mainly lactic acid, that contribute to a variety of flavours and textures. The Nham finally develops approximately 0.5 - 1.0% acidity as lactic acid and the pH is 4.45-4.55 (Comenuanta, 1966). Somathiti (1982) reported that coliform counts of Nham at the beginning and the early stages of fermentation were very high, but dropped significantly after 5 days of fermentation. The traditional process depends upon the bacteria present on the raw materials, equipment and the processing conditions. Hence, large variations in the nature and type of bacteria occur. The final Nham has a sausagelike flavour similar to that of a western sausage fermented with pediococci (Steinkraus et al., 1983).

1.6 RESEARCH ON NHAM FERMENTATION

Although early Nham makers did not understand fermentation, pH and microorganisms, they did realize that something happened during this stage of the process that affected the sausage and resulted in better colour, firmness and flavour. Although pH meters were nonexistent, the Nham makers could tell when this phase was completed by feeling the firmer texture, observing the redder colour and tasting the tang of the Nham. Lack of

control during the early phase could result in product failures due to undesirable odours and flavours.

The microbiological contributions to Nham fermentation did not gain significant attention until the 1960s. The application of early microbiological research to Nham was for identification of the microorganisms in Nham during the fermentation process and it was reported that the major microorganisms were lactic acid-producing bacteria (Comenuanta, 1966).

In 1975, Techapinyawat investigated the microorganisms during the Nham fermentation. She found that pork itself carried various types of flora mostly rods and cocci; both gram positive and negative. Added potassium nitrate was reduced to nitrite by naturally occurring nitrate-reducing micrococci, therefore contributing to the development of colour and flavour as well as to antimicrobial activity. Seasonings were added to improve flavour. The seasonings could contribute somewhat to the Nham preservation. Sensory testing by a taste panel showed that Nham was most acceptable at 3% sodium chloride. At the low (2%) and high (5%) sodium chloride levels, Nham became unacceptable because the acid was too low. She also recommended that the best consumption period was the fourth day after commencement of fermentation. During the first four days, rapid decrease in pH value and increase in acidity was found but pH and acidity changed only slightly after the fourth day. The final Nham pH was less than 4.5 and lactic acid content approximately 0.5%. She also found that there was a wide range of microorganisms during the initial period of fermentation, but after 24 hours, differential growth rate reduced the number of some types of microorganisms. During the first 24-72 hours homofermentative and heterofermentative lactobacilli and homofermentative cocci (P. cerevisiae) proliferated, resulting in a rapid rate of acid production. After 72 hours, homofermentative lactobacilli (L. plantarum) predominated with some Pediococcus and heterofermentative lactobacilli still growing. She found that on the fourth day most non-acid producing microorganisms were destroyed, including coliform bacteria. L. brevis was also found in large numbers along with L. plantarum but growing at a slow rate. When Nham was kept at room temperature longer than a week, it was found that the sourness increased and chewiness decreased; yeast (Candida sp.) was also found after seven days. The addition of 10% from the previous batch of Nham, five days old, shortened the fermentation period to 24-36 hours and reduced the pathogenic bacteria, but the texture of the finished Nham was softer.

Rojanasaroj et al. (1980) investigated the relationship between the total bacterial count and pH in Nham. They found that the most acceptable taste was on the third to fourth

day after production, at which time the pH was 4.55 -4.72, this 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.

Somathiti (1982) found that the initial coliform count was high in Nham approximately 10^7 cells per gram and decreased to 10^2 cells per gram on the fifth day. An investigation of Salmonella in Nham in the Bangkok market showed it was present in 56 samples (12%) out of 450 samples. In Nham produced in Chiang Mai, Chiang Rai, and Ubonratchathani, Salmonella was found in 25%, 42% and 11% of total samples respectively. However, Shigella sp. was not found in Nham bought from any of the markets.

H-kittikun et al. (1988) stated that one commercial Nham (A) contained only homofermentative lactobacilli which were predominantly L. plantarum, whereas in another commercial Nham (B) Pediococcus sp. were the major lactic acid bacteria but the amount of homofermentative lactobacilli increased significantly during storage. Only a few Streptococcus, Leuconostoc and heterofermentative lactobacilli were found during fermentation.

H- kittikun et al. (1988) used as single starter cultures pure inocula from the lactic acid bacteria associated with Nham, L. plantarum 50408 and 51006 from Nham (A) and L. plantarum 60412 and 61004 from Nham (B). They, found that, at the beginning of fermentation, the total count and lactic acid bacteria count with starter culture were more than one log cycle higher than those of the control (natural fermentation). The native Nham showed a high count of E. coli and had a positive test for Salmonella at the beginning of fermentation. In comparison, Nham with added starter culture showed a much lower count of E. coli and no Salmonella was detected. From sensory evaluation, the application of starter cultures in Nham fermentation seemed to offer no improvement on the natural fermentation, particularly in texture and colour development. However, this needs further testing because there are many variables that could affect the quality. This result stimulated the need for further research on the use of mixed starter cultures to improve the product quality and to give more standard quality.

The coliform and Salmonella counts may be high in the native Nham. Although a traditional means of achieving the product uniformity has been "slopping back"; i.e. at the end of fermentation a portion of one mix is retained and added at around the 5-10% level to a fresh batch, undesirable characteristics could be selected equally as well as desirable ones (Everson et al., 1970 (a); Techapinyawat, 1975; Bacus, 1984; Pearson and Dutson,

1986). Although the addition of a single starter culture in Nham did inhibit the growth of those pathogens, it seemed to be not better than the natural fermentation for product quality (H-kittikun et al., 1988). Therefore, these problems stimulated this study on mixed starter cultures to improve the product quality. The use of mixed starter cultures with L. plantarum, L. brevis, P. cerevisiae and Micrococcus varians in Nham may help to improve product safety, achieve consistency, reduce processing time and extend the shelf-life.

1.7 USE OF MIXED STARTER CULTURES IN MEAT PRODUCTS

The general concept of using a starter culture in meat products originated in the United States. Today, the primary genera of bacteria which are successfully utilized as meat starter cultures are Micrococcus (Niinivaara, 1955; Nurmi, 1966; Coretti, 1977), Lactobacillus (Nurmi, 1966; Everson et al., 1970(a); Bacus, 1984; Gilliland, 1985; Gibbs, 1987), and Pediococcus (Deibel and Niven, 1957; Bacus and Brown, 1981; Bacus, 1984; Gilliland, 1985 and Gibbs, 1987). The micrococci were selected and added for their nitrate-reducing activity and improvement in colour and flavour, whereas the lactobacilli and pediococci were added because of their acid production.

These selected microbial cultures are currently added to meat products to better ensure product safety, shorten fermentation schedules, and achieve unique product quality, consistency, and shelf-life. The product consistency and the unique quality attributes afforded by starter cultures were presented by Acton and Keller (1974), Acton (1977) Klettner and Baumgartner (1980). The safety aspects and shelf-life were dependent on rapid preservation techniques. The addition of salt, sugar, nitrate or nitrite, smoke and the subsequent holding of the product at reduced temperatures with a decrease in the oxidation-reduction potential i.e. in a casing, favoured the growth of lactic acid bacteria which fermented the sugar to primarily lactic acid, thus reducing the meat pH and providing prolonged stability against the proliferation of food pathogens. Further, harmful bacteria tend to die off when the finished product is stored (Bacus, 1984; Bacus and Brown, 1985 (a), (b)).

The use of starter cultures provides sufficient microbial numbers to ensure numerical dominance over the natural flora, including pathogens, and in a combination with the proper processing control guarantees the safety and quality of the final product (Bacus and Brown, 1981).

The ability of food pathogens to survive, initiate growth, and produce toxins depends upon their ability to overcome the inhibitory environment created during formulation and processing. Important components of the environment are 1) initial meat formulation and eventual changes in pH, brine, Aw, redox potential, and nitrite; 2) temperature, relative humidity, casing type, and rate of chemical and physical changes; 3) the initial numbers and types of pathogens; 4) the number of competing microflora in the sausage formulation, including any starter cultures (Table 1.1).

Table 1.1 General growth characteristics of food poisoning bacteria and moulds relevant to the processing of fermented meats (adapted from Genigeorgis, 1976).

Bacteria or moulds	Growth temperature range (°C)	Lowest pH permitting growth	Max. brine** concentration permitting growth (%)	Min. Aw permitting growth
Staphylococcus aureus	7-46	4.0 (+0_2) 4.6 (-0_2)	16-18 (+0_2) 14-16 (-0_2)	0.83-0.86(+0_2) 0.90 (-0_2)
S. aureus enterotoxin	10-45	4.0 (+0_2) 5.3 (-0_2)	10 (+0_2) 9.5 (-0_2)	0.90 (+0_2) 0.94 (-0_2)
Salmonella spp.	5-45	4.05	8	0.94
Clostridium perfringens	7-50	5.0	6	0.93-0.97
Clostridium botulinum				
types A	10-48	4.7	10	0.93-0.95
B	10-48	4.7	10	0.93-0.94
E	3-45	5.0-5.4	5-6	0.94-0.97
F	3	NK	NK	NK
Moulds*	-12 -65	1.7	20	0.62
Mycotoxin production	4-40	1.7	10	0.80-0.85

NK = not known

* Extreme conditions at which at least one species was able to grow and produce a mycotoxin

** Brine (%) = [NaCl (%)/ moisture (%) + NaCl (%)] x 100

Staphylococcus aureus. In general, staphylococci proliferate and produce enterotoxin during the initial stages of the sausage fermentation (Barber and Deibel, 1972; Lee et al., 1977). Therefore, proper control mechanisms in the successful production of fermented sausage must be applied to the initial fermentation stage. The beneficial effect of microbial starter cultures in inhibiting staphylococcal growth and enterotoxin production in fermented sausages has been readily demonstrated (Table 1.2). The large numbers of lactic acid bacteria provide a consistent controlled fermentation, accelerate the rate of acid development, and thus, indirectly retard the growth of staphylococci (Nurmi, 1966; Genigeorgis et al., 1971; Barber and Deibel, 1972; Daly et al., 1973; Haines and Harmon, 1973 (a), (b); Raccach, 1986).

Table 1.2 Staphylococcal enterotoxin development, dry sausage, 22-24°C (adapted from Niskanen and Nurmi, 1976).

	3 Days			7 Days		
Starter Culture	Log cfu.g^{-1}	pH	Enterotoxin	Log cfu.g^{-1}	pH	Enterotoxin
-	8.84	5.9	+	8.88	5.7	+
+	6.78	5.6	-	7.53	5.3	-

Salmonella. Lactic acid bacteria are definitely inhibitory to the growth of salmonellae with the relative effect dependent on the species, strain, ratio of lactic acid bacteria to salmonellae, the incubation temperature, and the degree and rate of acid production (Park and Marth, 1972). A process utilizing a commercial lactic acid starter culture reduced fermentation time and was the most restrictive to salmonellae survival while a natural lactic flora fermentation was the next most restrictive (Masters, 1979). Many researchers found that in meat products fermented with either Lactobacillus or Pediococcus, the number of salmonellae declined during fermentation (Goepfert and Chung, 1970; Baran and Stevenson, 1975; Smith et al., 1975 (a), (b).

Clostridium. The role of the lactic acid bacteria in inhibiting the toxin production of Clostridium botulinum in meat has been demonstrated (Table 1.3). Lactic starter cultures, in combination with either sucrose or dextrose, have proven effective through rapid acid production in preventing toxin production even in the absence of nitrite (Christiansen et al., 1975; Ivey and Robach, 1978; Tanaka et al., 1980). Tanaka et al.,

(1980) recommended that nitrite levels may be safely lowered in certain meat products if lactic cultures and carbohydrates are added. In fermented sausages, Clostridum perfringens is unlikely to thrive (Adams, 1986).

Table 1.3 Botulinal toxin development, summer style sausage at 27°C (adapted from Christiansen et al., 1975).

Nitrite (ppm)	Starter culture	Dextrose	Toxic/25*
0	-	+	8
0	+	-	22
0	+	+	2
50	+	+	0
150	-	-	14
150	+	+	0

* Number of samples with toxin in 25 samples.

The surface development of moulds on fermented sausages is favoured by the low pH and reduced water activity (Table 1.1). The most common moulds on fermented sausages are Penicillium. Toxin production in fermented meats by these naturally-occurring microorganisms does not appear to have been reported (Adams, 1986).

Microbial cultures contribute to the shelf-life of fermented meats mainly by consistent and controlled acidification. Fermented meat technology with the addition of starter cultures has also been applied to fish products to extend shelf-life (Herborg and Johansen, 1977; Schubring and Kuhlmann, 1978). Recently, microbial cultures were added to nonfermented meat products to prolong shelf-life and inhibit pathogens. Selected cultures have been successfully applied to ground beef, beef cuts, frankfurters, deboned poultry and shrimp (Reddy et al., 1970; Tezcan and Yuecel, 1975; Petaja, 1977; Raccach and Baker, 1978).

With regard to fermented meat products, Bacus and Brown (1981) presented an overview of the use of lactic cultures covering aspects of safety, shelf-life and the cultures used. Bacus (1984) also recommended the use of starter cultures in meat processing to prolong the shelf-life and improve the quality of fermented meat products.

Therefore, it could be possible to use mixed starter cultures in Nham to improve product quality, consistency, safety, shorten fermentation and shelf-life.

1.8 AN APPROACH TO NHAM DEVELOPMENT

In development of an improved Nham process, not only is there a need for the knowledge of modern scientific discoveries and technological developments but also the knowledge of consumer's needs and wishes. The final product must be acceptable to the consumer. A unified development system is required which combines scientific and consumer information to develop systematically the Nham product. This is the Product Development process.

In general, there are six stages in the Product Development Process (Booz-Allan and Hamilton Inc., 1980) as follows: exploration, screening, analysis evaluation or business analysis, formulation and process development, testing (storage, production and consumer/market trials), and commercialization. Methods for each step of the Product Development (PD) Process are many and varied, and can be found in the literature (Anderson, 1975; Chittaporn, 1977; Twiss, 1980, Anderson, 1981(a); Ngarmsak, 1983; Earle and Anderson, 1985; Sinthavalai, 1986; Lai Pai Wan, 1987; Chinprahast, 1988). Those stages may be combined or subdivided depending upon the needs of the particular project. However, this six-stage pattern is most common and represents the basic management process when product variations and innovations are considered (Sinthavalia, 1986).

This pattern has been applied to Thai foods. The product development techniques successfully used have included the quantitiative models proposed by Anderson (1975), Chittaporn (1977) , Ngarmsak (1983), and Sinthavalai (1986), each of which applied the quantitative systematic techniques in one or more of the PD step(s) of screening, development and testing. However, there is no published Thai product development investigation in the area of fermentation. It is believed that suitable techniques can be used for developing Nham by using mixed starter cultures and formal product development methods.

1.8.1 Experimental Designs in Product Development

Product development is based on scientific principles of systematic planning, experimentation, and analysis. Computer systems are available and practical application of statistical experimental planning in many industries has increased; there is considerable opportunity to use experimental designs in the development of formulation and processes.

The design of experiments is a body of knowledge, based on statistical and other scientific disciplines, for efficient and effective planning of experiments and for making sound inferences from the data (Joglekar and May, 1987). Experimental designs have been successfully applied in many areas: agricultural research (Fisher, 1941, 1960), chemical and allied industries (Box et al., 1978; Box and Draper, 1987; Miller and Miller, 1984), the electronic and automotive industries (Taguchi and Wu, 1980), and food products (Kissell, 1967; Anderson, 1981(a); Earle and Anderson, 1985; Floros and Chinnan, 1988). Today, design of experiments is viewed as a quality technology to achieve product excellence at the lowest possible overall cost. It is a tool to optimize product and process designs, to accelerate the development cycle, to reduce development costs, to improve transition of products from research and development (R & D) to manufacturing and to troubleshoot effectively manufacturing problems (Joglekar and May, 1987). This approach should be adopted from the early stages of overall project planning right through the in-depth experimentation for process optimization. There are 7 stages in a systematic approach to experimental design (Anderson, 1981(a); Earle and Anderson, 1985) as follows: problem definition, setting objectives, experimental planning, execution, analysis of results, drawing conclusions, and action.

There are many such design techniques which are relatively easy to formulate and to analyse. The purpose of this study was to design a development model for formulating and processing Nham. Systematic methods and also appropriate statistical techniques were used for the model. These were conducted mainly following the steps of the PD process described by Anderson (1981(a)) and Earle and Anderson (1985). The problem of product and process design optimization may be viewed as a two-stage one. In the first stage, the solution was approached through the application of the Plackett-Burman technique for screening the important variables (described in detail in Chapter 3). In the second stage, factorial experimental design techniques were used for optimization of the formulation and process (described in detail in Chapters 4, 5 and 6).

1.8.2 Sensory Testing in Product Development

Consumers have traditionally been used in product development for sensory evaluation. Consumer evaluation of the quality of any food product is based mainly on its sensory properties (Cooper, 1981). Thus, the sensory properties of a food are very important to its acceptance.

Sensory evaluation has several functions in the product development process. Blair (1978) and Moskowitz (1984) used sensory evaluation to identify the product attributes which are important and their influence on overall acceptability and/or purchase intent. Blair (1978) and Moskowitz and Rabino (1983) optimized a product formulation by systematic experimental design. Additionally, sensory evaluation methods were used to match the product concept to a standard; to differentiate the product prototype from a competitive product and also to compare a laboratory product prototype with a factory trial (Blair, 1978; Pangborn, 1980; Institute of Food Technologists, 1981). The estimation of product shelf-life by sensory testing during storage tests is also an important part of product development (Labuza and Schmidl, 1985; Sinthavalia, 1986; Lai Pai Wan, 1987).

The sensory tests can be divided into two major classifications - analytical and affective tests. The analytical tests are used for laboratory evaluation of products in terms of differences or similarities and for identification and qualification of sensory characteristics. Laboratory panels normally consist of 5-10 people who are used for the analytical test (Lai Pai Wan, 1987). They are carefully selected, highly trained (Amerine et al., 1965). Sensory evaluation with laboratory panels is carried out in a controlled environment (Moskowitz and Chandler, 1979).

On the other hand, the affective tests are used to evaluate preference and/or acceptance of products. Generally, a large number of untrained panelists, selected to represent target populations, are used (Anderson, 1981(b), Sinthavalai, 1986; Lai Pai Wan, 1987). Consumer panels consist of 100-200 people who have no special training or knowledge of the product being tested. The affective tests can be carried out as central location tests, market place tests and home use tests. Only central location tests are conducted under controlled conditions while market place and home use tests are conducted under uncontrolled conditions (Sinthavalai, 1986; Lai Pai Wan, 1987).

In product development, it is the consumer panels who are important in determining acceptability, and where trained panels are used, their data should be linked to consumer data.

The use of ideal absolute, ideal interval and ideal ratio scores has been successful in guiding product development during the past few years both at Massey University and in commercial studies. Ideal absolute scores have not proved very satisfactory in directing product development over a period of time. Ideal interval and ideal ratio scores have proved to be of greater use (Cooper et al., 1989). The use of ideal ratio scores has continued at Massey University in undergraduate and commercial product development projects in both New Zealand and Southeast Asia. The ideal product profile has been used with both large and small consumer panels throughout the product development process from the first product concept to the final consumer test (Sinthavalai, 1986; Lai Pai Wan, 1987). Further development by Beausire and Earle (1986) showed that the mean ratio scores could be used in factorial experimental designs to give empirical equations. These equations related product attributes to compositional or processing parameters and could be solved to indicate the formulation and processing conditions for the ideal product. Consumers may be free to choose their own ideal points (floating ideals) or the ideals may be set (fixed ideals). The advantage to the researcher of fixed ideals is more perceptual than mathematical. Consumers will often feel more at ease with a fixed ideal profile for use over a long period (Lai Pai Wan, 1987; Cooper et al., 1989).

1.9 AIM OF THE PROJECT AND PROJECT PLAN

This research aimed to develop a fermentation process for Nham using mixed starter cultures so that the sensory quality of Nham would be improved and more consistent. Additionally, the product would be safer and have a longer shelf-life than the traditionally made product.

Part of the systematic product development process, the prototype development and the product testing, was used as a model for the project. Experimental designs were used to study the effects of different mixture of the four starter cultures (Lactobacillus plantarum, Lactobacillus brevis, Pediococcus cerevisiae and Micrococcus varians) and of different carbon sources on the chemical, physical, microbiological and sensory characteristics of the Nham. The consumer acceptability was determined at different stages of the project to guide the selection of the final process and product.

The project plan was:

- To study the inoculum preparation of the mixed starter cultures.
- To identify the important formulation and process factors affecting the Nham characteristics.
- To establish suitable mixed starter cultures for Nham and study those in the Nham system.
- To investigate the effects of different carbon sources with mixed starter cultures.
- To study the effects of temperature and relative humidity with mixed starter cultures on the rate of fermentation.
- To study the shelf-life of the product at different temperatures.
- To test the acceptability of the product to the Thai people in Chiang Mai city and villages.
- To design the final Nham product and the suitable process.

CHAPTER 2

PROJECT METHODS

This chapter describes the materials and methods used for processing and testing Nham. The basic formulation and processing using mixed starter cultures are described. The physical, chemical, microbiological and sensory analyses used in testing the Nham are also described.

2.1 SOURCES OF MATERIALS

2.1.1 Meat System

The meat system of Nham production was lean pork meat and pork skin. Both were obtained from Kiwi Bacon Co. Ltd., Longburn, Palmerston North, New Zealand for experimentation in New Zealand, and from Chiang Mai Livestock Breeding and Research Centre, Ministry of Agriculture and Cooperatives, Chiang Mai, Thailand for experimentation in Thailand. In phase 1, in New Zealand, the lean pork and pork skin were transferred to Massey University, then frozen in a blast freezer at -20°C and kept in frozen storage (-20°C); in phase 2, in Thailand, the lean pork and pork skin were obtained fresh from Chiang Mai Livestock Breeding and Research Centre and used after holding at ambient temperatures for about three hours.

2.1.2 Curing Agents

The most common curing agents used for traditional Nham processing are sodium chloride and potassium nitrate but in this project, sodium nitrate was used, so that only the sodium ion was present in the system. Sodium tripolyphosphate was also used although is not used in the traditional fermentation. All curing agents used were food grade.

2.1.3 Seasonings

The seasonings used for Nham production were pepper and garlic. In order to get fresh good quality garlic, only small amounts were bought at a time from supermarkets.

2.1.4 Carbon Sources

Carbon sources used for Nham production were glucose and jasmine rice. Although glucose is not usually used in the traditional fermentation, it was used in food grade for this project. The jasmine rice was cooked before use in Nham formulation.

2.1.5 Starter Cultures

In this project, starter cultures used were Lactobacillus plantarum NHI 1100, Lactobacillus brevis, NZ Dairy Research Institute, Pediococcus cerevisiae, NZ Dairy Research Institute, and Micrococcus varians ATCC 15306.

2.1.6 Sausage Casings

Cylindrical plastic bags diameter 22.5 mm and length 30 cm were used in this project. There were made from laminated 15 micron uncoated nylon and 50 micron linear low density polyethylene, total thickness 67 micron including adhesive.

2.2 PREPARATION OF CULTURES

L. plantarum, L. brevis and P. cerevisiae were prepared as stock cultures in All Purpose and Tween Broth (APT) for 24 hours at 30°C for all experiments. The M. varians was prepared as a stock culture in Brain Heart Infusion Broth (BHI) for 48 hours at 30°C for all experiments.

The bacteria in broth were counted by using dilutions and then inoculated on LBS agar for lactic acid bacteria under microaerobic condition and on BHI agar for micrococci. This was done before using the cultures in the inoculation of Nham mass.

Monthly transfers were made of stock cultures. Young active cultures were prepared before each experiment and the numbers per gram were determined before production by means of a plating technique, as the actual numbers being added was set for each experiment. The calculation of inoculation was as below:

> If the Nham needs 10^6 cells per gram of L. plantarum starter. Therefore, 1 kg of Nham needs 10^9 cells. As L. plantarum stock culture has 10^{10} cells/ml, so 10^9 cells of culture should be in 0.1 ml.

However, 0.1 ml was difficult to mix in evenly, therefore the inoculum was diluted to 2 ml with sterile water and then added into the model system.

2.3 METHOD OF NHAM PROCESSING

Wongkhalaung and Boonyaratanakornkit (1986) recommended a formula which was slightly modified for this study and named "Basic Formulation", shown in Table 2.1.

Frozen lean pork was thawed at room temperature overnight and washed with cold water, the meat was dried with a clean cloth and then coarsely ground once through a 5-mm plate. The pork skin was cleaned with cold water, the hairs and fat removed and the skin boiled in water for 5 minutes. On removal from the water, the skin was dried with a clean cloth and the fat completely stripped and then the skin cut by hand into shreds (approximately 0.1 cm x 2-4 cm). Both minced pork and sliced pork skin were kept in a refrigerator (4°C) until the mixing process.

Thai jasmine rice was cooked for 20 minutes in water at the ratio of 1:1.5 wt/wt (rice/water), using an electric rice cooker. Cooked rice was allowed to cool it at room temperature for 2 hours. It was then minced once through a 5-mm plate.

The garlic was cleaned and peeled before mincing through a 5-mm plate.

Table 2.1 Basic formulation for Nham production.

Ingredients	Quantity
Meat system:	
Ground lean pork	80%
Sliced pork skin	20%
	% of meat system
Curing agents:	
Sodium chloride (NaCl)	3-4
Sodium nitrate ($NaNO_3$)	0.01-0.05
Sodium tripolyphosphate ($Na_5P_3O_{10}$)	0-0.3
Seasonings:	
Minced garlic	3-7
White pepper	0-0.05
Carbon source:	
Cooked rice	5-10
	cfu/g of meat system
Starter cultures:	
Lactobacillus plantarum	$0\text{-}10^6$
Lactobacillus brevis	$0\text{-}10^6$
Pediococcus cerevisiae	$0\text{-}10^6$
Micrococcus varians	$0\text{-}10^6$

The ground lean pork and sliced pork skin were mixed by blending for 2 mintues in a Kenwood Mixer Model A703C equipped with a K-beater for 1 kg of Nham, in a Peerless and Ericsson Birmingham 30 Mixer Model KNM1 equipped with a K-beater for more than 1 kg of Nham. The other ingredients were blended into the meat for 2 minutes at 40 rpm before adding starter cultures. The volume of stock cultures to give exact number for

each experiment were added to the Nham mixture. After addition of the starter cultures, the mixing was continued at the same speed for 1 minute. The mixture was stuffed into cylindrical plastic bags and the ends of the plastics bags were tied with string. The Nham was held in incubators (Qualtex, Andrew Thom Limited) with controlled temperature and relative humidity. Saturated potassium carbonate solution was used for 43% RH, saturated potassium sulphate solution for 97% RH and saturated strontium chloride solution for 70% RH. The incubators were sterilized before and after the experiment by using 70% ethanol as a sterilizing agent. This was done to ensure there were no microorganisms on the inside surfaces of the incubators before starting the experiment.

2.4 TESTING METHODS

Methods and procedures for quality evaluation in this project were divided into four classes on the properties being tested. These were physical, chemical, microbiological and sensory tests which were used through the project.

2.4.1 Physical Tests

2.4.1.1 Sampling for Testing

Two Nham samples were used for physical and chemical tests. The samples were blended together for 1 minute in a Waring Blender.

2.4.1.2 Instron Compression, Shear Force and Energy

The Instron Model 1140 with Compression Anvil Assembly was used for compression force measurement and the Warner Bratzler Meat Shear-Compression Type for shear force measurement. For compression force, the plate was driven onto the sample (2.25 cm diameter and 2 cm length) at 200 mm/min for both cross head and chart speed and the Nham was compressed until it broke down. The depth of driving was preset so that each test had the same mechanical parameters. The shear force was measured at the same speed both cross head and chart speed as the compression force; the same sized sample was shared by shear blade and eventually cut through. The depth of shearing was also preset. The maximum peak of both measurements were measured and converted to maximum force (Newtons) (Instron Limited, 1974). The areas of both peaks were measured by planimeter and converted to energy (Joules) (Appendix 2.1).

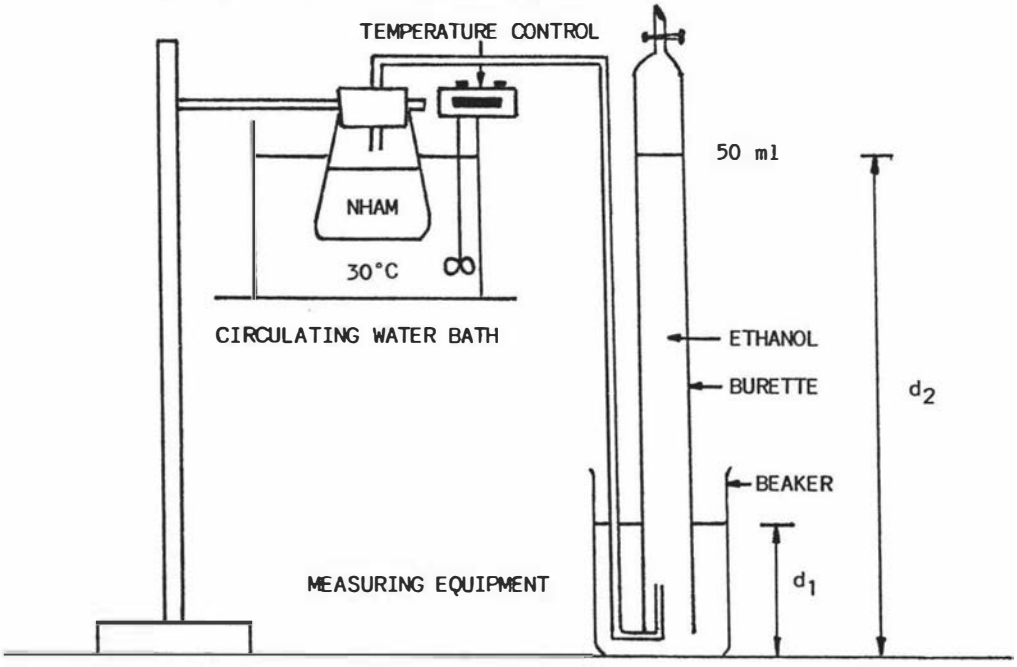
2.4.1.3 Reflective Colour

The CIE Tristimulus Values (x, y, z) were measured by the NEOTEC COLORIMETER, DU-COLOR MODEL 220. The standard used for the adjustment of x, y, z values was standard pink (x = 55.6, y = 47.8 and z = 44.5). The adjustment was done every 5-10 minutes. 25g sample was placed in a plastic container with a ring in the middle. The cover was of a plastic type for the sample and black cloth was used to protect the light (Sinthavalai, 1986).

2.4.1.4 Gas Formation

The gas formation was measured using a modified manometer made by staff of the Food Technology Department, Massey University. It consisted of a circulating water bath at a controlled temperature into which were placed 8-10 flasks containing the samples, and a gas measuring equipment that was a modified burette containing 98% ethanol.

100 g of fresh Nham were put into a 250 ml flask and placed in the circulating water bath at 30°C and left to equilibrate for 15-30 minutes before attachment to the gas measuring equipment. The flasks were then tightly closed and connected with the end of each burette so that the ethanol level was at 50 ml. The sample was incubated in the water bath and gas formation was recorded as the volume of carbon dioxide per 100 g of sample at atmospheric conditions. The gas volume was converted to normal temperature and pressure (NTP). The calculation is shown in Appendix 2.2.



GAS FORMATION MEASUREMENT DIAGRAM

2.4.1.5 Water Activity

The water activity was measured by using Aw-value Analyzer Model 5803 G. Lufft GmbH. 25 g sample was placed in the meter, which had previously been standardized with saturated barium chloride solution, Aw value 0.90 at 20°C. The meter was allowed to equilibrate over a period of four hours and then the water activity was recorded directly (Sinthavalai, 1986; Weerasooriya, 1987).

2.4.1.6 Weight Loss

After preparation of Nham, four fresh Nham sausages approximately 800 g were weighed on a weighing machine (Mettler PE 1600) which had two decimal place accuracy. The weight loss was determined by weighing the same four Nham sausages during the fermentation period. The weight loss was calculated as percentage of initial weight (Nurmi, 1966).

2.4.2 Chemical Tests

2.4.2.1 pH, Total Acidity, Volatile Acidity

The pH of Nham samples were measured using tissue slurries. Sample was removed from the casing by cutting with knife and the whole sausage was blended by Waring Blender for 1 minute. 10g sample was blended again with 100ml distilled water in the Waring Blender for 1 minute. The pH of the homogenate was measured using a pH-meter (pH M61 Laboratory pH meter). The meat slurry was then filtered and the total acidity and volatile acidity determined using the AOAC method (1984).

2.4.2.2 Residual Nitrite

The residual nitrite was determined using the standard AOAC method (1984) - nitrite in cured meat (colorimetric method). The standard curve of sodium nitrite is shown in Appendix 2.3.

2.4.2.3 Reducing Sugars and Cooked Rice

The methods of analysis for reducing sugars and cooked rice were modified from the standard AOAC method (1984) and the Dinitro Salicylic acid method (Miller, 1959; Wiriyacharee and H-kittikun 1984). The detail of the tests are described in Appendix 2.4 and 2.5.

2.4.3 Microbiological Tests

In this project, all microbiological tests were carried out immediately after sampling. Care was exercised in testing samples to make them representative of the items to be tested and to make certain that no contamination occurred prior to examination. Samples were taken under aseptic condition using sterilized knives, forcepts, etc.

2.4.3.1 Method of Sampling and Preparation of Sample

For microbiological examination, two Nham sausages were randomly sampled. From each sausage to be examined, a 4-5 mm thick slice was taken and cut into small pieces with sterile knives. 11 g were weighed into a sterile plastic bag, 99 ml of a sterile diluent (0.1% peptone) were added and the sample was homogenized with a stomacher machine (Sharpe and Jackson, 1972; Speck, 1976; Parry et al., 1982; Lowry, ca 1984; Kiss, 1984).

The necessary dilutions were then made by using 0.1 % peptone solution, the dilutions were selected so that the total number of colonies in a plate was between 30 and 300 (Breed and Dotterrer, 1916; Kiss, 1984). The inoculation was 1 ml of the dilutions in the plating method and 0.1 ml in the spread method.

The counts of bacteria were done after incubation at suitable time and temperature. When selective substrates were used, the number of colonies counted varied between 150 and 300. The final number of bacteria was obtained by calculating the weighed mean of two consecutive dilutions. The bacterial counts were stated per gram of sample and then converted to log number.

2.4.3.2 Mesophilic Aerobic Microorganisms

In order to determine the mesophilic aerobic microorganisms in Nham, plate count agar with suitable dilutions and the plating technique were used. The plates were incubated at 30°C for 48 hours before counting (Speck, 1976; Kiss, 1984).

2.4.3.3 Enterobacteriaceae

To determine the Enterobacteriaceae, selective agar, Neutral Red Bile Glucose Agar, was used by the pour plating technique and the plates were incubated at 37°C for 24 hours (Speck, 1976; Corry et al., 1982; Kiss, 1984).

2.4.3.4 Staphylococcus aureus

To determine this organism, 0.1 ml of the appropriate dilutions was spread evenly over the surface of dried Baird-Parker Agar plates (used within 48 hours of preparation) and incubated aerobically at 37°C for 24 hours. Counts were as presumptive S. aureus colonies which were black and shiny, had a narrow white margin, and were surrounded by a clear zone extending into the opaque medium. The position was marked of these colonies and the plates incubated for a further period so that the total length of incubation was 48 hours. At the end of the extended period of incubation all colonies with above appearance were counted. If the count exceeded 100, 10 were selected at random; submitting these colonies to coagulase-positive confirmation by using a fibrinogen-sensitized sheep red blood cells system (Staphylase^{R}, Oxoid Ltd., Basingstoke, England).

2.4.3.5 Yeasts and Moulds

To determine the yeasts and moulds, Potato Dextrose Agar and pour plating technique were used. The plates were incubated at 30°C for 3-5 days before counting (Speck, 1976; Kiss, 1984).

2.4.3.6 Starter Cultures

A suitable medium for counting the lactic acid bacteria such as L. plantarum, L. brevis and P. cerevisiae was Lactobacillus Selective Media (LBS). The cultures were incubated on LBS agar under microaerobic condition at 30°C for 2 days.

The L. plantarum was recorded by counting the large colonies with about 3.5-4.0 mm diameter white cream colonies whereas the L. brevis was also recorded by counting the smaller colonies with about 1.0 - 1.5 mm diameter white and translucent colonies at 2 days. On the other hand, the P. cerevisiae also grew in this agar at the same condition after 2 days, as very small colonies of about 0.1- 0.2 mm diameter white colonies. As a result of this, the time and characteristics of cultures were carefully counted.

A suitable medium for counting the M. varians was Ammonium Phosphate Agar. The culture was incubated on this agar at 30°C for 2 days. The colony was yellow with about 0.5-1.0 mm and changed the medium into yellow at 2 days.

For all cultures, gram strain technique was used to ensure the counting. The gas formation in MRS broth was also tested to identify and ensure the different characteristics between L. plantarum and L. brevis;only L. brevis produced gas in the broth.

2.4.4 Sensory Tests

In the sensory testing, a laboratory panel of ten Thai postgraduate students used the ideal profile method to identify the effects on the final Nham of different variables and their levels of treatment. The method used was an adaption of the ideal profile method in particular using the ideal ratio method of Moskowitz (1985), Sinthavalai (1986), Lai Pai Wan (1987), Chinprahast (1988). The method used was to:

- Organise the testing environment and methods
- Identify the Nham characteristics and select the important characteristics
- Develop the scaling method
- Set the ideal profile
- Select the sensory panel
- Train the sensory panel.

2.4.4.1 Testing Environment and Methods

All the sensory evaluation sessions were held in the sensory panel room in the Food Technology Department. Taste panel booths were used. The room was lit by white fluorescent light. Slices of Nham at 10°C, 0.5 cm thick, were placed on a plain white plate divided into sections. Each sample was given a random number code. A glass of water and a fork were provided. A maximum of 5 samples was presented at each testing. If more samples were to be tested in the experiment, 5 samples were tested and then after a break of 5 minutes, further samples were tested. It was not possible to have a larger break because of the limited time available to the panelists. Nham has a comparatively mild flavour, easy to test, and panelists did not have difficulty in testing this large number of samples at one sensory testing session. 6 panelists were invited to test in the morning (9.30 - 11.30 am) whereas the remainder (4 panelists) were invited to test in the afternoon (13.10-14.30 pm), the most suitable times available to each panelist. Discussions after the sensory testing were conducted in the consumer panel room in the Product Development Laboratory.

2.4.4.2 Identification of Nham Characteristics and Selection of the Important Characteristics

The panelists were given a blank form (Figure 2.1). The form divided the sensory testing of the Nham into four sections: appearance, texture, flavour and other characteristics. The panelists tested 3 samples of Nham and wrote down the Nham characteristics that they noticed from first looking at the Nham to the final swallow. Then all the panelists discussed the Nham characteristics, ranked them in importance and selected the important characteristics. The terminology required to describe the Nham characteristics was developed and agreed among the panelists. This was done again in the second session in order to ensure the terms developed to be correct and the panelists understanding of the terms. The final definitions to describe the important characteristics of Nham are shown in the Table 2.2.

Once the panelists identified the important characteristics, an ideal product profile was developed. In the first session the ideal point for each characteristic was also marked by the panelists in the blank form according to their own perception. Again, they were also asked for their ideal points in the second session with the defined characteristics. A group discussion was held to agree on a suitable profile. The approximate mean ideal scores for all characteristics were determined during this time.

Figure 2.1 A Nham characteristics identification questionnaire

PROFILE TEST

NHAM CHARACTERISTICS IDENTIFICATION

PRODUCT DEVELOPMENT SUB DEPARTMENT

MASSEY UNIVERSITY

NAME:_____ DATE:_____

Please taste the Nham sample and write in your words to describe each Nham characteristic. Put an (X) where you think the product fits on the scale. Also write in an (I) for your ideal.

After you have written all the attributes,return this sheet, and the samples, and wait for the next samples.

If you have any questions, need more water,etc., please ask experimenter.

The structured scale is shown below as an example.

WORD TO DESCRIBE
NHAM CHARACTERISTICS:

1. Firmness

LOW		MODERATE		HIGH
	X 825	X 766		I X 084

NOTE: 825 = sample A
766 = sample B
084 = sample C
I = your ideal (firmness of Nham)

Many thanks for your time and cooperation, your answer have been very useful.

Pairote Wiriyacharee

WORD TO DESCRIBE
NHAM CHARACTERISTICS:

	LOW	MODERATE	HIGH
A: APPEARANCE:			
B: TEXTURE:			
C: FLAVOUR:			
D: OTHERS:			

Table 2.2 Definitions to describe the important characteristics of Nham.

A: APPEARANCE:

1. Colour	: the intensity of red colour both internal and external of sample.
2. Visual Texture	: the overall distribution of lean pork and pork skin in the sample.
3. Air pockets	: the amount of air holes in the sample.

B: TEXTURE:

4. Firmness	: the overall force required to compress the sample between the molar teeth to give deformation or penetration.
5. Juiciness	: the total impression of liquid in the mouth just prior to swallowing. This includes water, meat juice, liquid fat, and saliva.
6. Fattiness	: the impression of a fatty or greasy mouth feel during the chewing process.
7. Stickiness	: the force required to remove the sample that adheres to the mouth, generally the palate, during the normal eating process.
8. Smoothness	: uniformity of texture that is the homogeneity of the sample whilst chewing, the degree to which both lean pork and pork skin are perceived to be present in chewed sample.

C: FLAVOUR:

9. Sourness	: the overall strength of fermented sour taste detected in the sample.
10. Saltiness	: the overall strength of salt taste detected in the sample.
11. Spiciness	: the overall strength of garlic and white pepper flavours detected in the sample.
12. Pork Flavour	: the overall strength of pork meat flavour detected in the sample.

D: OVERALL ACCEPTABILITY:

13. Acceptability of Nham	: take 5-6 bites of sample, chew in molar teeth until ready for swallowing. Swallow sample and evaluate overall texture, odour, flavour, and then give acceptability score of product sample.

2.4.4.3. Development of Scaling Method

It was necessary to develop an interval scale for each characteristic. A line (8 cm) with verbal anchors at each end was established. Each scale expressed the strength of each characteristic of Nham. The agreed mean ideal scores obtained from the panelists were also set on the scale. The panelists were again asked to agree on the positions of the ideal points. Additionally, the form also asked the panelists to list in order the six most important characteristics of Nham which affected their purchase of the product (Figure 2.2). This was to remove the least important characteristics of Nham.

Figure 2.2 Nham characteristics and ideal point questionnaire

NHAM CHARACTERISTICS AND IDEAL POINT

PRODUCT DEVELOPMENT SUB DEPARTMENT

MASSEY UNIVERSITY

NAME:_____ DATE:_____

GOOD MORNING / GOOD AFTERNOON

Now. I am doing some work on Thai people's attitudes towards traditional fermented pork sausages; Nham. I would like to test the Nham characteristics. You have been presented with_____samples of Nham coded_____.

I would like you to score each property of Nham. Please mark (X) where you think the product fits on scale. However, the ideal point of each attribute (I) was fixed on the scale. Do you agree to the positions of the ideal point? If not, please change the positions again.

NHAM CHARACTERISTICS:

A: APPEARANCE:

1. COLOUR	Dark red	Pale pink
2. VISUAL TEXTURE	Not smooth	Very smooth
3. AIR POCKETS	No air holes in sample	Plenty of air holes in sample

B: TEXTURE:

4. FIRMNESS	Not firm	Very firm
5. JUICINESS	Not juicy	Very juicy
6. FATTINESS	Not fatty	Very fatty
7. STICKINESS	Not sticky	Very sticky
8. SMOOTHNESS	Not smooth	Very smooth

C: FLAVOUR AND TASTE:

9. SOURNESS	Not sour	Very sour
10. SALTINESS	Not salty	Very salty
11. SPICINESS	Not spicy	Very spicy
12. PORK FLAVOUR	Absence of pork flavour	Very strong pork flavour

D: OVERALL ACCEPTABILITY:

13. ACCEPTABILITY OF NHAM

Dislike extremely ——— Like extremely

Please list in order the six most important attributes of the Nham which will affect your purchase of this product.

1._____
2._____
3._____
4._____
5._____
6._____

Many thanks for your cooperation.

Pairote Wiriyacharee

2.4.4.4 Setting the Ideal Profile

The ideal profile of Nham was discussed after sensory testing among the panelists to ensure that the group agreed on the profile. The mean ideal scores were determined and used as fixed ideal points on the scale for all attributes. The fattiness and stickiness - the least important attributes affecting the panelists' purchase of the Nham were dropped from the questionnaire (See Appendix 2.6). The final sensory panel form is shown in Figure 2.3.

Figure 2.3 Standard questionnaire tor Nham sensory testing.

STANDARD FORM FOR NHAM TESTING

NHAM CHARACTERISTICS

PRODUCT DEVELOPMENT SUB DEPARTMENT
MASSEY UNIVERSITY

NAME:_____ DATE:_____

NHAM CHARACTERISTICS:

A: APPEARANCE:

1. C LOUR	Dark red	Pale pink
2. VISUAL TXTUR	Nt s m oh	Very s m oh
3. AIR F KETS	N air holes in sample	Planty of air holes in sample

B: TEXTURE:

4. Fi f NESS	Nt firm	Very firm
5. JUICINESS	Nt juicy	Very juicy
6. S K TNESS	Nt s m oh	Very smooth

C: FLAVOUR AND TASTE:

7. SURESS	Nt sour	Very sour
S. SALTINSS	Nt salty	Very salty
9. SPICINSS	Nt spicy	Very spicy
10. P R FLAVOUR	Absence of prk flavour	Very strong prk flavour

D: OVERALL ACCEPTABILITY:

11. A a EPTASILi l OF NHAM

Dislike extre m ly — Like extre m ly

MANY T ANKS FOR YOR TI M AND C OP R TION. YOUR ANWE R H VE B EN VE R USEFUL.

PAI R TE WIRIYA C ARE

2.4.4.5 Sensory Panel Selection

Ten Thai postgraduate students at Massey University were invited to be the panelists. During the setting up of the ideal profile of Nham, two of the panelists had vastly different ideas. Therefore, only 8 Thai students were chosen as the sensory panel.

2.4.4.6 Training of the Sensory Panel

The 8 Thai students at Massey University were trained to improve their ability to describe the Nham characteristics and to use the scale. During the training period the sensory panels were held in two sessions. Three formulations of Nham were presented (Appendix 2.7). The definitions of the characteristics were given to each of the panelists at each session until they were familiar with the method of evaluation. Group discussions were held following these sessions to ensure the proper understanding of the terms and scales used in the form.

2.4.4.7 Sensory Panel in Thailand

A further panel of 10 Thai undergraduate students at Chiang Mai University were used for sensory testing in Thailand. There were also trained. The form and definitions used in New Zealand were translated into Thai (Appendix 2.8). The form and definitions were used to train the panelists in Thailand.

CHAPTER 3

IDENTIFYING THE IMPORTANT FACTORS AFFECTING THE CHARACTERISTICS OF NHAM

3.1 INTRODUCTION

The purpose of the experiments described in this chapter was to determine which of the ingredients and process variables are responsible for the production of Nham of high quality.

L. plantarum, P. cerevisiae, L. brevis and M. varians were used together as mixed starter cultures in order to investigate the possibility of improving Nham quality. These cultures have been found in native Nham (Techapinyawat, 1975; Srisomwong, 1985; Wongkhalaung and Boonyaratanakornkit, 1986).

Sodium chloride, sodium nitrate, sodium tripolyphosphate, cooked rice, white pepper and raw garlic used in the Nham formulations, were studied to identify the important variables affecting the Nham system. Although some research has shown the effect of sodium chloride in the product (Techapinyawat, 1975), the effects of the other ingredients have not been studied.

A Plackett-Burman design (Stowe and Mayer, 1966; Anderson, 1981(a); Earle and Anderson, 1985) was selected for use in this experiment as Plackett-Burman designs are the most efficient for screening large number of variables.

Chemical, physical and microbiological tests were used as response variables in order to screen the important factors by using t-test analysis.

3.2 PLACKETT-BURMAN DESIGN

Plackett-Burman designs can screen N-1 variables in N experiments, that is, only 16 experiments are required to screen 15 variables. Use of this type of design will not in general give accurate estimates of the main effects, because of all the interactions confounded with them. Thus, there might be a tendency to ignore the confounding

because it is impossible to measure the effects of interactions and this is not usually important in screening as the main objective is to isolate the important variables (Stowe and Mayer, 1966; Anderson, 1981(a)).

Plackett and Burman have provided the first row of the design matrix for investigating various numbers of factors (Earle and Anderson, 1985). The remainder of the design matrix is generated by shifting this first row one space to the left N-2 times, where N is the number of factors to be studied. The final row of the matrix, a row of minus signs, is added to the bottom of the generated matrix.

In this experiment, 10 variables were used as input variables. The most appropriate Plackett-Burman design (N=16) was selected and provided five dummy variables from which the error was estimated. If the design (N=12) was used, only one dummy variable would be available to estimate the error.

Table 3.1 shows the full design matrix with each variable at two levels "+" denoting high level, and "-" low level. The 16 runs were done in random order, so that no "order" bias was introduced.

To calculate the effect of any variable, the average of the results at the low level of that variable was subtracted from the average of the results at the high level of the same variable. For example, the effect of Micrococcus varians was calculated as follows:

$$\text{Effect (MV)} = \frac{\left[X_1 + X_2 + X_3 + X_4 + X_6 + X_8 + X_9 + X_{12}\right]}{8} - \frac{\left[X_5 + X_7 + X_{10} + X_{11} + X_{13} + X_{14} + X_{15} + X_{16}\right]}{8}$$

where X_n = value of the response variable in run n

n = number of run.

Table 3.1 Plackett-Burman matrix for determining the effects of 10 variables at two levels using 16 runs.

TREATMENTS	EXPERIMENTAL ORDER	Input variables														
		M.varians	P.cerevisiae	L.plantarum	L.brevis	NaCl	$NaNO_3$	Cooked rice	$Na_5P_3O_{10}$	White pepper	Raw garlic	Dummy	Dummy	Dummy	Dummy	Dummy
1	(9)	+	+	+	+	-	+	-	+	+	-	-	+	-	-	-
2	(2)	+	+	+	-	+	-	+	+	-	-	+	-	-	-	+
3	(11)	+	+	-	+	-	+	+	-	-	+	-	-	-	+	+
4	(10)	+	-	+	-	+	+	-	-	+	-	-	-	+	+	+
5	(6)	-	+	-	+	+	-	-	+	-	-	-	+	+	+	+
6	(16)	+	-	+	+	-	-	+	-	-	-	+	+	+	+	-
7	(5)	-	+	+	-	-	+	-	-	-	+	+	+	+	-	+
8	(13)	+	+	-	-	+	-	-	-	+	+	+	+	-	+	-
9	(4)	+	-	-	+	-	-	-	+	+	+	+	-	+	-	+
10	(15)	-	-	+	-	-	-	+	+	+	+	-	+	-	+	+
11	(7)	-	+	-	-	-	+	+	+	+	-	+	-	+	+	-
12	(8)	+	-	-	-	+	+	+	+	-	+	-	+	+	-	-
13	(3)	-	-	-	+	+	+	+	-	+	-	+	+	-	-	+
14	(12)	-	-	+	+	+	+	-	+	-	+	+	-	-	+	-
15	(14)	-	+	+	+	+	-	+	-	+	+	-	-	+	-	-
16	(1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ = high level; - = low level

Similar calculations were made for each of the effects including those of the dummy variables. The calculated effects of the dummy variables were used to test the significance of the real effects.

The standard error was calculated as:

$$\text{Standard error (SE)} = \sqrt{\frac{\sum(Ed)^2}{n}}$$

where Ed = dummy effect

n = number of dummy effects

The t- values were calculated for each of the effects as: $t = \frac{\text{effect}}{SE}$ and were then compared with the t-table value(two tailed test) whose number of degrees of freedom was equal to the number of dummy effects making up the error term. These calculations were performed using the Massey Plackett-Burman computer package (Van Tel, 1988).

3.3 STARTER PRODUCTION

Although various strains of lactic acid bacteria and micrococci were available in frozen and freeze-dried forms for use as meat starter cultures (Everson et al., 1970 (a), (b); Nordal and Slinde, 1980; Bacus and Brown, 1981; Liepe, 1983; Bacus, 1984; Raccach, 1984; Gilliland, 1985; Gibbs, 1987), for this project the starter cultures were used as broth cultures because controlled amounts with known activities were to be used in the model system. They were usually added to the sausage mix to achieve a minimum microbial count of one million organisms per gram of product (Bacus, 1984).

Firstly, a study was made of suitable media for growth of the cultures; five media were used; Nutrient Broth (NB), Lactobacillus Selective Broth (LBS), Lactobacillus Broth (MRS), All Purpose and Tween Broth (APT), and Brain Heart Infusion Broth (BHI). Those were obtained from BBL, Division of Becton, Dickinson and Company, U.S.A.. 0.1 ml of each late log phase culture was added to separate tubes containing 10 ml of sterile medium. A 30°C incubation temperature was used for all cultures.

Cultures were sampled and counted by plating suitable dilutions (0.1 % peptone water) on the same five media in agar. The results are shown in Table 3.2.

Table 3.2 The numbers of starter cultures in different media after incubation at 30°C.

Time (hours)	Media	P. cerevisiae (log N)	L. plantarum (log N)	L. brevis (log N)	M. varians (log N)
24	NB	3.42	7.52	6.07	7.68
	BHI	7.77	9.41	8.78	8.07
	APT	9.13	10.15	9.76	8.05
	MRS	8.99	10.20	9.74	6.48
	LBS	4.08	9.10	8.54	NG
48	NB	6.74	7.74	7.17	8.23
	BHI	8.36	9.63	8.69	8.55
	APT	9.03	10.07	8.76	7.94
	MRS	9.59	10.84	9.46	7.01
	LBS	7.21	10.09	9.21	NG
72	NB	5.97	7.75	7.54	8.32
	BHI	8.55	9.49	9.17	8.36
	APT	8.63	9.70	8.50	7.62
	MRS	9.47	9.16	8.93	7.11
	LBS	8.81	9.94	9.51	NG

NG = No Growth

A suitable medium and incubation time was selected for each culture. As can be seen in Table 3.2. P. cerevisiae and L. brevis grew quickly in APT broth for 24 hours up to log N = 9.13 and 9.76 respectively and then the numbers declined slowly. The L. plantarum grew well in MRS broth and APT broth to log N = 10.20 and 10.15 respectively. After this its number increased a little in MRS but decreased slowly in APT. As a result of this and for ease of working, P. cerevisiae, L. plantarum and L. brevis were prepared as stock cultures in APT broth incubated for 24 hours for each experiment.

M. varians grew well in BHI broth and increased to log N = 8.55 after 48 hours and then slowly decreased. Therefore, M. varians was prepared as a stock culture in BHI incubated for 48 hours for each experiment.

Monthly transfers were made of stock cultures. All inocula for experiments were prepared freshly from actively-growing cultures and standardized before use.

3.4 SAUSAGE PREPARATION FOR PLACKETT-BURMAN DESIGN

The Nham formulation is shown in Table 3.3. The sausage preparation and processing were as described in Section 2.3. The volume of starter culture added was calculated to give 1×10^6 cfu.g^{-1} of each organism. After production, the Nham was held for 60 hours in an incubator at 30°C and relative humidity of 43%.

Table 3.3 Nham formulation in Plackett-Burman experiment.

Ingredients	Quantity	
Meat system:		
minced lean pork	80%	
sliced pork skin	20%	
	% of meat system	
	low (-)	high (+)
Curing agents:		
sodium chloride (NaCl)	1	4
sodium nitrate ($NaNO_3$)	0.01	0.03
sodium tripolyphosphate ($Na_5P_3O_{10}$)	0	0.5
Seasonings:		
minced raw garlic	3	7
white pepper powder	0	0.05
Carbon source:		
minced cooked rice	5	8
	cfu/g of meat system	
	low (-)	high (+)
Starter cultures:		
Lactobacillus plantarum	0	10^6
Lactobacillus brevis	0	10^6
Pediococcus cerevisiae	0	10^6
Micrococcus varians	0	10^6

3.5 TEST METHODS

After 60 hours, the sausages were randomly sampled for testing. The tests used were:

pH, total acidity and volatile acidity (see Section 2.4.2).

Water activity, weight loss, compression force and energy, shear force and energy, colour (CIE tristimulus values) and gas formation (see Section 2.4.1).

Enterobacteriaceae determination (see Section 2.4.3).

3.6 RESULTS: CHEMICAL, MICROBIOLOGICAL AND PHYSICAL CHARACTERISTICS OF NHAM

Tables 3.4 and 3.5 show that the pH, total acidity, water activity, weight loss, Enterobacteriaceae and gas formation varied widely with different treatments after 60 hours of fermentation. The pH varied from 4.35 to 5.99. The physical characteristics also varied widely during fermentation; the colour (x, y, z) from respectively 19.2, 16.6, 14.6 to 35.2, 31.8, 31.9; the compression force and energy from 8.47 Newtons to 85.11 Newtons and 0.09 Joules to 0.58 Joules respectively. The shear force and energy also varied widely. An important observation was that addition of starter cultures inhibited the growth of Enterobacteriaceae and gas formation, in fact that Enterobacteriaceae was reduced approximately 7 log cycles and gas formation decreased from 147 to 15 ml CO_2 per 100 g at NTP. Addition of starter cultures increased the compression force and energy, except when L. brevis was added alone.

Table 3.4 The chemical and microbiological characteristics after 60 hours of Nham fermentation at 30°C and 43% relative humidity.

Treatments	Final pH	Total acidity (%)	Volatile acidity (%)	Water activity (A_w)	Weight loss (%)	Enterobacteriaceae (log cfu/g)	Gas formation ml CO_2/100g at NTP
1	5.30	0.87	0.04	0.98	1.46	6.94	86.52
2	4.62	1.08	0.18	0.96	2.20	1.95	26.77
3	4.44	1.24	0.09	0.98	3.54	5.24	58.89
4	4.72	1.08	0.12	0.97	1.43	5.81	17.49
5	5.25	0.91	0.06	0.95	0.19	3.91	19.62
6	4.40	1.28	0.14	0.98	3.47	2.14	58.58
7	4.55	1.14	0.16	0.98	3.70	6.62	44.39
8	4.60	1.10	0.17	0.95	1.28	4.34	23.85
9	5.38	0.85	0.16	0.98	0.72	7.71	113.04
10	4.35	1.52	0.02	0.98	2.37	5.03	49.42
11	4.80	1.04	0.10	0.97	0.42	4.13	15.48
12	5.15	0.93	0.08	0.96	0.10	4.87	14.56
13	5.99	0.45	0.08	0.97	0.12	4.15	23.50
14	4.94	1.04	0.02	0.96	0.21	5.03	29.05
15	4.52	1.20	0.16	0.95	3.09	3.03	25.02
16	5.00	0.97	0.08	0.98	0.37	8.61	146.93
Range							
Min.	4.35	0.45	0.02	0.95	0.10	1.95	14.56
Max.	5.99	1.52	0.18	0.98	3.70	8.61	146.93

Table 3.5 The physical characteristics after 60 hours of Nham fermentation at 30°C and 43% relative humidity.

Treatments	Response variable						
	Colour			Compression		Shear	
	CIE Tristimulus values						
	X	Y	Z	Force (N)	Energy (J)	Force (N)	Energy (J)
1	24.1	21.2	20.7	39.75	0.28	18.17	0.43
2	23.9	20.6	20.2	85.11	0.55	31.86	0.57
3	28.6	25.0	24.3	36.31	0.28	20.29	0.48
4	35.2	31.8	31.9	75.31	0.50	33.20	0.69
5	20.7	17.4	16.4	61.84	0.30	29.41	0.45
6	28.2	24.6	24.4	42.26	0.30	18.54	0.40
7	30.6	27.2	26.9	33.47	0.27	20.00	0.46
8	31.5	28.2	27.5	79.10	0.58	28.23	0.65
9	23.2	20.4	19.2	42.31	0.13	19.67	0.43
10	25.4	22.4	22.2	51.09	0.29	19.11	0.43
11	25.0	21.5	21.0	51.23	0.29	22.34	0.47
12	26.2	22.8	21.7	55.87	0.34	21.96	0.46
13	19.2	16.6	14.6	8.47	0.09	15.98	0.20
14	24.9	21.5	20.1	49.84	0.32	23.60	0.45
15	27.6	24.4	23.9	77.21	0.54	27.38	0.59
16	21.8	19.0	18.2	21.58	0.27	20.90	0.36
Range							
Min.	19.2	16.6	14.6	8.47	0.09	15.98	0.20
Max.	35.2	31.8	31.9	85.11	0.58	33.20	0.69

3.7 DISCUSSION

3.7.1 Starter Cultures Affecting the Characteristics of Nham

Table 3.6 shows that all four starter cultures affected the Nham system in terms of colour and firmness development. M. varians increased significantly the tristimulus values (y, z) and increased slightly the x value. L. plantarum also increased the z value significantly and slightly increased the x and y values whereas the L. brevis decreased the x, y, z values. These results are consistent with the work of Klettner and Baumgartner (1980) who stated that to ensure good colour development and subsequent retention of colour in fermented meat products the oxygen content within the sausages should be minimal and pH be low. They also showed that the nitrate was reduced to nitrite by micrococci at a pH about 5.6. If the pH was too low at the beginning, the micrococci would be inhibited such that the nitrate remained unconverted. Thus, M. varians played an important role in stabilizing the colour of Nham during the initial period of ripening before the pH reduction.

L. plantarum produced acid causing a significant pH reduction. Both L. plantarum and P. cerevisiae increased the weight loss and increased significantly the compression energy of Nham. According to Klement et al. (1974) the development of firmness is directly influenced by acid development in the region of pH 5.3, approaching the gel point of meat protein. The protein forms a gel, with a consequent rapid increase in firmness. However, Klettner and Baumgartner (1980) have also stated that the rate of water loss and increase in firmness depended upon the water vapour gradient between the sausage and the ripening room.

In this experiment, L. plantarum showed an effect on the colour development in Nham. The reduction of the nitrite to nitric oxide can be accelerated by low pH and high temperature. Thus the acid condition also enhanced colour development, as nitric oxide reacted with myoglobin in meat forming a red pigment (Klettner and Baumgartner, 1980).

Table 3.6 The main effects and significance levels of starter cultures on the characteristics of Nham.

Response variables	Input variables			
	M. varians	P. cerevisiae	L. plantarum	L. brevis
1 pH	-0.099	-0.231	-0.401[a]	0.304
2 Total acidity (%)	0.021	0.057	0.212	-0.128
3 Volatile acidity (%)	0.037	0.032	0.002	-0.021
4 Water activity (Aw)	0.001	-0.004	0.001	-0.001
5 Tristimulus values X	3.212	0.988	2.962	-2.888
Y	3.075[a]	0.800	2.850	-2.800
Z	3.325[a]	1.075	3.425[a]	-3.250
6 Weight loss (%)	0.463	0.887[b]	1.399[b]	0.116
7 Compression force (N)	12.661	14.661	12.167	-11.847
8 Compression energy (J)	0.074	0.105[a]	0.096[a]	-0.106[a]
9 Shear force (N)	1.652	3.088	1.636	-3.070
10 Shear energy (J)	0.088	0.085	0.063	-0.084
11 Enterobacteriaceae (LogN)	-0.180	-0.900	-0.800	-0.400
12 Gas formation (ml CO_2/100 g. at NPT)	4.510	-17.730	-8.550	10.690

a = t-test significance level at 90%
b = t-test significance level at 95%

L. brevis decreased significantly the firmness of Nham at 90% confidence level. It also produced gas and this would lead to poor Nham texture, owing to the presence of gas bubbles. Additionally, the tristimulus value was reduced slightly and lactic acid was apparently not produced. L. brevis is a heterofermentative lactic acid bacterium, producing not only lactic acid but also acetic acid, ethanol and carbon dioxide (Klettner and Baumgartner, 1980; Bacus, 1984). It is possible that the growth of L. brevis requires metabolic products of other lactic acid bacteria and may also require a lower starting pH as in the sauerkraut fermentation (Fleming et al., 1985).

3.7.2 Ingredients Affecting the Characteristics of Nham

Table 3.7 shows that sodium chloride strongly decreased the weight loss and water activity and increased the firmness of Nham. The salt was added to Nham to achieve the desired water binding, flavour, and preservative qualities. The three studies of Klement et al. (1973; 1974; 1975) showed that firmness development in fermented meat sausage was related more to the effect of pH on the myofibrillar proteins than on the sarcoplasmic proteins. Salt also enhanced the denaturation of scarcoplasmic proteins under fermentation conditions, but its primary role was probably in determining texture aspects related to binding the meat mass together, as was suggested by Palumbo et al. (1976).

The salt also inhibited the growth of Enterobacteriaceae and the gas formation. Bacus (1984) stated that sodium chloride was a major component that allowed the lactic acid bacteria to predominate and inhibited many undesirable microorganisms, including pathogens.

In general, fermented sausages are formulated with 2.0 to 3.5% salt, depending on the nature of the product. Techapinyawat (1975) indicated that Nham was most acceptable to a taste panel at 3% salt level. At the low (2%) and high (5%) levels, Nham became unacceptable because the sourness was insufficiently developed at 2% salt and the product was too salty at 5%. Zaika et al. (1978) noted that no major differences in fermentation rate were observed up to 3% salt.

Sodium nitrate had no significant effect in this screening experiment. It perhaps served simply as a reservoir from which nitrite could be produced by micrococci.

Table 3.7 The main effects and significance levels of curing agents on the characteristics of Nham.

Response variables	Input variables		
	NaCl	$NaNO_3$	$Na_5P_3O_{10}$
1 pH	0.196	0.221	0.196
2 Total acidity (%)	-0.141	-0.143	-0.026
3 Volatile acidity (%)	0.009	-0.036	-0.041
4 Water activity (Aw)	-0.018[b]	0.002	-0.003
5 Tristimulus values X	0.288	1.438	-3.662[a]
Y	0.250	1.325	-3.625[a]
Z	-0.075	1.150	-3.775[a]
6 Weight loss (%)	-0.928[b]	-0.338	-1.169[b]
7 Compression force (N)	21.841[b]	-13.781	7.915
8 Compression energy (J)	0.138[b]	-0.073	-0.039
9 Shear force (N)	6.574[b]	-2.448	0.198
10 Shear energy (J)	0.077	-0.031	-0.019
11 Enterobacteriaceae (LogN)	-1.670[a]	0.760	-0.040
12 Gas formation (ml CO_2/100 g. at NPT)	-47.900[b]	-22.940	-6.790

a = t-test significance level at 90%
b = t-test significance level at 95%

Sodium tripolyphosphate strongly decreased the weight loss of Nham, but also reduced the colour development. Sodium tripolyphosphates are used commonly in meat products to improve the texture of the product, to give better slicing characteristics and to provide higher processing yield (Cole and Lawrie, 1975). Bacus (1984) found that the use of phosphate increased the pH in most meat products and provided a buffer to retard any pH decrease via starter cultures. Depending upon the respective levels of phosphate and a fermentable carbohydrate, the culture fermentation would overcome the buffer, but the

pH decline would proceed more slowly. This in turn would influence the colour and keeping qualities of Nham, because a lower pH enhances cure colour development (Terlizzi et al., 1980; Klettner and Baumgartner, 1980) and reduces growth of spoilage microorganisms. Bacus (1984) indicated that generally, in fermentation processes using starter cultures, phosphate was omitted, or at least the phosphate level reduced, to expedite the fermentation. Molins et al. (1984) stated that sodium tripolyphosphate had only a slight effect on starter cultures. It appears that in the system under study sodium tripolyphosphate addition could be beneficial in reducing weight loss.

Cooked rice and raw garlic both increased the weight loss significantly at 95% confidence level (Table 3.8). Cooked rice also inhibited the growth of Enterobacteriaceae (Table 3.8), this was probably because the starter cultures used cooked rice as carbon source for acid production and the raw garlic stimulated acid production, thus inhibiting the Enterobacteriaceae. Many researchers have shown that natural spices, typically those used in meat formulations, can have a direct effect on the rate of fermentation by stimulating acid production by the bacteria (Ingolf and Skjelkvale, 1982). Recent studies by Zaika and Kissinger (1979, 1984) have identified manganese as the factor in spices responsible for enhancement of the acid production. Manganese is required by lactic acid bacteria for various enzyme activities, including the key enzyme of glycolysis, fructose 1-6 diphosphate aldolase. Oleoresin preparations devoid of manganese had no effect on the fermentation, though the natural spices from which they were derived stimulated growth and acid production in selected commercial starter cultures (Ingolf and Skjelkvale, 1982). Extracts of manganese from various spices have been shown to be strongly stimulatory, the effect increasing with increasing manganese level (Zaika and Kissinger, 1984). Since naturally ground spices retain their flavour over a longer period of time than do oils or oleoresins, the natural spices were used in this study of the Nham formulation.

Table 3.8 The main effects and significance levels of carbohydrate and seasonings on the characteristics of Nham.

Response variables		Input variables		
		Cooked rice	White pepper	Raw garlic
1 pH		-1.184	0.164	-0.269
2 Total acidity (%)		0.096	-0.060	0.169
3 Volatile acidity (%)		0.005	0.004	0.007
4 Water activity (Aw)		0.000	0.000	-0.002
5 Tristimulus values	X	-0.988	0.788	2.488
	Y	-1.100	1.050	2.400
	Z	-1.075	1.100	2.300
6 Weight loss (%)		0.745[b]	-0.359	0.669[b]
7 Compression force (N)		0.547	4.773	4.956
8 Compression energy (J)		0.003	0.009	0.020
9 Shear force (N)		-1.964	-0.310	-1.272
10 Shear energy (J)		-0.039	0.033	0.045
11 Enterobacteriaceae (LogN)		-2.310[b]	0.350	0.530
12 Gas formation (ml CO_2/100 g. at NPT)		-28.810	-4.290	-3.310

b = t-test significance level at 95%

3.8 CONCLUSION

According to this experiment, it can be concluded that the use of mixed starter cultures has the potential to produce good quality of Nham. The Nham without starter cultures has the highest gas formation, numbers of Enterobacteriaceae and lower compression force than the Nham with mixed starter cultures. When the product using single culture,

particularly L. brevis, the pH was quite high and low acidity; firmness and colour developed slower than the Nham using mixed starter cultures. The L. plantarum, P. cerevisiae and M. varians improved the firmness and colour and also inhibited the growth of Enterobacteriaceae and gas formation in the Nham system. The four starter cultures were used in further experiments, although the L. brevis seemed to be a poor acid producer; it might produce important specific flavours.

The level of sodium tripolyphosphate was reduced for the further experiments because it decreased the colour but reduced the weight loss in the Nham system. The Thai Food Regulations limit use 0.3% sodium tripolyphosphate in meat products (Food and Drug Administration, Ministry of Public Health, 1984). Sodium chloride was set for further experiments at the level of 3% as suggested by Techapinyawat (1975) and Zaika et al. (1978). Sodium nitrate had no significant effect in this study and was set at low level (200 ppm) throughout this project. The Food Regulations in Thailand permitted 500 ppm (Food and Drug Administration, Ministry of Public Health, 1984).

There was an indication that cooked rice had some effects and there was also studied in the further experiments,particularly as cooked rice may be the main carbohydrate source for acid production. Many researchers have suggested the use of glucose to supply the carbohydrate for meat sausage fermentation (Acton et al., 1977; Andersen and Ten Cate, 1965; Bacus, 1984). Therefore, in further experiments, glucose was used with cooked rice as the source of carbon.

Garlic had also some effects in the Nham system; it was set at the high level (7%) throughout this project for flavouring purposes. White pepper had no significant effect in this study and was set at high level (0.05%) throughout this project as the same purposes as garlic.

Thus, for the further studies, the important factors affecting the Nham characteristics and being concentrated to study were the four mixed starter cultures and carbon sources. The other ingredients were set at suitable level throughout this project.

CHAPTER 4

EFFECTS OF MIXED STARTER CULTURES ON THE CHARACTERISTICS OF NHAM

4.1 INTRODUCTION

This study was designed to investigate further the effects of the mixed starter cultures on Nham fermentation and to establish the optimum mix of microorganisms. The organisms used were L. plantarum, P. cerevisiae, M. varians and L. brevis. The particular factors studied were:

- The role of M. varians in the development of Nham characteristics, particularly the prevention of colour faults.
- The role of lactic acid-producing bacteria: L. plantarum, P. cerevisiae and L. brevis in the acceleration of the ripening process, in the inhibition of Enterobacteriaceae and in the formation of faulty products.

A mathematical model was fitted for each of the response variables to study the effects of the four independent variables.

4.2 EXPERIMENTAL DESIGN

The most elementary class of factorial experiments is the 2^k factorial, that is k variables each at two levels. This type of design is extremely useful in cases where the experimental situation is adequately represented by a first order relationship (Myers, 1971; Ramsay, 1981). A full factorial 2^k design, in this case required, $2^4(=16)$ experimental runs.

Factor A = Micrococcus varians
a_1 = 10^2 cells/gram (low level)
a_2 = 10^6 cells/gram (high level)
Factor B = Pediococcus cerevisiae
b_1 = 10^2 cells/gram (low level)
b_2 = 10^6 cells/gram (high level)
Factor C = Lactobacillus plantarum
c_1 = 10^2 cells/gram (low level)
c_2 = 10^6 cells/gram (high level)
Factor D = Lactobacillus brevis
d_1 = 10^2 cells/gram (low level)
d_2 = 10^6 cells/gram (high level)

The 16 treatment combinations were:

(1)	a	b	ab	c	ac	bc	abc
d	ad	bd	abd	cd	acd	bcd	abcd

It was not possible because of time limitation to do the complete experiment at one time, so the design was divided into two blocks, each of 8 experimental run plus two centrepoints as shown in Table 4.1. The first block was a 1/2 factorial, aliasing ABCD.

Table 4.1 The full 2^4 factorial experiment with blocks, including four centrepoints, for investigating the effects of mixed starter cultures on the characteristics of Nham.

Blocks	Treatments	EXPERIMENTAL ORDER	Coded level	Factors (cultures) cfu.g^{-1}			
				MV	PC	LP	LB
			+	10^6	10^6	10^6	10^6
			0	10^4	10^4	10^4	10^4
			-	10^2	10^2	10^2	10^2
	(1)	8		-	-	-	-
	ab	10		+	+	-	-
	ac	6		+	-	+	-
	bc	5		-	+	+	-
I	ad	2		+	-	-	+
	bd	7		-	+	-	+
	cd	1		-	-	+	+
	abcd	4		+	+	+	+
	cp1	3		0	0	0	0
	cp2	9		0	0	0	0
	a	7		+	-	-	-
	b	4		-	+	-	-
	c	3		-	-	+	-
	abc	9		+	+	+	-
II	d	1		-	-	-	+
	abd	8		+	+	-	+
	acd	10		+	-	+	+
	bcd	6		-	+	+	+
	cp1	2		0	0	0	0
	cp2	5		0	0	0	0

MV = Micrococcus varians (a)
PC = Pediococcus cerevisiae (b)
cp = Centrepoint
LP = Lactobacillus plantarum (c)
LB = Lactobacillus brevis (d)
(1) = CONTROL

4.3 SAUSAGE PREPARATION FOR 2^4 FACTORIAL DESIGN

From the Plackett-Burman results (Chapter 3), the basic Nham formulation used in this study is presented below:

Ingredients		Quantity (%)
Meat system:	minced lean pork	80
	sliced pork skin	20
		% of meat system
Cooked rice		8
Glucose		1
Sodium chloride		3
Sodium tripolyphosphate		0.3
Sodium nitrate		0.02
Minced raw garlic		7
White pepper powder		0.05

The sausage mixes were prepared as described previously (Section 2.3) and the bacterial cultures were grown by the same method as in the previous experiment (Section 2.2). The volume added was calculated to give $1x10^6$, $1x10^4$ and $1x10^2$ cfu.g^{-1} of Nham (Section 2.2.1) depending upon each treatment (Table 4.1). The mixture was stuffed into cylindrical plastic bags as in the screening experiment. The Nham was held in an incubator at 30°C and 43% relative humidity for 14 days.

4.4 TEST METHODS

Two Nham sausages were randomly sampled from each treatment and microbiologically examined as in Section 2.4.3 during 14 days storage, for M. varians, P. cerevisiae, L. plantarum, L. brevis and Enterobacteriaceae counts.

Weight loss, gas formation, compression force and energy, colour measurement, pH and residual nitrite were determined in duplicate using the methods in Sections 2.4.1 and 2.4.2.

A trained panel consisting of 8 Thai postgraduate students at Massey University was used for sensory evaluation (Section 2.4.4). The Nham was evaluated for appearance,

texture, taste and flavour and overall acceptability using the trained panel for Nham testing questionnaire (Appendix 4.1). The mean ideal ratio scores were calculated, using the fixed ideal scores.

4.5 ANALYSIS OF RESULTS

4.5.1 Empirical Equations Relating Dependent and Independent Variables

An empirical mathematical model incorporating each of the coded linear, first order interaction terms was fitted to each of the dependent variables by multivariate linear regression, using a locally written statistical package; MUTAB (Boag, 1988). As this experiment was blocked by aliasing ABCD, the block variable was included in the matrix (See Appendix 4.2, Table A2.1). The models were determined for 3, 7, 10 and 14 days of the fermentation period.

Coefficients of each variable with the Student's t-statistic not significant at the 95% level were discarded and only those with significant values of t contributed to the final model.

The adequacy of fit of each of the models to the observed data was assessed using the F-ratio between the lack of fit mean square and the experimental error mean square.

$$\text{Therefore, } F(n_1, n_2) = \frac{\text{MS lof}}{\text{MS pure}}$$

where MS lof = mean sum of squares due to lack of fit

MS pure = mean sum of squares due to pure error

n_1, n_2 = degree of freedom for lack of fit and pure error respectively.

The pure error sum of squares was found by computing the sum of squares between the observations at the centre of the design (Myers, 1971). Thus:

$$\text{SS pure error} = (0_1^2 + 0_2^2 + 0_3^2 + 0_4^2) - (0_1 + 0_2 + 0_3 + 0_4)^2/4$$

where $0_1 - 0_4$ = observations at the centre of the design.

An example for calculating the goodness of fit is presented in Appendix 4.3.

If this ratio was not significant, the conclusion was made that the errors about the fitted model (lack of fit) were on the same order of magnitude as those accounted for by error

of observation (experimental error) and the model was an adequate representation of the data.

4.5.2 Decoded Models

The equations from multiple regression above were manipulated to produce models which gave the predicted response using uncoded independent variables (for example L. plantarum in log number of L. plantarum). Decoded models are generally easier to apply in practical work (Ramsay, 1981).

The coded models were determined by using this factor in the coded models (Myers, 1971; Boag, 1985).

$$\text{Coded value} = \frac{\text{Actual value} - (\text{high} + \text{low})/2}{(\text{high} - \text{low})/2}$$

4.5.3 Analysis of Variance

The analysis of variance with combined analysis between treatments and time was used. To identify the significance among treatments, orthogonal comparison test was applied (Appendix 4.5).

4.6 RESULTS

4.6.1 Sensory Evaluation of Nham

4.6.1.1 Overall Acceptability of Nham

As can be seen from Table 4.2, the mean ideal ratio scores of overall acceptability of most treatments increased at 7 days and then decreased until 14 days. The Nham with added high level of P. cerevisiae with the low levels of other cultures had the highest score at 7 days and still maintained the score until 10 days. However, the Nham added high level of L. plantarum with the low levels of other cultures which had the highest score at 3 days was still high at 7 days and then the score decreased quickly until 14 days. In addition, the M. varians or L. brevis added at the high level with the low levels of other cultures gave a lower score of overall acceptability.

Table 4.2 The mean ideal ratio scores of overall acceptability during 3-14 days of fermentation affected by starter cultures.

Treatments	OA_3	OA_7	OA_{10}	OA_{14}
a	0.61	0.78	0.71	0.78
b	0.79	0.85	0.84	0.80
c	0.86	0.85	0.68	0.62
d	0.61	0.61	0.63	0.68
ab	0.68	0.84	0.77	0.74
ac	0.78	0.80	0.76	0.77
ad	0.71	0.72	0.58	0.56
bc	0.71	0.81	0.80	0.60
bd	0.71	0.65	0.59	0.53
cd	0.62	0.82	0.67	0.75
abc	0.86	0.80	0.78	0.74
abd	0.66	0.65	0.66	0.66
acd	0.67	0.77	0.67	0.66
bcd	0.76	0.76	0.66	0.66
abcd	0.70	0.66	0.53	0.60
(1)	0.80	0.75	0.71	0.70
cp_1	0.76	0.78	0.56	0.55
cp_2	0.74	0.72	0.62	0.56
cp_3	0.76	0.75	0.68	0.71
cp_4	0.71	0.74	0.67	0.69

OA_3 = overall acceptability at 3 days; CP = centrepoint; (1) = control.
a = M. varians, b = P. cerevisiae, c = L. plantarum, d = L. brevis

4.6.1.2 Correlation of Overall Acceptability with All Sensory Attributes

From the sensory evaluation results, the mean ideal ratio scores of overall acceptability were regressed with all sensory attributes of Nham in order to identify which affected the overall acceptability scores. The results are presented in Table 4.3.

Table 4.3 Correlations of overall acceptability with the sensory attributes of Nham during 3-14 days fermentation.

Fitted equations	R^2 (%)
3 days; Overall acceptability [(a)] =	
- 0.221 + 0.460 (Visual texture) + 0.600 (Sourness)	71.19
7 days; Overall acceptability =	
- 1.571 + 0.504 (Firmness) - 0.672 (Juiciness)	
+ 1.455 (Smoothness) + 0.578 (Sourness) + 0.638 (Spiciness)	67.86
14 days; Overall acceptability =	
1.290 - 0.487 (Air pockets) - 0.355 (Juiciness)	
- 0.801 (Saltiness) + 2.242 (Spiciness) - 1.051 (Pork flavour)	86.06

(a) = mean ideal ratio score.

Generally, sourness, firmness, smoothness, spiciness and visual texture increased acceptability scores, while juiciness, air pockets, saltiness and pork flavour reduced the scores (See Table 4.3).

The sourness of Nham showed highly significant correlation ($P<0.01$) with the pH values at 3, 7 and 10 days ($r = -0.73, -0.61, -0.88$ respectively) and significant correlation ($P<0.05$) at 14 days ($r = -0.47$).

Significant correlations were also found between the air pockets of Nham and gas formation ($r = 0.55, 0.54$; $P<0.05$ at 3 and 7 days; $r = 0.70$; $P<0.01$ at 14 days). Additionally, significant correlations between firmness and gas production were found ($r = -0.54$; $P<0.05$ at 3 days). The firmness also correlated with compression energy significantly ($r = 0.46$, $P<0.05$ at 3 days, $r = 0.53$; $P<0.05$ at 14 days).

4.6.1.3 Relationship between Overall Acceptability and the Starter Cultures

The multiple regression models for overall acceptability with all independent variables during Nham fermentation are shown in Appendix 4.4; Table A4.1.

The models developed for the overall acceptability of Nham during fermentation are presented in Table 4.4

Table 4.4 Fitted models for overall acceptability during 3-14 days of fermentation affected by starter cultures.

	Fitted equations	R^2 (%)	Lack of fit (F-ratio)
3 days;	Overall acceptability [(a)] = 0.725 + 0.026 (PC) +0.037 (LP) - 0.028 (LB) + 0.024 (MVPCLP) + 0.028 (PCLPLB)	66.09	5.27^{ns}
7 days;	Overall acceptability = 0.755 + 0.038 (LP) - 0.041 (LB) - 0.021 (MVLP) - 0.021 (PCLP) + 0.021 (LPLB)	76.86	2.78^{ns}
10 days;	Overall acceptability = 0.678 + 0.036 (PC) + 0.026 (LP) - 0.044 (LB) -0.027 (PCLB) + 0.033 (MVPCLB)	71.37	0.84^{ns}
14 days;	Overall acceptability = 0.668 + 0.023 (MV) - 0.028 (LB) - 0.028 (MVLB) + 0.033 (LPLB) + 0.039 (MVPCLB) - 0.094 (MVPCLPLB) + 0.072 (BLOCK)	81.38	0.04^{ns}

(a) = mean ideal ratio score; ns = not significant

LP = L. plantarum, PC = P. cerevisiae, LB = L. brevis, MV = M. varians

As can be seen from Table 4.4, the use of starter cultures increased the overall acceptability, with the exception of L. brevis, which had the opposite effect. The L. plantarum increased the score significantly during 3-10 days whereas the L. brevis decreased it significantly during 3-14 days. The P. cerevisiae increased the scores at 3, and 10 days as did the M. varians at 14 days. In addition, the starter culture interactions affected the overall acceptability.

From Table 4.5, analysis of variance of overall acceptability showed that the Nham with added P. cerevisiae at the high level and with the other cultures at the low level (b. treatment) had the highest acceptability.

Table 4.5 Analysis of variance of overall acceptability during fermentation affected by starter cultures (described in detail in Appendix 4.5).

SOV		df	SS	MS	F-TEST
Treatment		14	1.820	0.130	20.28**
b	vs the rest	1	0.421	0.421	65.70**
abc	vs the rest	1	0.287	0.287	44.79**
ac	vs the rest	1	0.219	0.219	34.18**
ab	vs the rest	1	0.183	0.183	28.56**
c	vs the rest	1	0.178	0.178	27.78**
bc	vs the rest	1	0.116	0.116	18.10**
a	vs the rest	1	0.089	0.089	13.89**
cd	vs the rest	1	0.107	0.107	16.70**
bcd	vs the rest	1	0.120	0.120	18.73**
acd	vs the rest	1	0.088	0.088	13.73**
abd	vs the rest	1	0.024	0.024	3.74^{ns}
ad	vs the rest	1	9.6×10^{-3}	9.6×10^{-3}	1.49^{ns}
d	vs the rest	1	3.4×10^{-3}	2.4×10^{-3}	0.53^{ns}
bd	vs (1)	1	1.5×10^{-3}	1.5×10^{-3}	2.3×10^{-4} ns
Pooled error		392	2.512	6.4×10^{-3}	

** significant at 99% level; ns = not significant

a = M. varians; b = P. cerevisiae; c = L. plantarum, d = L. brevis

The product profile of the most acceptable Nham is shown in Figure 4.1. The mean ideal ratio scores of all attributes of Nham including overall acceptability were plotted. It is easy to compare between the overall acceptability and all attributes of Nham. The visual display is accomplished by using a same angle for the various dimensions on a series of lines that radiate from a centrepoint. According to the distance away from the ideal product, most attributes of this Nham were nearly the ideal product profile at 3, 7, 10 and 14 days except for the air pockets, firmness and sourness attributes. As the fermentation time increased, the sourness increased and was higher than the ideal at 14

days, the firmness increased upto 7 days but then decreased, the air pockets decreased and were near ideal at 14 days.

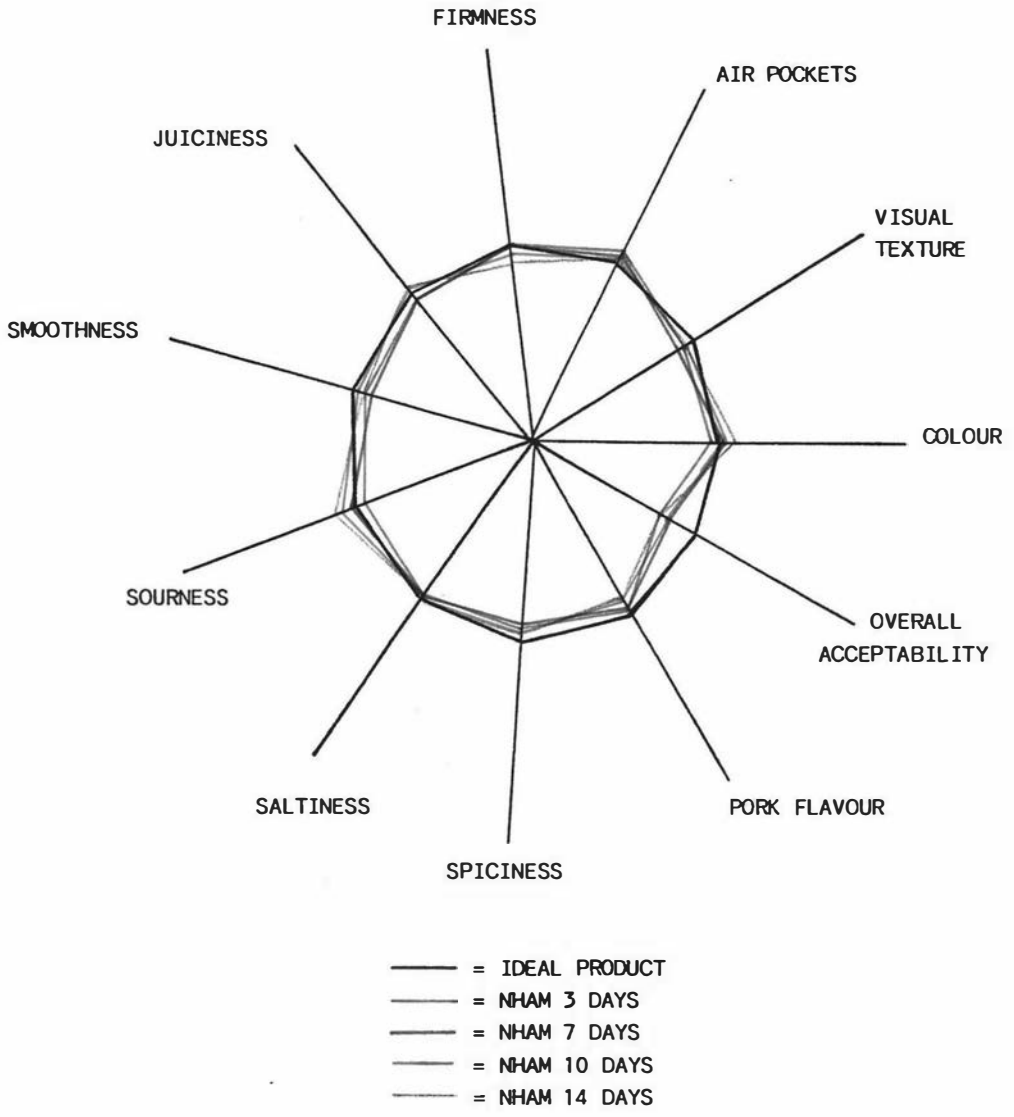


Figure 4.1 Ideal ratio profile of Nham added P.cerevisiae at the high level with the low levels of the other cultures tested by trained panel.

4.6.2 Bacteriology

During 3 days of fermentation, there was an increase in lactic acid bacteria counts. The numbers were highest on the third day of fermentation, with a steady decrease in the later period of fermentation (Figure 4.2). There is a same trend for all treatments, therefore only main effects (a, b, c and d) are plotted and presented in graph.

The log number of L. plantarum was 3.2 - 6.5/g on the first day and was highest on the third day with 7.7-9.1/g then decreasing slowly to 7.4-8.5/g at 14 days (Figure 4.2 and Appendix 4.2; Table A2.13).

The log number of L. brevis increased from 2.9 - 6.3/g on the first day to 6.9 - 8.6/g on the third day and then decreased slightly to 7.0 - 7.7/g at 14 days (Figure 4.2 and Appendix 4.2; Table A2.14).

Similarly, the log number of P. cerevisiae was 2.6 - 6.2/g on the first day and was highest on the third day with 6.5 - 7.8/g gradually declining to 6.3 - 7.1/g at 14 days (Figure 4.2 and Appendix 4.2; Table A2.15).

However, the log number of M. varians was 6.2 - 6.6/g on the first day and decreased approximately 2 log cycles by the third day and then declined slowly to 1.0 - 2.3/g at 14 days (Figure 4.2 and Appendix 4.2; Table A2.16).

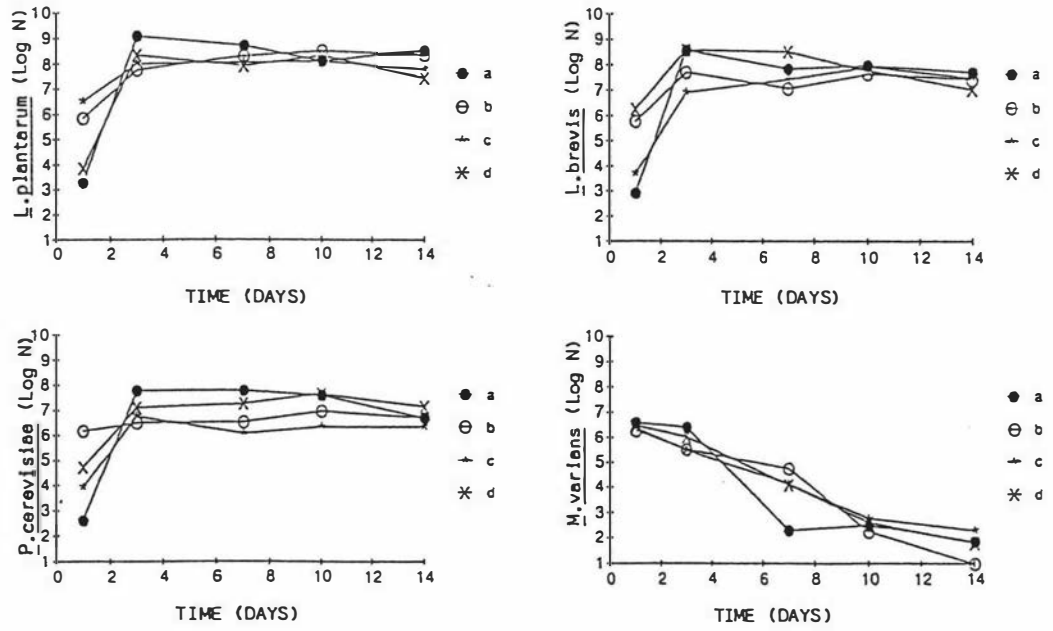


Figure 4.2 The numbers of L.plantarum, L.brevis, P.cerevisiae and M.varians during 14 days of fermentation using combinations of the four cultures (a= M.varians, b= P.cerevisiae, c= L.plantarum and d= L.brevis)

The multiple regression models for each starter culture with all independent variables are shown in Appendix 4.4; Table A4.2. In the initial fermentation, the models for each culture depended upon the inoculated level of that microorganism . In the middle period of the Nham fermentation, the relationship between M. varians and the other cultures was significant at 3 days; the M. varians concentration was dependent on the number of M. varians inoculated. On the other hand, the level of L. plantarum inoculated decreased the number of M. varians at 3 days. In this periods, there were also some interactions of the cultures affecting the number of M. varians in Nham system. In the later stages of fermentation only the model for the level of L. plantarum appeared significant. It thus appears that the ultimate concentrations of the lactic acid bacteria in Nham are practically independent of the levels inoculated into the raw meat mix. However, the rate of development during the early stages is influenced by the level and composition of the inoculum.

4.6.3 pH Change During Fermentation

There was a significant correlation between the sourness and pH of Nham, the investigation of pH was very important. There was a similar trend of pH reduction for all treatments, therefore only the main effects are presented. The original pH of the raw Nham mix was 5.9 - 6.2 and reduced to pH 3.9 - 4.6 on the third day. The pH remained essentially constant after 7 days (Figure 4.3 and Appendix 4.2; Table A2.17).

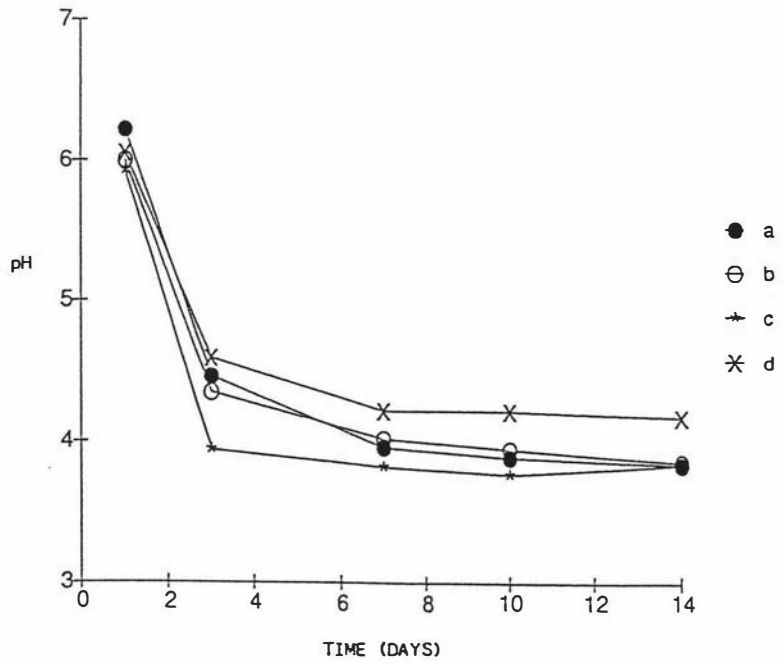


Figure 4.3 pH values during 14 days of fermentation using combinations of four cultures- M.varians (a), P.cerevisiae (b), L.plantarum (c) and L.brevis (d).

The multiple regression models for pH with all independent variables during Nham fermentation are shown in Appendix 4.4; Table A4.3. The models developed for pH during 3-14 days of the Nham fermentation are presented in Table 4.6.

Table 4.6 Fitted models for pH during 3-14 days of fermentation affected by starter cultures.

	Fitted equations	R^2 (%)	Lack of fit (F-ratio)
3 days;	pH = 4.218 - 0.162 (LP) + 0.058 (LB)	70.93	0.39^{ns}
7 days;	pH = 4.029 - 0.138 (LP) + 0.077 (LB)	71.49	0.55^{ns}
14 days;	pH = 4.034 - 0.115 (LP) + 0.100 (LB) + 0.230 (MVPCLPLB) - 0.157 (BLOCK)	78.21	0.10^{ns}

ns = not significant

LP = L. plantarum, LB = L. brevis, PC = P. cerevisiae, MV = M. varians

As shown by the equations in Table 4.6, the pH during fermentation was partially dependent upon the levels of L. plantarum and L. brevis inoculated into the meat mix. The L. plantarum decreased the pH and then changed the product to be suitable for consumption. As can be seen in Figure 4.4, as the pH decreased the overall acceptability increased until the pH was less than 4.05 when the overall acceptability decreased. Thus, the pH which the panelists preferred was 4.05.

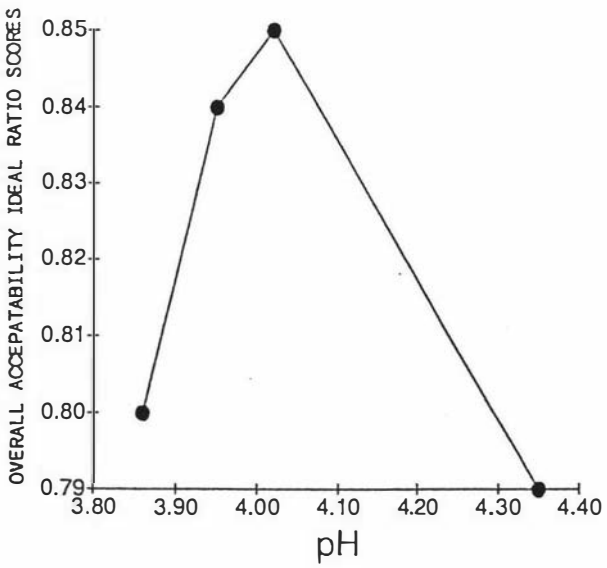


Figure 4.4 The ralationship between overall acceptability, ideal ratio scores and pH of Nham product.

4.6.4 Gas Production During Fermentation

There is a similar trend for gas formation of all treatments during fermentation. Only the main effects are presented in graph. As can be seen from Figure 4.5, the Nham with P. cerevisiae at the high level in treatment (b) produced least gas. L. brevis at the high level (d) increased the gas formation quickly to 6 days of the Nham fermentation with 192 ml/100g at NTP. After 6 days, the gas formation was constant until 14 days of fermentation except treatment (b). The gas increased slightly until 14 days.

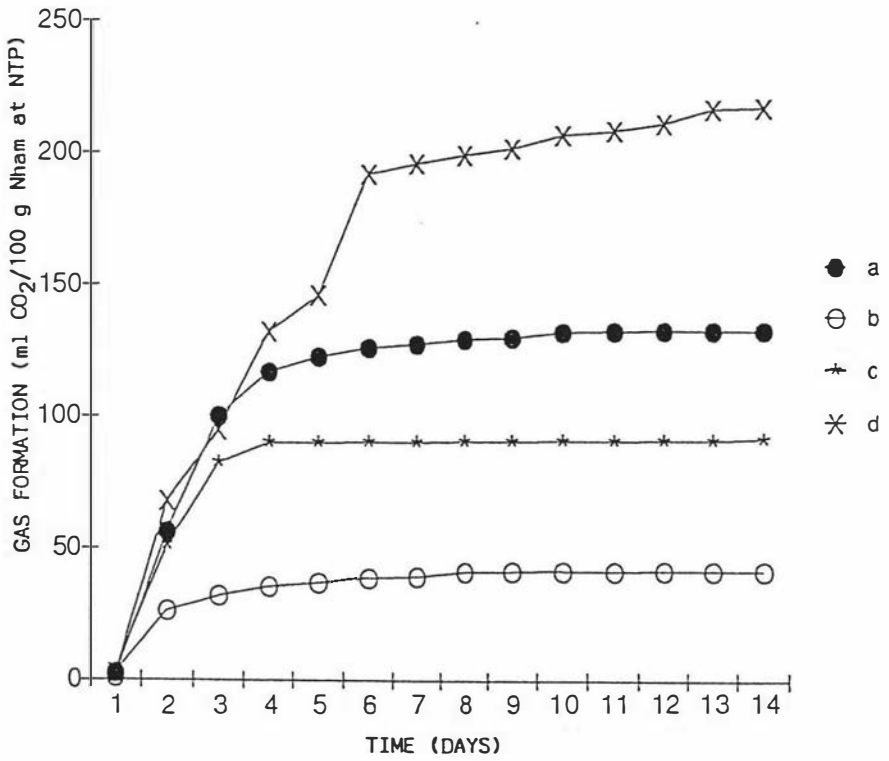


Figure 4.5 Gas formation during 14 days of fermentation using combinations of four cultures- M.varians (a), P.cerevisiae (b), L.plantarum (c) and L.brevis (d).

Significant correlations between gas formation and pH at 7, 10 and 14 days were found ($r = 0.55, 0.49$; $P<0.05$ at 7, 10 days respectively and $r = 0.57$; $P<0.01$ at 14 days). Gas production was retarded when the pH decreased.

The multiple regression models for gas production with all independent variables are seen in Appendix 4.4; Table A4.4.

The models developed for gas production during 2-6 days of Nham fermentation are presented in Table 4.7.

Table 4.7 Fitted models for gas production (G) during 2-6 days of fermentation affected by starter cultures.

	Fitted equations	R^2 (%)	Lack of fit (F-ratio)
2 days;	G* = 48.054 - 11.777 (PC) - 12.333 (LP) - 12.339 (LPLB)	66.35	4.29^{ns}
3 days;	G = 68.192 - 18.548 (PC) - 18.236 (LP) + 10.008 (PCLP) + 11.979 (PCLB) - 12.896 (LPLB)	86.32	3.05^{ns}
4 days;	G = 78.818 - 20.842 (PC) - 24.366 (LP) + 8.248 (LB) + 12.810 (PCLP) + 14.275 (PCLB) - 13.744 (LPLB)	95.66	1.51^{ns}
5 days;	G = 83.550 - 20.539 (PC) - 27.189 (LP) + 11.974 (LB) + 12.177 (PCLP) + 15.363 (PCLB) - 14.867 (LPLB)	94.63	2.28^{ns}
6 days;	G = 88.280 - 23.136 (PC) - 29.712 (LP) + 16.368 (LB) + 14.282 (PCLP) + 12.919 (PCLB) - 16.527 (LPLB)	94.88	3.21^{ns}

ns = not significant
LP = L. plantarum, PC = P. cerevisiae, LB = L. brevis
* = ml CO_2/100g at NTP.

As can be seen from Table 4.7, the effects of P. cerevisiae and L. plantarum were to reduce gas formation, whereas L. brevis resulted in increased gas production. Interaction terms were significant.

4.6.5 Firmness Development During Fermentation

The firmness development of all treatments had a similar trend during fermentation. The firmness of the product, expressed as compression force and compression energy, increased from 10-17 Newtons compression force and 0.06-0.08 Joules compression energy on the first day to 68-181 Newtons compression force and 0.4-1.3 Joules compression energy on the seventh day. Thereafter, firmness decreased slowly to 44-176 Newtons compression force and 0.2-1.1 Joules compression energy (Figure 4.6 and Appendix 4.2; Table A 2.19 and 2.20).

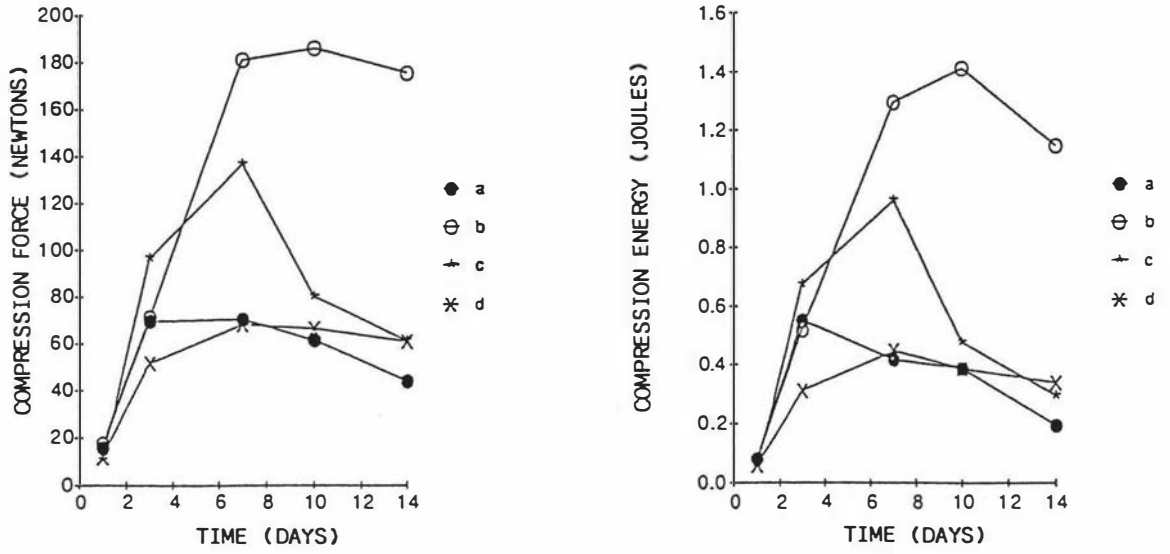


Figure 4.6 Compression force and compression energy during 14 days of fermentation using combinations of four cultures- M.varians (a), P.cerevisiae (b), L.plantarum (c) and L.brevis (d).

Significant correlations were found between the compression force and pH on the third day ($r = -0.76$; $P<0.01$) and gas production ($r = -0.48, -0.57, -0.56$; $P<0.05$) at 7, 10 and 14 days respectively.

The multiple regression equations of the compression force and energy with all independent variables during Nham fermentation are shown in Appendix 4.4; Table A 4.5, A 4.6.

The models developed for compression force and energy during the middle and late periods of fermentation are presented in Table 4.8.

Table 4.8 Fitted models for the compression force (CF) and compression energy (CE) during 3-10 days of fermentation affected by starter cultures.

	Fitted equations	R^2 (%)	Lack of fit (F-ratio)
3 days;	CF (N) = 79.325 + 12.326 (LP) - 5.014 (LB) - 6.614 (LPLB) + 6.168 (MVPCLB)	73.46	2.67^{ns}
	CE (J) = 0.495 + 0.088 (LP) - 0.066 (LB) - 0.042 (LPLB)	66.08	1.52^{ns}
10 days;	CF (N) = 83.452 + 13.838 (PC) - 11.472 (LB) - 13.441 (PCLB) + 11.866 (MVPCLP) - 11.839 (MVLPLB)	71.13	6.56^{ns}

ns = not significant

LP = L. plantarum, PC = P. cerevisiae, LB = L. brevis, MV = M. varians

The major effects on texture were caused by inclusion of L. plantarum and P. cerevisiae, which tended to increase compression force and energy, while the inclusion of L. brevis, with consequent gas evolution, resulted in production of a swollen, spongy product with a softer texture.

4.6.6 Colour Development During Fermentation

The pink colour of Nham developed during fermentation. The colour of the product was expressed as the tristimulus values (x, y, z). The values changed quickly until the third day and then decreased slightly at the last period of fermentation (Figure 4.7 and Appendix 4.2; Table A2.21). All treatments had the same patterns of colour development.

Significant correlation between the number of M. varians and the tristimulus values (x, y, z) on the first day was found (r = 0.79, 0.71, 0.79; $P<0.01$ respectively).

On the other hand, the level of nitrite reduced during fermentation. There was a quick reduction of nitrite at 3 days and then it slowly decreased until 14 days (Figure 4.7 and Appendix 4.2; Table A 2.22).

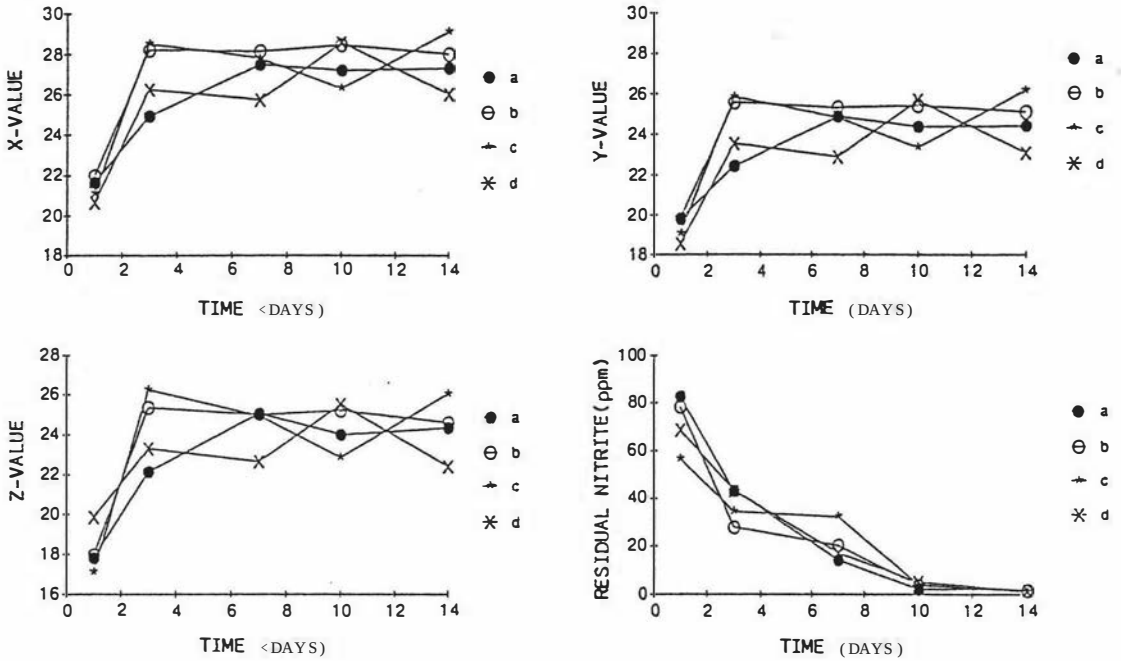


Figure 4.7 Tristimulus values (X Y Z) and residual nitrite during 14 days of fermentation using combinations of four cultures- M.varians (a), P.cerevisiae (b), L.plantarum (c) and L.brevis (d).

The multiple regression models for the tristimulus values and residual nitrite with all independent variables during Nham fermentation are shown in Appendix 4.4; Table A4.7 and A4.8.

The models developed for the tristimulus values and residual nitrite during fermentation are presented in Table 4.9.

As can be seen from Table 4.9, M. varians increased significantly the tristimulus values on the first day whereas L. brevis decreased them significantly. However, interaction terms had also the effects on the values. The L. plantarum increased the values later until the last period of fermentation (Appendix 4.4; Table A 4.7).

On the other hand, the M. varians increased significantly the residual nitrite whereas L. plantarum decreased it on the first day. On the third day, the L. plantarum and L. brevis decreased significantly the value. In the last period of fermentation, the L. brevis increased the residual nitrite in Nham system. However, interaction terms affected significantly the residual nitrite as well (Table 4.9).

Table 4.9 Fitted models for the tristimulus values (x, y, z) and residual nitrite (RN) during 1-10 days of fermentation affected by starter cultures.

	Fitted equations	R^2 (%)	Lack of fit (F-ratio)
1 day;	x = 22.302 + 0.625 (MV) - 0.362 (LB) + 0.344 (MVLP) + 0.506 (MVPCLP) + 1.150 (MVPCLPLB)	83.91	1.33^{ns}
	y = 20.010 + 0.569 (MV) - 0.356 (LB) + 0.262 (MVLP) + 0.450 (MVPCLP) + 0.906 (MVPCLPLB)	75.08	1.50^{ns}
	z = 18.300 + 0.666 (MV) - 0.497 (LB) + 0.328 (MVLP) + 0.284 (MVPCLP) + 1.059 (MVPCLPLB)	80.02	1.24^{ns}
1 day;	RN* = 58.844 + 8.109 (MV) - 5.445 (LP) + 8.563 (PCLPLB)	67.93	7.74^{ns}
3 days;	RN = 39.043 - 14.385 (LP) - 5.920 (LB) + 6.937 (MVPC) + 6.875 (LPLB) - 5.617 (MVPCLP) - 11.933 (MVPCLB)	87.85	4.49^{ns}
10 days;	RN = 4.463 + 0.948 (LB) + 0.373 (MVPC) - 0.428 (PCLB) + 0.471 (MVPCLP) + 0.653 (PCLPLB) + 0.850 (MVPCLPLB)	88.68	3.48^{ns}

ns = not significant

* = (ppm)

LP = L. plantarum, PC = P. cerevisiae, LB = L. brevis, M. varians

4.6.7 Inhibition of Enterobacteriaceae

Initial counts of Enterobacteriaceae were 10^6 to 10^8 cfu.g^{-1}. By the seventh day of fermentation, the numbers were less than 10^5 cfu.g^{-1} in all cases (Figure 4.8) and in several treatments Enterobacteriaceae were undetectable in the finished product except the control treatment and treatment ad and bd that the Enterobacteriaceae counts were 10^1-10^3 cfu.g^{-1} at 14 days (Appendix 4.2; Table A2.23).

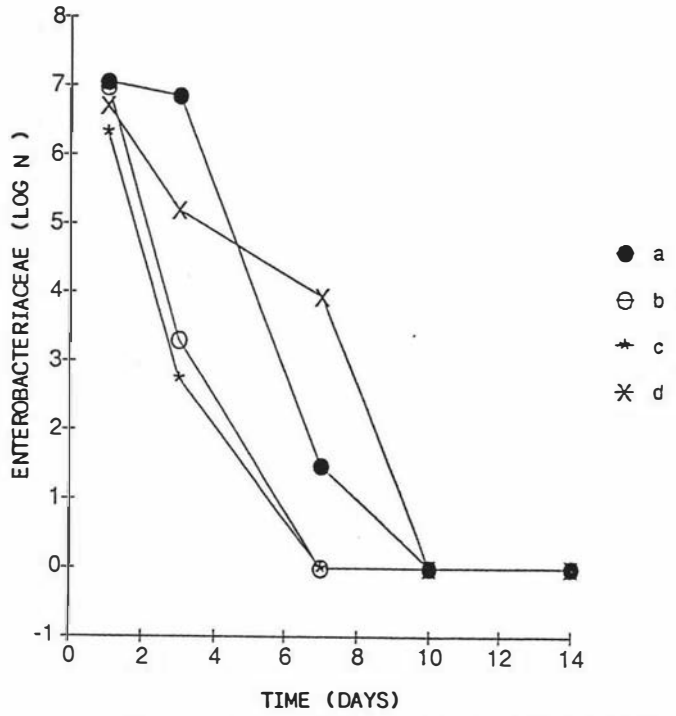


Figure 4.8 The numbers of Enterobacteriaceae during 14 days of fermentation using combinations of four cultures- M.varians (a),P.cerevisiae (b), L.plantarum (c) and L.brevis (d).

The multiple regression models for the Enterobacteriaceae in Nham with all independent variables during fermentation period are shown in Appendix 4.4; Table A4.9.

The L. plantarum had a significant effect on the decrease of Enterobacteriaceae. The Enterobacteriaceae might be inhibited by pH directly as the L. plantarum markedly decreased the pH during fermentation (Table 4.6). However, the L. brevis did not inhibit the numbers of Enterobacteriaceae.

4.6.8 Weight Loss During Fermentation

The cumulative weight loss during fermentation increased quickly up to tenth day and then slowly increased during the last period of fermentation (Figure 4.9 and Appendix 4.2; Table A2.24). In fact the weight loss at 3 days was 1.3-4.4% changed to 5-8% at 10 days and slowly increased up to 6-9% at 14 days.

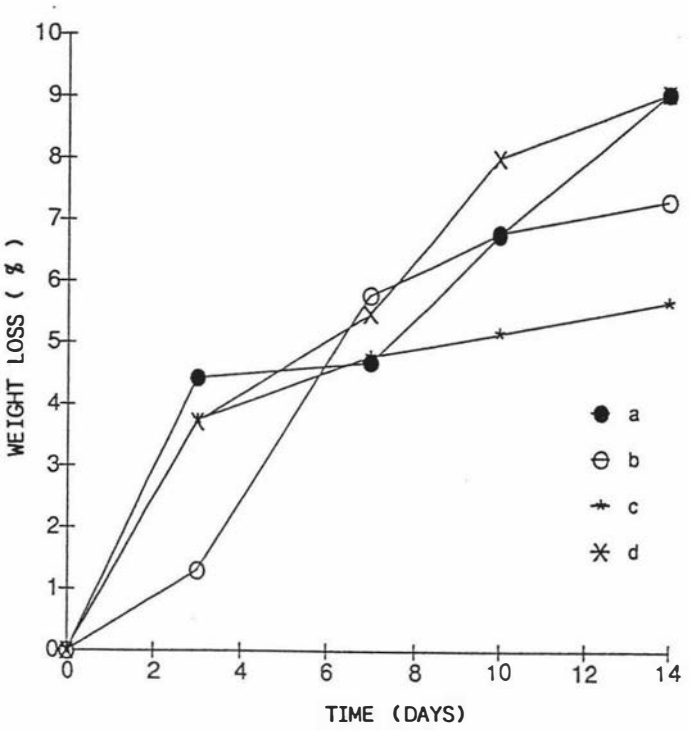


Figure 4.9 Weight loss during 14 days of fermentation using combinations of four cultures- M.varians (a), P.cerevisiae (b), L.plantarum (c) and L.brevis (d).

The multiple regression models for weight loss with all independent variables during fermentation as shown in Appendix 4.4; Table A4.10.

The models developed for weight loss during later period of fermentation (7-14 days) are presented in Table 4.10.

Table 4.10 Fitted models for weight loss (WL) during 7-14 days of fermentation affected by starter cultures.

Fitted equations	R^2 (%)	Lack of fit (F-ratio)
7 days; WL (%) = 7.054 + 0.703 (PC) + 0.836 (LP) + 1.354 (LB) + 0.693 (LPLB)	83.09	0.31^{ns}
10 days; WL (%) = 8.762 + 1.541 (LB) + 0.938 (LPLB)	71.09	2.50^{ns}
14 days; WL (%) = 9.468 + 1.466 (LB) + 0.979 (LPLB)	69.16	2.71^{ns}

ns = not significant

LP = L. plantarum, PC = P. cerevisiae, LB = L. brevis

It can be seen from Table 4.10, that L. brevis increased the weight loss during 7-14 days of the fermentation. The L. plantarum and P. cerevisiae also increased the weight loss at 7 days. L. plantarum showed an interaction with L. brevis in affecting weight loss.

4.7 DISCUSSION

4.7.1 Effects of Starter Cultures

L. plantarum caused a rapid decrease of the pH of the Nham and this was related to increased sourness perceived by the taste panel. The desired firmness also developed rapidly. Klettner and Baumgartner (1980) stated that firmness development is directly influenced by acid development. 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 protein forms a gel, with a consequent rapid increase in firmness (Klement et al., 1974). Thus L. plantarum influenced directly the development of firmness.

L. plantarum is homofermentative - producing only lactic acid from carbohydrate metabolism but not producing gas (Bacus, 1984). In fact L. plantarum also inhibited gas production during fermentation. This aided firmness development as gas production correlated negatively with the firmness.

An important aspect of the use of L. plantarum was that it decreased the number of Enterobacteriaceae during fermentation, probably as a result of the rapid decrease of the pH value caused by the lactic acid production. Bacus (1984) and Gilliland (1985) stated that the lactic acid bacteria ferment the sugar to primarily lactic acid thus reducing the meat pH and providing prolonged stability against the proliferation of food pathogens. Nurmi (1966) found that the number of coliform bacteria was distinctly reduced by the lactobacilli. Gibbs (1987) also reported the inhibition of meat spoilage by lactic acid bacteria. Addition of lactic acid bacteria caused the pH to decline to 4.0-4.5 with generally reduced growth of non-lactic flora. The growth of the gram-negative flora was markedly inhibited in the presence of glucose plus either of the lactic acid bacteria (Gibbs, 1987).

The pink colour of Nham developed during the ripening process. L. plantarum played an important part in the improvement of the colour of Nham during the middle and later periods of fermentation. The most favourable pH range for the reduction of nitrate is 5.9-6.1 (Nurmi, 1966). The nitrite formed is decomposed spontaneously in acid surroundings into nitric oxide which subsequently reacts with myoglobin to form a red compound, nitrosomyoglobin. This reaction takes place best in pH range 5.5-5.0

(Niinivaara, 1955). In this study, L. plantarum decreased significantly the amount of residual nitrite in the Nham system on the first and third day because the acid produced accelerated the decomposition of the nitrite. During the middle and late periods of fermentation, L. plantarum increased the x, y and z tristimulus values, probably by its effect on nitrite reduction.

The Nham added the high level of L. plantarum had a higher sourness as the L. plantarum had strong effect in terms of pH reduction; the pH dropped quickly (=3.90) at 3 days. This caused the lower overall acceptability as the pH which panelists preferred was 4.05.

P. cerevisiae caused an increase in firmness during the later period of fermentation. The optimum growth of the P. cerevisiae is at pH 5.0 (Buchanan and Gibbons, 1974). The conditions during the last period of fermentation allowed good growth and acid production causing the increase in firmness. In this work P. cerevisiae suppressed the gas formation.

L. brevis was found to be unsuitable as a starter organism. The presence of this organism in the starter mixture resulted in a higher final pH value and consequent reduction in sourness. As it is heterofermentative, L. brevis increased the gas formation in the product and thus caused a reduction in firmness and visual texture score. L. brevis decreased colour development, probably as a result of its elevation of the product pH and consequent slowing of nitrite decomposition. It was found that L. brevis increased the amount of nitrite at the last period of fermentation. There was no effect on numbers of Enterobacteriaceae, but the growth of L. brevis was strongly correlated with weight loss during fermentation.

When nitrate was used in the formulation, the formation of colour required the presence of nitrate-reducing bacteria, especially micrococci (Niinivaara, 1955; Niinivaara et al., 1964). The nitrate is reduced to nitrite by micrococci at pH about 5.6 (Klettner and Baumgartner, 1980). In the present study, M. varians played an important role in reducing nitrate to nitrite, increasing the residual nitrite at the first day of fermentation. It stabilized the colour of Nham during the initial period of fermentation, causing an increase in the tristimulus values. If the pH is too low at the beginning the micrococci will be inhibited such that the nitrate remains unconverted and unused in the sausage (Klettner and Baumgartner, 1980). The addition of micrococci together with lactobacilli also prevents the development of flavour faults caused by lactobacilli producing hydrogen peroxide (Nurmi, 1966; Bacus, 1984; Gilliland, 1985; Gibbs, 1987).

4.7.2 Selection of Inoculum Levels for Future Experiments

L. plantarum strongly decreased the pH of Nham during the first 3 days of fermentation, making the product to be sour and affecting the firmness development. The M. varians developed the colour at the beginning of fermentation. Therefore, the predictions of pH and tristimulus values are very important to select the suitable inoculum levels for the next experiment.

According to decoded regression models of pH and the tristimulus values - ignoring the interaction terms, as below:

7 days; pH = 4.305 - 0.069 (Log N of L. plantarum)

1 day; x = 21.052 + 0.312 (Log N of M. varians)

y = 18.872 + 0.284 (Log N of M. varians)

z = 16.968 + 0.333 (Log N of M. varians).

Values calculated from these equations suggest that suitable inoculum levels of the three chosen starter cultures will be as follows:

L. plantarum	10^3 cells/g to reduce pH to about 4.1 by day 7
M. varians	10^3 cells/g to achieve colour development (x = 22, y = 20, z = 18) on the first day of fermentation.
P. cerevisiae	10^6 cells/g

4.7.3 Fermentation Time

The fermentation time was also very important for Nham production. The starter cultures were highest on the third day of fermentation. The lactic acid bacteria, particularly L. plantarum, caused a rapid decrease of the pH of Nham on the third day. Consequently, the desired firmness was also developed rapidly until 7 days. The Enterobacteriaceae decreased markedly on the 7 days. In addition, the colour developed quickly on the 3 days. Thus, the first 7 days of fermentation should be carefully studied for the next experiment in order to investigate more information during that time.

4.8 CONCLUSION

From this experiment, it can be concluded that the use of mixed starter cultures consisting of L. plantarum, P. cerevisiae and M. varians has a potential to produce good quality Nham, achieving a consistent quality and suppressing the Enterobacteriaceae. The

overall acceptability of Nham increased significantly with the use of L. plantarum and P. cerevisiae whereas the L. brevis decreased acceptability. The P. cerevisiae added at the high level with the other cultures at the low level gave the highest acceptability of the Nham. However, if the pH was too low i.e. less than 4.0, the overall acceptability of the Nham decreased. The mixed starter cultures produced lactic acid rapidly, causing the rapid reduction of pH and satisfactory development of texture and colour during the first 7 days of fermentation. M. varians was also necessary to reduce nitrate and improve colour during the initial fermentation. From these results it appears that mixed starter cultures composed of L. plantarum, P. cerevisiae and M. varians should be used in Nham formulation. The L. brevis should be left out as it increased the gas formation - causing the softer texture of the product. The effect of L. plantarum was considered to be worthy of further investigation because it caused a large pH reduction in the Nham system.

CHAPTER 5

EFFECTS OF CARBON SOURCES AND L. PLANTARUM ON THE pH REDUCTION IN NHAM

5.1 INTRODUCTION

The purpose of this study was to investigate the effects of carbon sources, particularly cooked rice and glucose, on the pH reduction and firmness development. Three independent variables were chosen for this study - cooked rice, glucose and L. plantarum. The L. plantarum was specifically to investigate as the lactic acid bacterium used the carbon sources producing acid in the Nham. The changes in pH, reducing sugars and cooked rice were studied in detail during the first 48 hours of fermentation and then every day up to 7 days. The weight loss, colour, firmness and sensory properties were determined from 3 to 7 days.

It was hoped that the detailed study of the acid development during the initial fermentation period could be related to the final properties of the Nham, particularly in firmness and colour development and the sensory properties.

5.2 EXPERIMENTAL DESIGN

The system was examined by fitting a first order model with the three factors at two levels. A full factorial 2^3 design requires 8 experimental runs (Myers, 1971):

Factor A = Cooked rice (CR)
- a_1 = 8% (low level)
- a_2 = 10% (high level)

Factor B = Glucose (G)
- b_1 = 0.5% (low level)
- b_2 = 1.0% (high level)

Factor C = Lactobacillus plantarum (LP)
- c_1 = 10^2 cells/gram (low level)
- c_2 = 10^3 cells/gram (high level)

Two centrepoints were added to the 8 treatment combinations to allow for estimation of error giving a total of 10 treatments as shown in Table 5.1.

Table 5.1 2^3 factorial design for investigating the effects of carbon sources and L. plantarum on the pH reduction in Nham.

Treatments	Random order of testing	Coded			Uncoded		
		CR	G	LP	CR	G	LP
(1)	5	-	-	-	8	0.5	2
a	2	+	-	-	10	0.5	2
b	4	-	+	-	8	1.0	2
ab	7	+	+	-	10	1.0	2
c	1	-	-	+	8	0.5	3
ac	10	+	-	+	10	0.5	3
bc	6	-	+	+	8	1.0	3
abc	8	+	+	+	10	1.0	3
cp_1	3	0	0	0	9	0.75	2.5
cp_2	9	0	0	0	9	0.75	2.5

CR = Cooked rice (%); G = Glucose (%); LP = L. plantarum (Log N)
cp = centrepoint

5.3 SAUSAGE PREPARATION, TESTING AND ANALYSIS FOR 2^3 FACTORIAL DESIGN

The formulation used for the Nham sample was the same as in Chapter 4, except that the levels of glucose and cooked rice were varied as the factors for this study (Table 5.1). Two kilograms of Nham were prepared for each treatment, following the procedure developed in Section 2.3. The volume of starter cultures added was calculated to give $1x10^6$ cfu.g^{-1} for P. cerevisiae, $1x10^3$ cfu.g^{-1} for M. varians and $1x10^2$, $1x10^3$ and $1x10^{2.5}$ cfu.g^{-1} for L. plantarum as a factor of this study. After production, the Nham was held in an incubator at 30°C and relative humidity of 43% for 7 days.

Two Nham sausages were randomly sampled from each treatment for chemical, physical and sensory analyses at intervals during the 7 days of fermentation. pH, reducing sugars and starch (as cooked rice) were measured at 6, 12, 18, 24 hours and then daily up to 7 days. The weight loss, compression force and colour measurement were measured using

the methods from Sections 2.4.1 and 2.4.2. The sensory evaluation was made at 3 days and 7 days of fermentation, using the method described in Section 2.4.4.

The models were developed by the methods used in Chapter 4. The coefficients in the developed models were significant at more than 90% confidence level. As change of pH with time was not linear, a model was developed by stepwise multiple regression and transformation using Stat-Packets (Walonick, 1987) (detail in Appendix 5.2; Table A2.1).

5.4 RESULTS

5.4.1 Chemical and Physical Changes During the First 48 hours and their Relation to the Nham Quality.

As can be seen in Figure 5.1, the cooked rice decreased quickly during the first 24 hours and then decreased slowly to 2.21-3.52% at 48 hours (Appendix 5.1; Table A1.4).

The changes in reducing sugars during the first period of Nham fermentation are shown in Figure 5.1. In all cases, there was an increase in the reducing sugars in the Nham system during the first 12 hours of fermentation followed by a quick decrease to 24 hours and then a steady decrease to 1.00-1.54% at 48 hours (Appendix 5.1; Table A1.3).

The initial pH value in the Nham sausage was approximately 6.9. The pH changed slightly during the first 12 hours of fermentation and then dropped rapidly to 5.10-5.64 at 18 hours. The final pH of all samples was about 4.4 at 48 hours (Figure 5.1 and Appendix 5.1; Table A1.2).

The decline in cooked rice coincided with the initial increase in reducing sugars and the subsequent decrease in reducing sugars corresponded to the rapid lowering of pH.

The sequence of events suggests that the cooked rice was hydrolysed to reducing sugars, which were then consumed by the microorganisms to produce lactic acid. Thus the important period of the Nham fermentation in this experiment was at 12-24 hours, where the acid production was very rapid while the reducing sugar concentrations dropped quickly.

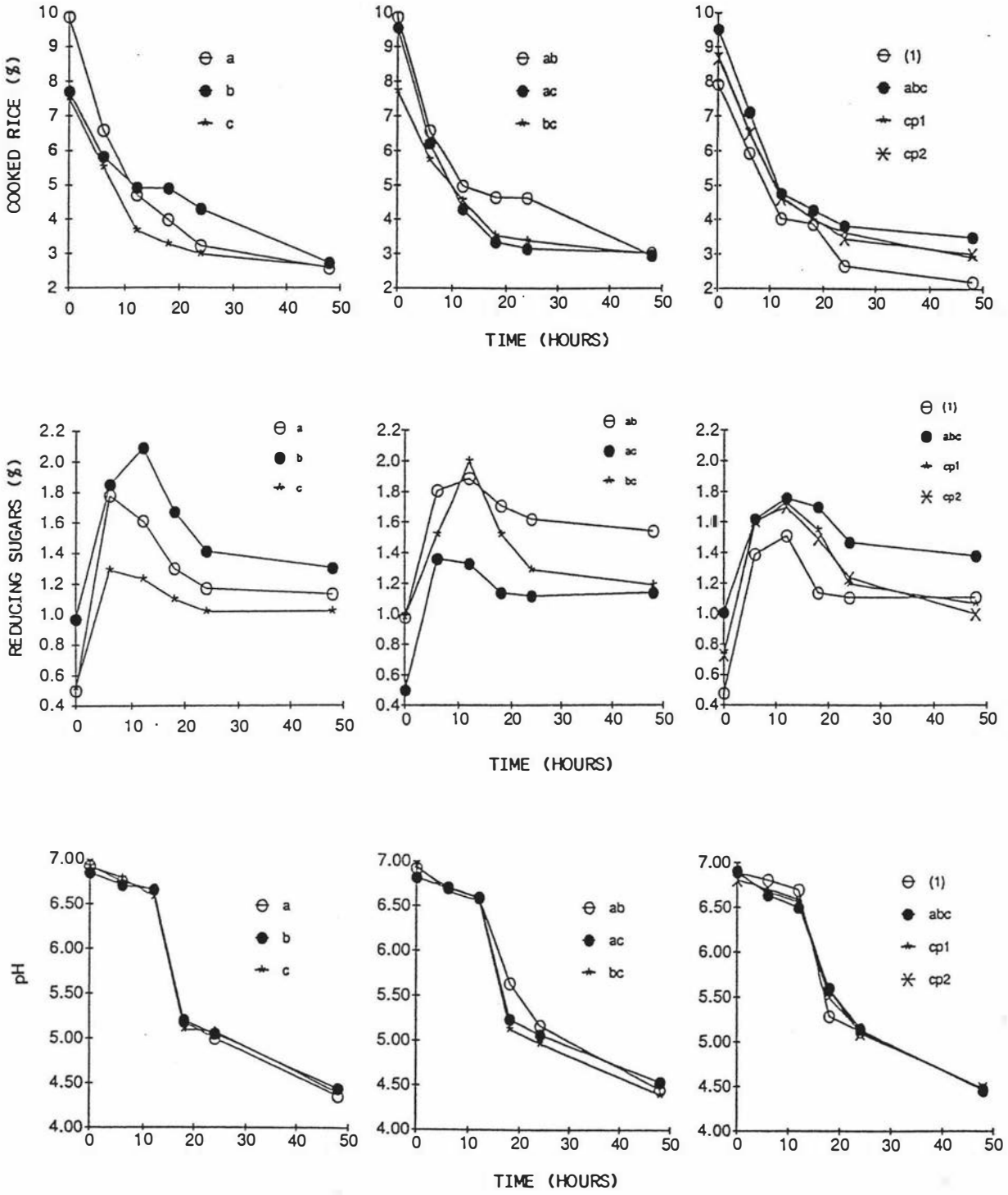


Figure 5.1 Cooked rice, reducing sugars, and pH during the first 48 hours of fermentation affected by the concentrations of cooked rice (a),glucose (b) and L.plantarum (c).

Table 5.2 Cooked rice, reducing sugars and pH at 18 hours of Nham fermentation.

Treatments	Cooked rice (%)	Reducing sugars (%)	pH
(1)	3.88	1.14	5.29
a	3.97	1.30	5.18
b	4.88	1.67	5.20
ab	4.65	1.71	5.64
c	3.26	1.10	5.10
ac	3.35	1.14	5.24
bc	3.55	1.52	5.13
abc	4.28	1.70	5.60
cp_1	3.99	1.55	5.50
cp_2	4.05	1.49	5.58

(1) = control; a= cooked rice; b = glucose; c = L. plantarum
cp = centrepoint

Although the pH values at 18 hours were quite similar, there were differences which appeared to be related to the formulation. In samples where either cooked rice or glucose were at the high level, pH reduction was more rapid than where both were high. The greatest fall occurred where L. plantarum was at the high level and cooked rice and glucose were at the low levels (Table 5.2).

During the first period of Nham fermentation, there was little weight loss at 24 hours but at 48 hours it was 0.54-1.20% (Table 5.3). The highest weight loss at 48 hours was with high cooked rice, high glucose and low level L. plantarum, the lowest weight loss with low cooked rice, low glucose and high L. plantarum.

The compression force increased markedly between 24 hours and 48 hours, from 51-82 Newtons to 88-140 Newtons (Table 5.3). There were differences between treatments at both 24 hours and 48 hours, the treatment with all factors at a low level giving the lowest compression force. The treatments with only L. plantarum at high level (c) and also with high glucose (bc) gave the highest compression force.

Table 5.3 The weight loss and compression force at 24 and 48 hours of fermentation.

Treatments	Weight loss (%)		Compression force (Newtons)	
	24 hours	48 hours	24 hours	48 hours
(1)	0.11	0.78	51.74	87.98
a	0.09	0.56	65.68	105.98
b	0.09	0.90	70.67	98.10
ab	0.09	1.20	56.61	101.21
c	0.07	0.54	78.75	139.81
ac	0.09	1.08	76.74	96.02
bc	0.11	1.04	81.89	136.55
abc	0.08	0.80	59.46	112.84
cp_1	0.07	0.60	54.34	93.74
cp_2	0.08	0.75	51.01	98.94

(1) = control; a = cooked rice; b = glucose; c = L. plantarum
cp = centrepoint

In all case the pink colour of Nham developed quickly between 24 and 48 hours, as a logical result of the nitrosomyoglobin formation (Table 5.4). The Nham with low level L. plantarum and glucose but high level cooked rice had slightly higher colour values than the rest of the Nham samples at 48 hours, but there were no large differences in colour.

Table 5.4 The tristimulus values (x, y, z) at 24 and 48 hours of fermentation.

Treatments	24 hours			48 hours		
	x	y	z	x	y	z
(1)	20.45	17.95	16.80	24.35	21.60	20.95
a	22.65	20.05	18.85	25.45	22.85	22.20
b	22.00	19.08	18.70	25.00	22.40	21.50
ab	23.45	21.05	19.45	24.75	22.15	21.20
c	21.30	18.95	17.50	24.60	21.85	21.35
ac	22.85	20.60	19.50	24.15	21.60	20.65
bc	23.05	20.85	19.10	24.95	22.05	21.50
abc	22.05	20.25	18.80	25.35	22.65	22.00
cp_1	22.65	20.65	19.30	25.20	22.60	21.80
cp_2	22.65	20.40	19.10	25.05	22.45	21.85

(1) = control; a = cooked rice; b = glucose; c = L. plantarum
cp = centrepoint

In summary, significant changes occurred during the first 48 hours of fermentation: the pH fell and compression force increased. During this time the colour also became more intense.

5.4.2 The Empirical Models of Dependent Variables During the First 48 Hours of Fermentation

The multiple regression models for pH with all independent variables with time during the first period of fermentation are shown in Appendix 5.2; Table A 2.1.

The developed model for pH during the first period of Nham fermentation was

$$\frac{1}{pH} = 0.0186 + 0.1207\sqrt{Time + 1} \qquad R^2 = 90.75\%$$

According to the equation, only time affected significantly the pH of Nham, i.e. during the first 48 hours of fermentation, the glucose, cooked rice and L. plantarum had no

statistically significant effect on the pH reduction, which was dependent only upon the fermentation time.

The multiple regression models for reducing sugars and cooked rice with all independent variables during the first period of Nham fermentation are shown in Appendix 5.2; Table A2.2 and A2.3 respectively.

The developed models for the reducing sugars and cooked rice during the first period of Nham fermentation are presented in Table 5.5 and 5.6 respectively.

Table 5.5 Fitted models for the reducing sugars (RS) during 6-24 hours of fermentation affected by carbon sources and L. plantarum.

Fitted equations	R^2 (%)	Lack of fit F-Ratio
6 hrs; RS(%) = 1.582 + 0.062 (CR) + 0.122 (G) - 0.130 (LP)	84.87	51.80 ns
12 hrs; RS(%) = 1.685 + 0.258 (G) - 0.098 (LP)	88.87	27.98 ns
18 hrs; RS(%) = 1.432 + 0.053 (CR) + 0.024 (G) - 0.045 (LP)	94.35	3.13 ns
24 hrs; RS(%) = 1.265 + 0.069 (CR) + 0.171 (G) - 0.051 (LP)	95.07	3.55 ns

ns = not significant
CR = Cooked rice; G = Glucose; LP = L. plantarum

As can be seen from Table 5.5, the addition of cooked rice to the Nham base contributed significantly to the measured concentration of reducing sugars during the first 24 hours of fermentation. The addition of glucose (itself a reducing sugar) had a greater influence. The L. plantarum used up reducing sugars during 6-24 hours.

Table 5.6 Fitted models for the residual level of cooked rice (CR) during 12-48 hours of fermentation affected by carbon sources and L. plantarum.

Fitted equations	R^2 (%)	Lack of fit (F-Ratio)
12 hrs; CR(%) = 4.520 + 0.197 (CR) + 0.312 (G) - 0.165 (LP)	87.76	11.24 ns
18 hrs; CR(%) = 3.986 + 0.363 (G) - 0.368 (LP)	86.52	30.57 ns
24 hrs; CR(%) = 3.529 + 0.186 (CR) + 0.514 (G) - 0.181 (LP)	83.67	6.20 ns
48 hrs; CR(%) = 2.860 + 0.192 (CR) + 0.225 (G) + 0.210 (LP)	94.45	2.28 ns

ns = not significant
CR = Cooked rice; G = Glucose; LP = L. plantarum

Table 5.6 shows that the residual level of cooked rice was reduced by the activity of L. plantarum but that the inclusion of glucose in the formulation decreased the utilization of cooked rice.

The model developed for weight loss on the first day of Nham fermentation did not adequately fit the data, no doubt because the weight losses were so low (Appendix 5.2; Table A2.4). The fitted model for the second day was:

$$\text{Weight loss (\%)} = 0.825 + 0.122\ (G) - 0.162\ (CR\ G\ LP)$$
$$(R^2 = 70.81\%)$$

The lack of fit of the model was not significant (F-ratio = 1.86) and the model therefore adequately represented the observed data. The model showed that the glucose increased significantly the weight loss at 48 hours.

The developed models for the compression force during 24-48 hours of Nham fermentation are presented in Table 5.7 (Appendix 5.2; Table A 2.5).

Table 5.7 Fitted models for the compression force (CF) during 24-48 hours of fermentation affected by carbon sources and L. plantarum.

	Fitted equations	R^2 (%)	Lack of fit (F-ratio)
24 hours;	CF = 64.689 + 6.518 (LP) - 6.052 (CRG)	51.45	17.79 ns
48 hours;	CF = 107.117 + 11.494 (LP) - 11.076 (CRLP)	72.27	9.47 ns

ns = not significant
CR = Cooked rice; G = Glucose; LP = L. plantarum
* = (Newtons)

It can be seen from Table 5.7 that the L. plantarum increased significantly the compression force at 24 and 48 hours whereas the carbohydrate interaction terms (cooked rice * glucose) and (cooked rice * L. plantarum) decreased it significantly at 24 hours and 48 hours respectively.

Table 5.8 Fitted models for the tristimulus values (x, y, z) during 24-48 hours of fermentation affected by carbon sources and L. plantarum.

	Fitted equations	R^2 (%)	Lack of fit (F-Ratio)
24 hours;	x = 22.30 + 0.525 (CR) + 0.412 (G) - 0.413 (CRG) - 0.387 (CRLP)	88.97	0.00 ns
	y = 20.055 + 0.550 (CR) + 0.550 (G) - 0.387 (CRG) -0.287 (CRLP)	82.27	11.31 ns
	z = 18.710 + 0.563 (CR) + 0.425 (G) - 0.450 (CRG)	80.36	13.48 ns
48 hours;	x = 24.885 + 0.188 (G) + 0.200 (GLP) + 0.275 (CRGLP)	75.14	6.89 ns
	y = 22.220 + 0.169 (CR) + 0.169 (G) + 0.294 (CRGLP)	65.44	10.56 ns
	z = 21.500 - 0.144 (CRLP) + 0.244 (GLP) + 0.344 (CRGLP)	75.88	80.44 ns

ns = not significant
CR = Cooked rice; G = Glucose; LP = L. plantarum

As can be seen in Table 5.8, the cooked rice and glucose increased significantly the tristimulus values (x,y,z) at 24 hours. However, there were two-factor interaction effects on the values at this time. At 48 hours, the cooked rice and glucose increased the y-value significantly. The glucose also increased the x-value. Additionally, there were the effects of interaction terms on the tristimulus values.

5.4.3 Chemical and Physical Changes During 2-7 Days of Fermentation and their Relation to the Nham Quality.

As can be seen in Figure 5.2 the properties of the Nham continued to change, but more slowly than during the first 48 hours. In all treatments, the cooked rice concentration decreased to between 1.3 and 2.2% at 7 days (Appendix 5.1; Table A1.4).

The reducing sugars steadily decreased in most treatments except ab (the high levels of cooked rice and glucose) in which a slow increase was noted from day 4. However, with treatment abc (the high levels of all factors) the reducing sugars increased markedly to 4 days and then decreased up to 7 days. Similar behaviour was exhibited by the centrepoint treatments (Figure 5.2 and Appendix 5.1; Table A1.3).

The pH continued to fall in all samples, reaching about 4.0 by day 7, indicating that the fermentation was complete by this time (Figure 5.2).

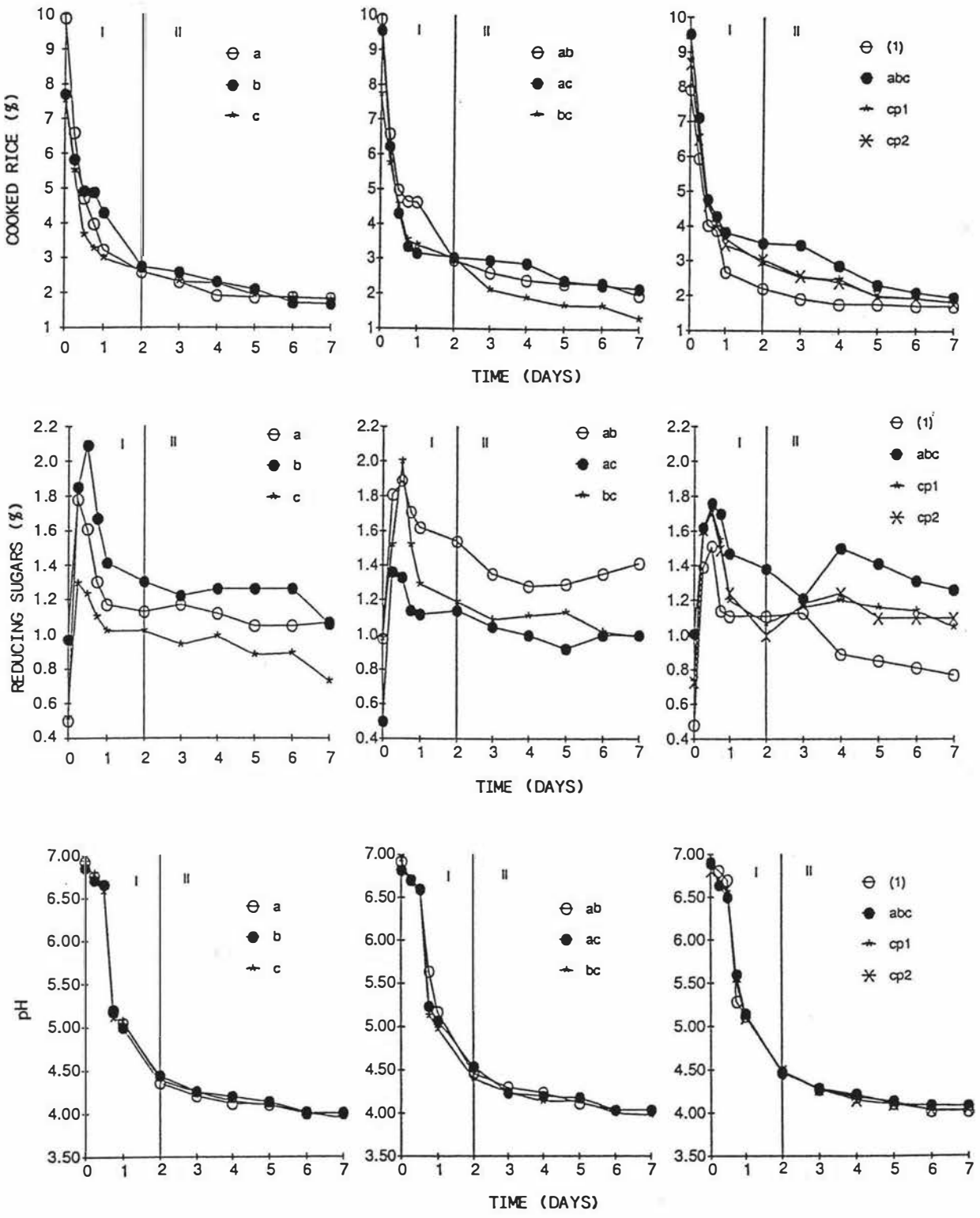


Figure 5.2 Cooked rice, reducing sugars, and pH during 1-2 days (I) and 2-7 days (II) of fermentation affected by the concentrations of cooked rice (a),glucose (b) and L.plantarum (c).

In all samples the cumulative weight loss increased signficantly during 2-7 days to between 5.1-7.8% on the seventh day (Figure 5.3 and Appendix 5.1; Table A 1.5). As can be seen in Figure 5.3, a high level of glucose appeared to promote weight loss, while L. plantarum had the opposite effect.

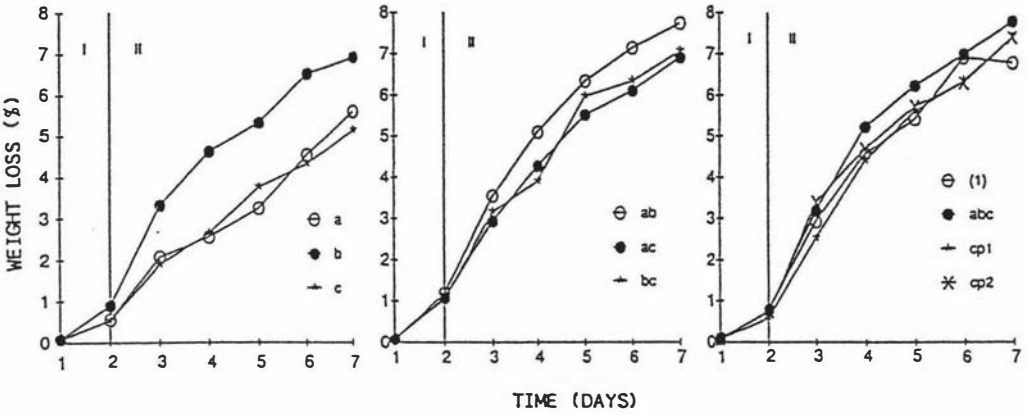


Figure 5.3 Weight loss during 1-2 days (I) and 2-7 days (II) of fermentation affected by the concentrations of cooked rice (a),glucose (b) and L.plantarum (c).

The Nham quality in terms of texture and colour was observed during 2-7 days of fermentation. In all samples the compression force increased during the first 2 days continued until about 4 days, when minor decreases occurred. At 7 days there were significant differences in compression force between the treatments. When only single factors were high, the final compression force was about 125 Newtons. However, the combinations ab, ac and abc all produced Nham with a lower final compression force of about 100 Newtons (Figure 5.4).

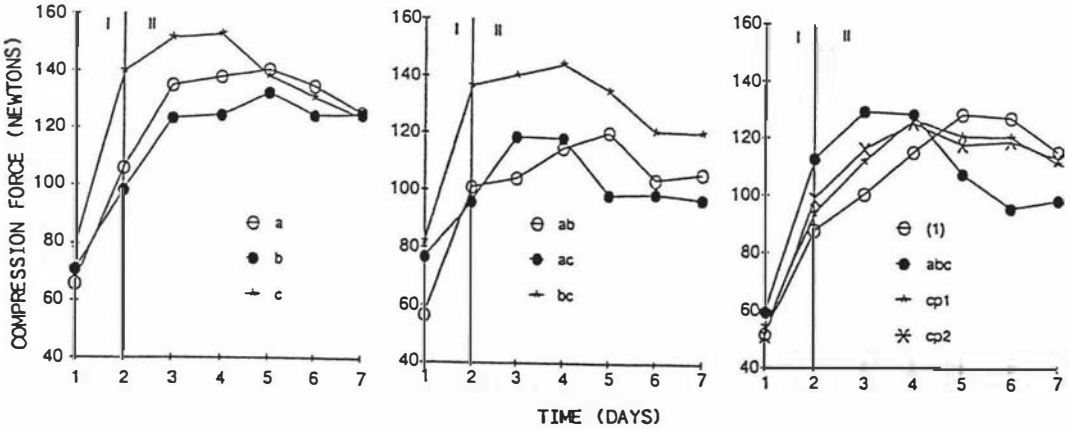


Figure 5.4 Compression force during 1-2 days (I) and 2-7 days (II) of fermentation affected by the concentrations of cooked rice (a),glucose (b) and L.plantarum (c).

The pink colour developed quickly until on the third day of fermentation and remained constant during 3-6 days of fermentation and then slightly decreased at 7 days (Figure 5.5 and Appendix 5.1; Table A1.7).

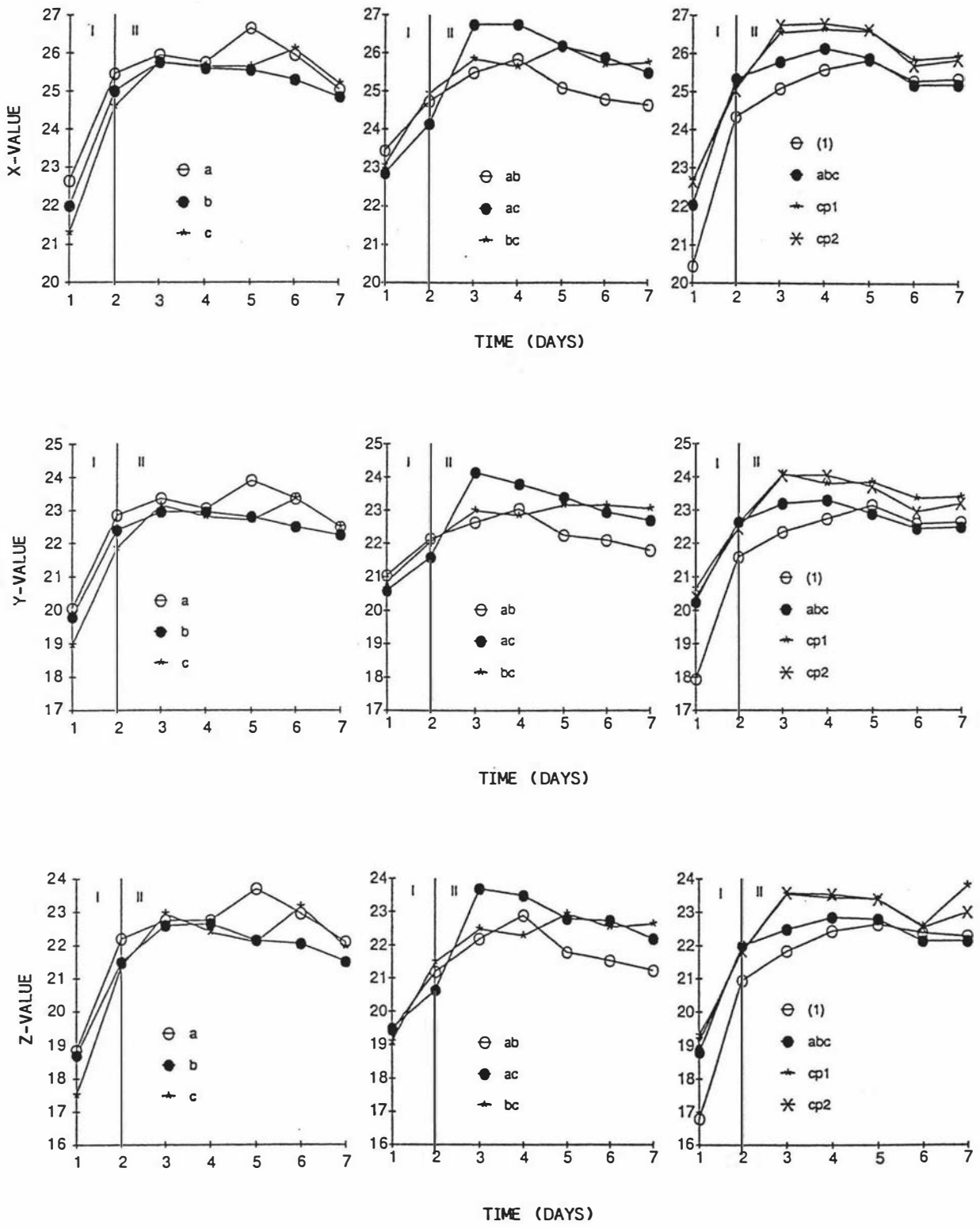


Figure 5.5 Tristimulus values (X Y Z) during 1-2 days (I) and 2-7 days (II) of fermentation affected by the concentrations of cooked rice (a),glucose (b) and L.plantarum (c).

5.4.4 Sensory Evaluation of Nham

From the mean ideal ratio scores, all attributes of Nham were near the ideal scores at 3 days and 7 days (Appendix 5.1; Table A1.8 (a) and Table A1.8 (b)).

As can be seen from Table 5.9, the overall acceptability of some treatments were not high (0.85 - 0.90) at 3 days of fermentation but the values increased at 7 days. The treatment a and c which had higher scores at 3 days were still high (0.94) at 7 days. It was clear that there were two groups of Nham processes, Nham from some treatments could be consumed at 3 days, but Nham from other treatments could only be consumed at 7 days. If the fast fermentation were desirable, treatment a and c could be used.

Table 5.9 The overall acceptability mean ideal ratio scores of Nham fermented for 3 and 7 days.

Treatments	Overall acceptability mean ratios	
	3 days	7 days
(1)	0.85	0.91
a	0.93	0.94
b	0.90	0.92
ab	0.90	0.91
c	0.93	0.94
ac	0.85	0.91
bc	0.92	0.92
abc	0.91	0.90
cp_1	0.89	0.89
cp_2	0.90	0.89

(1) = control; a = cooked rice; b = glucose; c = L. plantarum; cp = centrepoint

On the third day, overall acceptability of Nham depended upon sourness and spiciness (equation below). The colour also affected significantly the overall acceptability. From the multiple regression, the equation was:

Overall acceptability = -1.822 - 0.441 (Colour) + 0.892 (Sourness)
(3 days) + 2.474 (Spiciness)
(R^2 = 89.51%)

On the seventh day, overall acceptability of Nham depended significantly upon the firmness, visual texture and smoothness. The multiple regression equation was:

Overall acceptability = 0.713 - 0.438 (Visual texture) +
(7 days) 0.266 (Firmness) + 0.370 (Smoothness)
(R^2 = 87.55%)

The multiple regression models of overall acceptability with all independent variables at 3 and 7 days are shown in Appendix 5.2; Table A 2.7, but only the developed model at 3 days was signficant.

Overall acceptability = 0.898 + 0.009 (G) - 0.021 (CRLP)
(3 days) + 0.019 (CRGLP)
(R^2 = 95.62%)

In comparisons of sensory attributes and objective measurements, significant correlation between compression force and firmness was found ($r = 0.64$; $P<0.05$ at 3 days and $r = 0.89$; $P<0.01$ at 7 days). The perceived colour of Nham also correlated with the tristimulus values ($r = 0.95, 0.96, 0.90$ for x, y and z respectively; $P<0.01$ at 3 days and $r = 0.71$; $P<0.05$ for x-value, $r = 0.78$; $P<0.01$ for y-value, $r = 0.72$; $P<0.05$ for z value at 7 days). The pH correlated significantly with sourness ($r = -0.97; -0.98$; $P<0.01$ at 3 and 7 days respectively).

5.5 DISCUSSION

5.5.1 Chemical and Physical Changes

The fermentation was very active during the first 48 hours while the second stage (2-7 days) was slow (Figure 5.6). The effects of carbon sources and L. plantarum were seen during the first 48 hours of fermentation.

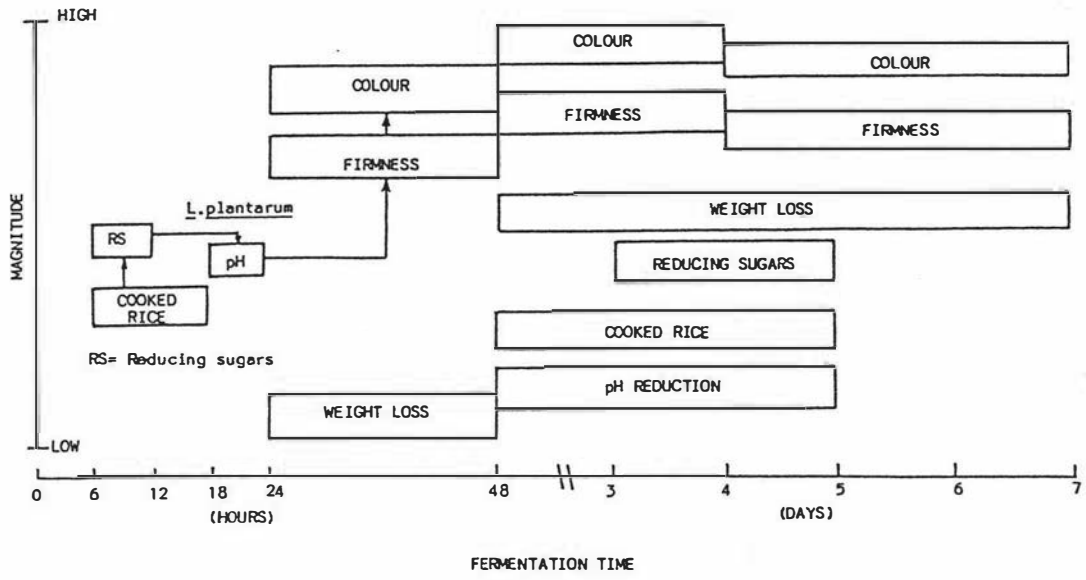


Figure 5.6 The chemical and physical changes during the first 48 hours and 2-7 days of fermentation affected by carbon sources and L. plantarum.

The pH of Nham decreased slightly during the first 12 hours of fermentation and then dropped rapidly until 18 hours to a pH of 5.1-5.6.

The cooked rice and glucose were added to the Nham formulation to provide the necessary fermentation substrates for the production of lactic acid. There was an increase in the content of reducing sugars during 6-12 hours as the cooked rice starch was hydrolysed to reducing sugars. This might be because the enzyme amylase produced by starter cultures or other organisms in Nham hydrolysed the cooked rice. These changes were shown to be affected by the concentration of L. plantarum present initially. Consistent with this result, the cooked rice concentration decreased quickly early in the fermentation and this coincided with the increase of the reducing sugars. As the reducing sugars declined, the pH in the Nham system also fell. It appears that the presence of glucose in the Nham retards the conversion of cooked rice to reducing sugars, presumably because glucose is preferentially metabolised by L. plantarum. Deibel (1974) stated that the conversion of either sucrose or glucose to lactic acid is due primarily to the homofermentative lactic acid bacteria. Selective growth of this group of bacteria is favoured by the anaerobic nature of meat mixtures and the presence of salt (Pezacki and Szostak, 1962; Andersen and Ten Cate, 1965; Deibel, 1974; Bacus, 1984; Gibbs, 1987). However, at the beginning of fermentation when both cooked rice and glucose were used

at the high levels, the rate of pH reduction was slower. Therefore, to have rapid decrease to a pH about 5.1 after 18 hours, low levels of cooked rice and glucose need to be used.

During the second period of Nham fermentation (2-7 days), the pH dropped slowly because the pH had already dropped nearly as far as it could go. The cooked rice and reducing sugars decreased slowly which correlates with the slow pH reduction during this time. Nham containing a high level of carbohydrate certainly showed a slower rate of acid production than the Nham containing a low level of carbohydrate. With high levels of cooked rice and glucose, there is a continuing hydrolysis of cooked rice which gives levels of reducing sugars which the low level of L. plantarum cannot use up. Even at the high level of L. plantarum, there is an increase in reducing sugars up to 4 days but after that the L. plantarum is able to use up.

Glucose added to the Nham formulation made a statistically significant increase in the weight loss at 48 hours. However, the actual loss was only about 1%. The sausages were stuffed without vacuuming the mix and therefore a high oxygen concentration was present. As the growth of micrococci is stimulated by oxygen, the micrococci may contribute to a more complete oxidation of the available carbohydrate. The result could have been the production of some carbon dioxide and water in the first 2 days of fermentation and thus a weight loss occurred.

$$C_6H_{12}O_6 + 6O_2 \longrightarrow \underset{(29\%)}{6\,H_2O} + \underset{(71\%)}{6\,CO_2}$$

According to the equation, a 71% yield of carbon dioxide based on glucose and oxygen system is released from the Nham sausage during the initial fermentation while there is only 29% water loss. DeKetelaere et al. (1974) stated that oxidative dissimilation of available carbohydrate does not appear to occur if sausage mixes are vacuumized.

The weight loss increased markedly during the second period of fermentation. The glucose increased the weight loss significantly during this time. The residual sugars are used by part of the surviving active flora, particularly heterofermentative lactic acid bacteria, yielding carbon dioxide and ethanol (Wood, 1985). According to equation as below:

$$C_6H_{12}O_6 \longrightarrow \underset{(50\%)}{CH_3CHOHCOOH} + \underset{(26\%)}{C_2H_5OH} + \underset{(24\%)}{CO_2}$$

A 24% yield of carbon dioxide based on glucose consumption is released from the Nham system by the heterofermentative lactic acid bacteria. However, Pezacki and Jaroszewski

(1963), Pezacki and Fiszer (1966), and Fiszer (1970) reported that during the last period of the ripening process, all fermentation processes ceased and the remaining glucose was oxidized yielding carbon dioxide and water. The oxidation was accompanied by an equivalent oxygen uptake from the air surrounding the sausage. Consistent with these reports , the weight of Nham during the second period of fermentation decreased quickly because of carbon dioxide production in the Nham system.

During the first active phase of Nham fermentation, the firmness and colour were developed. L. plantarum increased the firmness significantly during 24-48 hours of fermentation, because these bacteria produced lactic acid and influenced directly the firmness of Nham similar to the results from Chapter 4. The glucose and cooked rice increased significantly the tristimulus values during 24-48 hours of fermentation. The probable explanation is that the glucose and cooked rice were used by micrococci, particularly by the M. varians present in the early stages of fermentation, and then these bacteria reduced nitrate to nitrite. Under the acid condition, nitrite reaction with myoglobin was accelerated to form nitrosomyoglobin in the Nham system. The quality of Nham, in terms of its firmness and colour, was maintained during 3-5 days and then decreased slowly up to 7 days. In the fast fermentation, the firmness was maintained longer than the slow fermentation. The L. plantarum appeared to increase the firmness during the initial 3-4 days but then caused a decrease during the last period of fermentation. Cooked rice had the same effect. This might be more likely softening due to gas production. However, the glucose had a significant effect on the loss of colour during the last period of fermentation. The glucose could have been used during the last period of fermentation either by non-lactic acid bacteria or in an oxidation pathway and this would have affected the acid condition in the Nham system - affecting the colour. On the other hand, the L. plantarum increased significantly the tristimulus values during the last period of fermentation, producing acid which then accelerated the nitrite decomposition to combine with myoglobin (Klettner and Baumgartner, 1980; Bacus, 1984) similar to the results from Chapter 4.

There were also 1.0-1.3% reducing sugars and 2.0-3.0% cooked rice in the Nham after being fermented for 3 days. These residual carbohydrates could be used by undesirable organisms such as heterofermentative lactic acid bacteria, non-lactic acid bacteria, yeasts and moulds during storage. Pezacki and Jaroszewski (1963), Pezacki and Fiszer (1966), and Fiszer (1970) reported that the residual sugars remaining in final sausage is oxidized by the surviving active flora at the last period of fermentation. Rice (1971) recommended that the range in carbohydrate content for sausage products is generally 1.2-1.7% in finished state. The residual carbohydrate content following the active fermentation phase

could be much lower (Acton, 1977). Therefore, the carbon source levels in Nham formulation should be low. Tandler (1963) stated that acid production was more rapid in fermentations with monosaccharides as compared to disaccharides or polysaccharides. The amount of acid produced was dependent on the quantity of simpler carbohydrates such as glucose, initially available in the formulation. So, the glucose was maintained at 0.5% in the Nham formulation but the level of cooked rice could be reduced. As the higher molecular weight of the carbohydrate substrate increased, a longer period was needed to attain an adequately fermented product (Pycrz and Pezacki, 1974; Pezacki, 1978; Acton, 1977). It was difficult to use cooked rice for the starter culture. The high level of cooked rice also retarded the pH reduction during fermentation. On increasing the level of cooked rice, the firmness of Nham decreased during the last period of fermentation. Thus, the Nham which has low level of cooked rice with 0.5% glucose should be used if a fast fermentation is desired. The time period and temperature of fermentation should be also suited to the type or source of carbohydrate furnished. Therefore, the cooked rice level could be reduced to 6% for avoiding the risk of the microbiological spoilage and this may also improve the firmness.

5.5.2 Acceptability of Nham

On the basis of overall acceptability ideal ratio scores, the Nham with 10^3 cfu.g^{-1} L. plantarum + 8% cooked rice + 0.5% glucose (treatment c) was most acceptable (ratio score = 0.93). This treatment produced a rapid pH reduction and had the highest compression force during the first 48 hours of fermentation. It was clear that the treatment was a fast fermentation and there was a sign that fermentation was complete at 3 days as the pH was 4.2. Similarly, the Nham with 10^2 cfu.g^{-1} L. plantarum + 10% cooked rice + 0.5% glucose (treatment a) was also ready for consumption at 3 days as the overall acceptability mean ideal ratio score was 0.93. However, the pH reduction and firmness development were slower than the treatment c. The other treatments required 7 days fermentation when the overall acceptability ideal ratio scores came up to 0.89-0.92. The overall acceptability ratio score at 3 days related to glucose in the Nham system and it was also related to sourness attribute. Acid production was more rapid in fermentation with glucose than cooked rice. Additionally, the firmness attribute of Nham influenced the overall acceptability ratio score at 7 days. This was because of the acid production effect in the Nham system. In this study, the cooked rice at the high level with the high level of L. plantarum (treatment ac) needed 7 days to attain a ripened Nham. This result also agreed with the result of Pycrz and Pezacki (1974). Thus, the Nham which has low levels of cooked rice and glucose with the higher level of L. plantarum should be used if a fast fermentation is desired.

5.6 CONCLUSION

Two methods of producing satisfactory Nham emerge from these experiments: fast fermentation for 2-4 days, followed by chilling, or slow fermentation for 4-7 days and then chilling. During the first 48 hours of fermentation, the Nham system was actively changing, affecting the Nham quality. The firmness and colour were quickly developed between 24-48 hours and there was little weight loss in the Nham system during this period.

During the second period of Nham fermentation from 2-7 days, the pH dropped slowly. The presence of cooked rice and glucose retarded significantly the pH reduction from the need of the first period of fermentation until 7 days.

L. plantarum caused a desirable increase in the compression force, while the cooked rice and glucose decreased it. Additionally, the L. plantarum improved the colour development during the last period of fermentation, whereas glucose and cooked rice increased at an earlier stage.

The most desirable Nham was formulated with 10^3 cfu.g^{-1} L. plantarum +8% cooked rice + 0.5% glucose. This produced a rapid fermentation and would therefore be quicker and cheaper to make, particularly since the level of cooked rice could be reduced to 6% for firmness improvement and also for avoiding the microbiological spoilage using the residual cooked rice in the Nham system during storage.

This formulation was used as the basis for the next study, and testing over a period of 72 hours. The effect of different temperatures and relative humidities of fermentation was also investigated for process improvement.

CHAPTER 6

EFFECTS OF TEMPERATURE AND RELATIVE HUMIDITY ON THE RATE OF NHAM FERMENTATION

6.1 INTRODUCTION

This experiment was designed to investigate further the processing of Nham, particularly the effects of temperature and relative humidity and to establish the optimum environmental conditions for a fermentation lasting only 3 days.

The purposes of this study were:

- to investigate the total process of Nham fermentation.
- to determine the rate of Nham fermentation particularly in terms of pH reduction, firmness development and colour development.
- to determine a suitable process to inhibit the growth of pathogenic bacteria.

A mathematical model was fitted for each of the response variables to study the effects of temperature and relative humidity.

6.2 EXPERIMENTAL DESIGN

In this experiment, a 2^2 factorial design was used to investigate the effects of temperature and relative humidity. Therefore, the experiment was composed of 4 treatment combinations with 2 centrepoints as follow:

Factor A = Temperature (T)

a_1 = 20°C (low level)

a_2 = 30°C (high level)

Factor B = Relative humidity (RH)

b_1 = 43% (low level)

b_2 = 97% (high level)

The temperatures selected for this experiment were 20°C and 30°C because the average minimum daily temperature in Northern Thailand during the year was 20°C and the average maximum temperature was 30°C. Additionally, the average minimum relative humidity in Northern Thailand during the year was 43% and the average maximum relative humidity was 97%.

The design of this study is shown in Table 6.1.

Table 6.1 The 2^2 factorial design with centrepoints for investigating the effects of temperature and relative humidity on the rate of Nham fermentation.

Treatments	Coded level	Factors: Temperature (°C)	Factors: Relative humidity (%)
	+	30	97
	0	25	70
	-	20	43
(1)		-	-
a		+	-
b		-	+
ab		+	+
cp_1		0	0
cp_2		0	0

6.3 SAUSAGE PREPARATION, TESTING AND ANALYSIS FOR 2^2 FACTORIAL DESIGN

The basic formulation used in this study was developed from the results in Chapters 4 and 5.

Ingredients		Quantity (%)
Meat system:	minced lean pork	80
	sliced pork skin	20
		% of meat system
Cooked rice		6
Glucose		0.5
Sodium chloride		3
Sodium tripolyphosphate		0.3
Sodium nitrate		0.02
Minced raw garlic		7
White pepper powder		0.05

	cfu/g of meat system
Starter cultures:	
L. plantarum	10^3
P. cerevisiae	10^6
M. varians	10^3

The sausage preparation was the same as previously (Section 2.3) and the bacterial cultures were grown by the same method as in the previous experiments (Section 2.2). The Nham was held in the incubators at different temperatures and relative humidities (Table 6.1). Two Nham sausages were randomly sampled from each treatment for physical, chemical, microbiological and sensory analyses during the 3 days fermentation period. pH, weight loss, compression force and colour measurement were determined using the methods in Sections 2.4.1, and 2.4.2. Counts of the M. varians, P. cerevisiae and L. plantarum, Enterobacteriaceae, Staphylococcus aureus and aerobic mesophiles counts were studied using the methods described in Section 2.4.3. A trained panel of 8 Thai postgraduate students at Massey University was also used for sensory evaluation (Section 2.4.4).

At intervals of 0, 6, 12, 18, 24, 48 and 72 hours pH was determined to investigate the fast fermentation. Weight loss, compression force and colour measurement were determined at 1, 2, and 3 days, sensory evaluation at 3 days, bacterial counts at 1 and 3 days. The models were analysed by the methods used in previous chapters.

6.4 KINETICS OF NHAM FERMENTATION

In order to investigate the rate of Nham fermentation, the Arrhenius equation was applied to calculate the rate constant of pH reduction, firmness and colour development. The reaction constants (k-values) were determined using a linear regression model. The basic equation was a regression of ln C (the dependent variable) against time (the independent variable). It was assumed that the rate constant of pH reduction, firmness and colour development were the first order relationships, so the following equation was used (Saguy and Karel, 1980; Hill and Grieger-Block, 1980; Wells and Singh, 1988).

$$\ln C = \ln Co \pm kt$$

where C = concentration at time t

C_o = concentration at time = 0

k = rate constant

t = time

To determine the goodness of fit of the data, R^2 was computed and interpreted as fraction of the variation in ln C that could be explained by means of the straight-line prediction equation. The temperature dependence of the rate of quality changes of Nham was determined using the linear models. The rate constants (k values) were plotted against the reciprocal of the temperature in ^oK (1/T), to investigate the relationship between the rate and temperature.

6.5 RESULTS

6.5.1 pH Change During Fermentation

The initial pH of Nham was 6.66-6.75 and began to change rapidly at 12-18 hours of fermentation, particularly at the high temperature (30°C). The pH continued to change markedly until 48 hours of fermentation to a pH of about 4.55 at 30°C and 5.1 at 20°C and then slowly decreased to a pH of about 4.4 at 30°C and 4.70 at 20°C after 72 hours (Figure 6.1 and Appendix 6.1; Table A1.2).

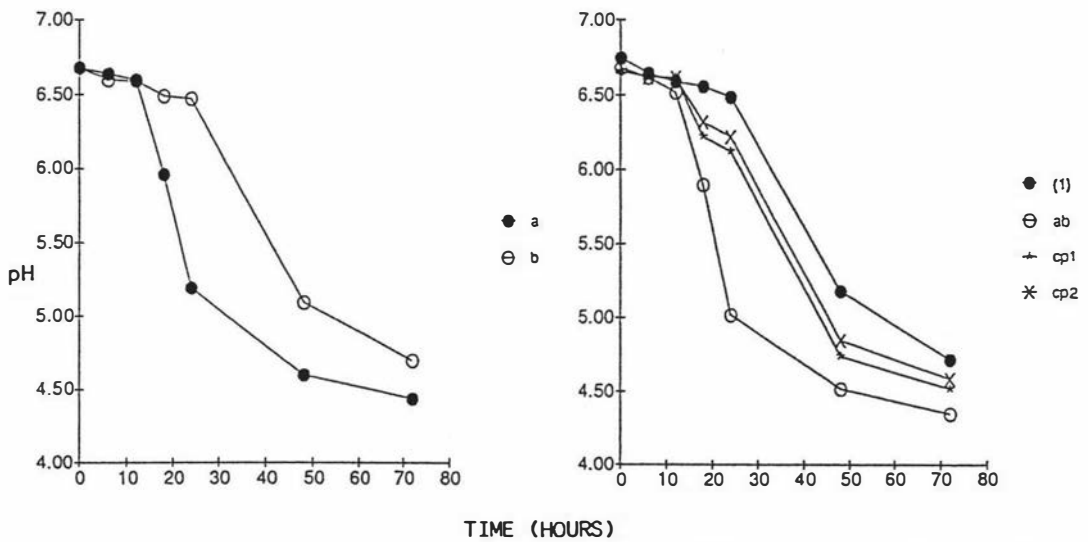


Figure 6.1 pH change during 72 hours fermentation at different temperatures and relative humidities.

The multiple regression models for pH with all independent variables during 72 hours of fermentation are shown in Appendix 6.2; Table A2.1.

The developed models for pH during Nham fermentation are presented in Table 6.2. As can be seen in Table 6.2, the temperature was significantly related to the decrease in pH during 18-72 hours of Nham fermentation, whereas no effect of relative humidity was observed.

Table 6.2 Fitted models for pH during 18-72 hours of fermentation affected by temperature.

Fitted equations	R^2 (%)	Lack of fit (F-ratio)
18 hrs; pH = 6.242 - 0.298 (T)	96.81	0.47ns
24 hrs; pH = 5.918 - 0.688 (T)	90.02	13.67ns
48 hrs; pH = 4.830 - 0.287 (T)	95.12	0.60ns
72 hrs; pH = 4.553 - 0.157 (T)	93.67	0.58ns

ns = not significant

T = temperature

As the temperature had a strong effect on the decrease in pH, the variation in rate constant of pH reduction at different temperatures were calculated (Table 6.3).

Table 6.3 Calculation of the rate constants of pH reduction in Nham at different temperatures.

Temperatures		1/T	-k	R^2
°C	°K	($°K^{-1}x10^{-3}$)	($hr^{-1}x10^{-3}$)	(%)
20	293	3.41	5.480	93.46
25	298	3.36	6.135	93.99
30	303	3.30	6.628	87.45

As can be seen in Table 6.3, the higher the temperature during Nham fermentation, the greater the rate of fermentation. The linear plot between 'k' values and 1/T for the rate constant of pH reduction of Nham at different temperatures (20-30°C) is presented in Figure 6.2.

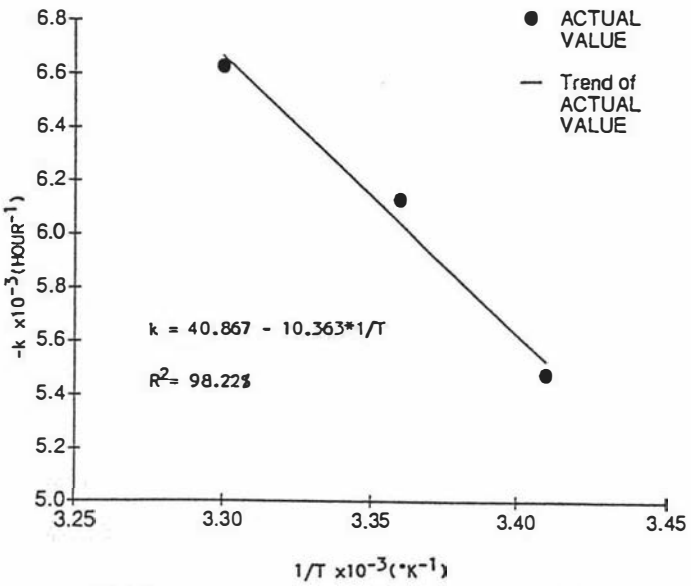


Figure 6.2 The rate of pH reduction during fermentation at 20°C, 25°C and 30°C.

The trend of actual value fitted very well as R^2 was 98.22%. The equation in Figure 6.2 could be used to predict pH in final Nham between the temperature of 20°C and 30°C.

6.5.2 Weight Loss During Fermentation

Cumulative weight loss of each treatment is shown in Figure 6.3. It can be seen that weight loss was greatest at high temperature and in low relative humidity conditions (Appendix 6.1; Table A 1.3). However, there was little weight loss on the first day of Nham fermentation. The weight loss increased slowly during 2 days in all treatments except treatment a. After 2 days, the weight loss increased quickly in all treatments particularly in treatment a, but the treatment b still increased slowly.

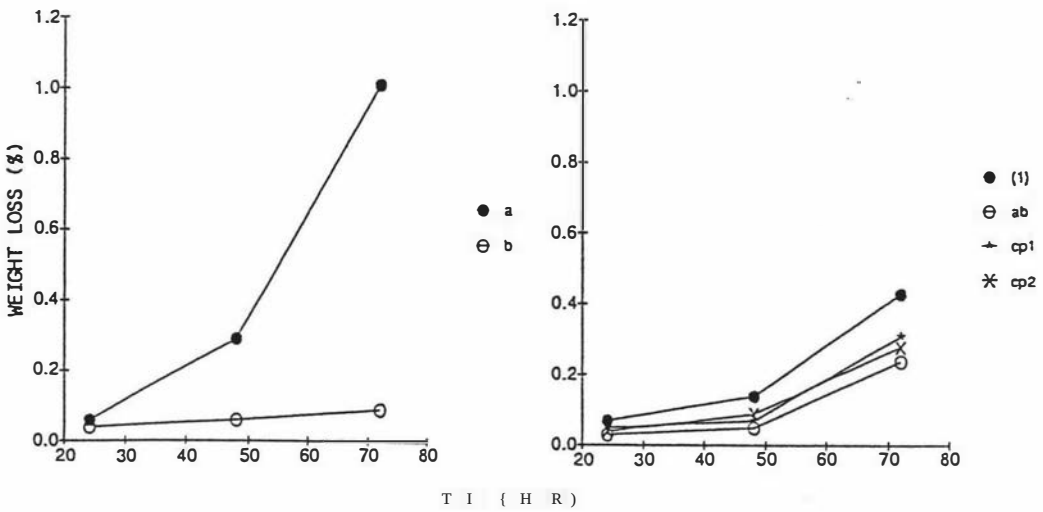


Figure 6.3 Weight loss during 72 hours fermentation at different temperatures and relative humidities.

The developed models of weight loss during the Nham fermentation are presented in Table 6.4 (from Appendix 6.2; Table A 2.2). It can be seen that both temperature and relative humidity of the fermentation environment have significant effects on the cumulative weight loss over the three-day fermentation period.

Table 6.4 Fitted models for weight loss (WL) during 72 hours of fermentation affected by temperature and relative humidity.

Fitted equations	R^2 (%)	Lack of fit (F-ratio)
24 hrs; WL (%) = 0.048 - 0.015 (RH)	83.08	0.89ns
48 hrs; WL (%) = 0.117 - 0.080 (RH)	62.24	26.33ns
72 hrs; WL (%) = 0.393 + 0.183 (T) - 0.277 (RH)	85.36	81.72ns

ns = not significant

T = Temperature, RH = Relative humidity.

6.5.3 Firmness Development During Fermentation

The compression force of Nham immediately after production was 10.38 Newtons, which increased during fermentation. As can be seen in Figure 6.4 all treatments showed significant rises in compression force, but the treatments at 30°C showed a more rapid increase than those at 20°C. Relative humidity appeared to have little effect (Appendix 6.1; Table A 1.4).

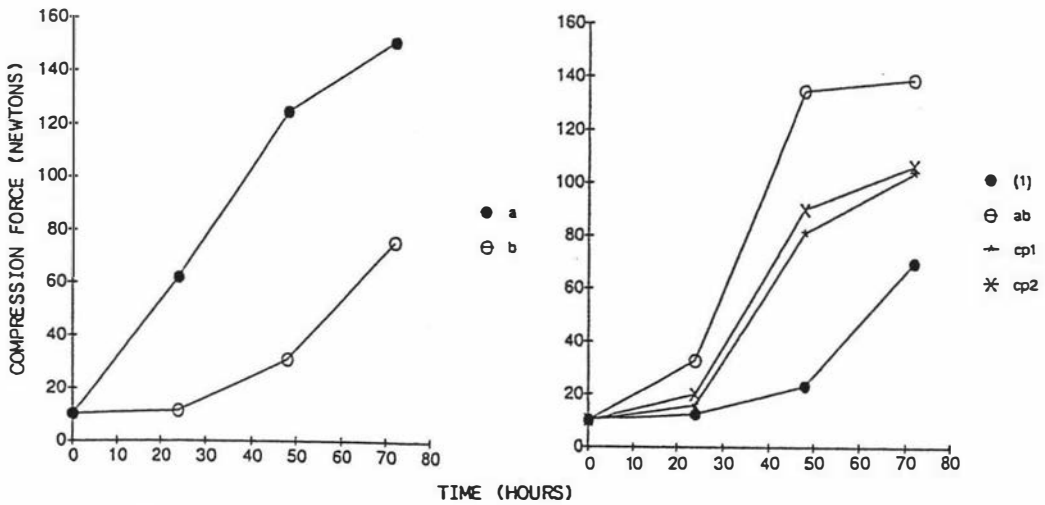


Figure 6.4 Compression force during 72 hours fermentation at different temperatures and relative humidities.

This was confirmed by the multiple regression models, which are presented in Table 6.5.

Table 6.5 Fitted models for the effect of temperature on the compression force (CF) during 72 hours of fermentation.

Fitted equations	R^2 (%)	Lack of fit (F-ratio)
24 hrs; CF* = 25.872 + 17.677 (T)	67.24	22.68ns
48 hrs; CF = 80.850 + 51.202 (T)	98.22	1.18ns
72 hrs; CF = 107.582 + 36.095 (T)	97.86	9.65ns

ns = not significant; T = Temperature; * = (Newtons)

As the temperature had a significant effect on the increase of firmness of Nham. The rate constant for increase of firmness with temperature was calculated (Appendix 6.3; Table A 3.1).

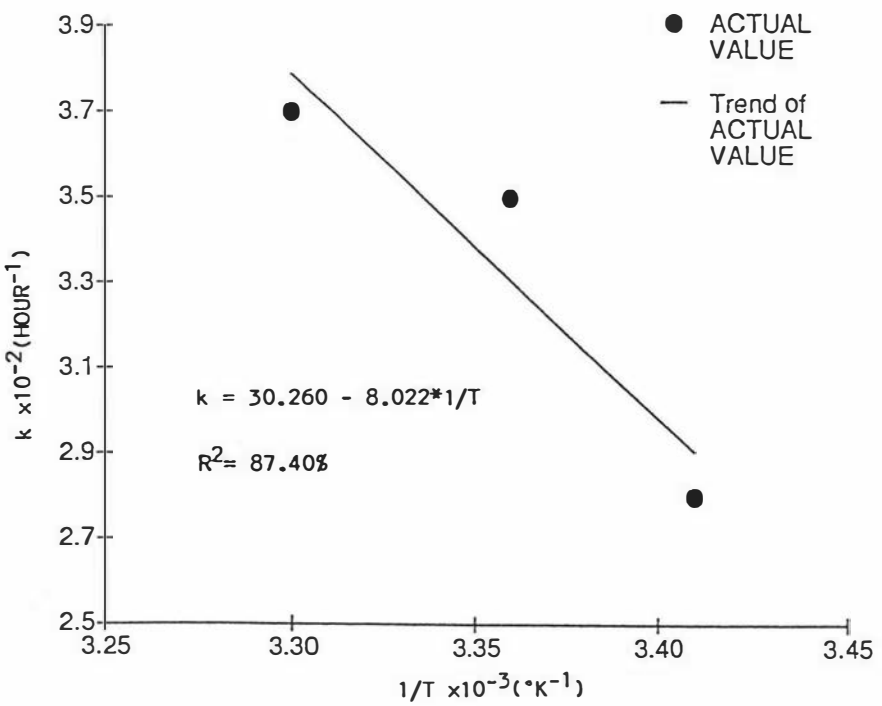


Figure 6.5 The rate of firmness development during fermentation at 20°C, 25°C and 30°C.

It can be seen from Figure 6.5 that the higher the temperature during Nham fermentation, the greater the rate of firmness development. The trend of actual data did not fit very well as R^2 was only 87.40%. This should be carefully considered to investigate for future experiment. Two or three points between 20°C and 30°C should be studied.

6.5.4 Colour Development During Fermentation

The initial colour of Nham was quite pale. The typical colour of finished Nham is pink, resulting from formation of nitrosomyoglobin. In these experiments the colour expressed as tristimulus values (x, y, z) developed quickly in all treatments over the 3 days fermentation period (Figure 6.6). Colour development was most rapid and the final colour most intense at the high temperature. As can be seen from Figure 6.6, relative humidity of the fermentation surroundings appeared to have little effect on the rate or extent of colour change (Appendix 6.1; Table A 1.5).

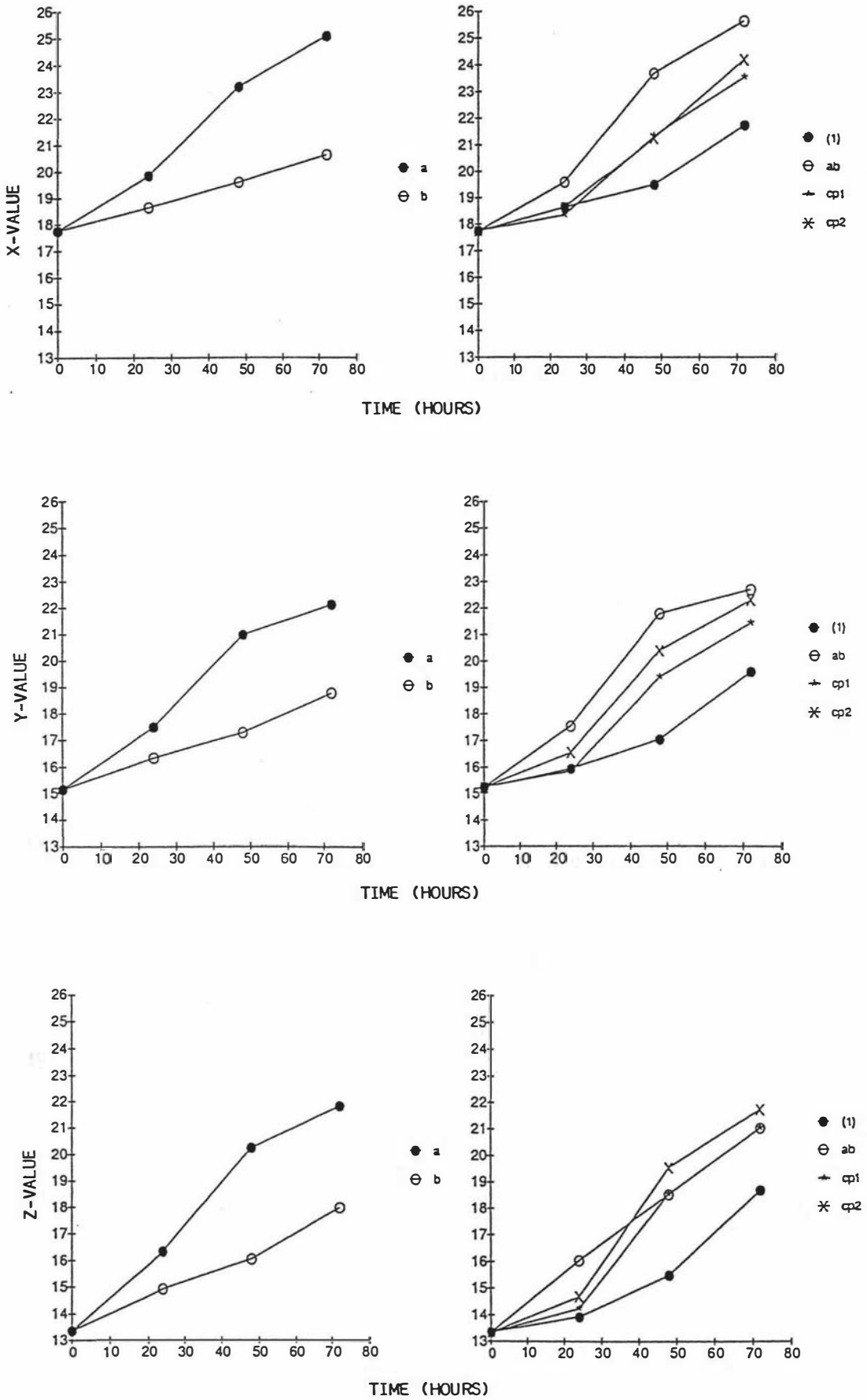


Figure 6.6 Tristimulus values (X Y Z) during 72 hours fermentation at different temperatures and relative humidities.

The multiple regression models for the tristimulus values (x, y, z) during 72 hours Nham fermentation are shown in Appendix 6.2; Table A 2.4.

The developed models for the tristimulus values during Nham fermentation are presented in Table 6.6. These models show that, in these experiments, only temperature had a significant effect on colour development.

Table 6.6 Fitted models for the tristimulus values (x, y, z) at 48-72 hours of fermentation affected by temperature.

Fitted equations		R^2 (%)	Lack of fit (F-ratio)
48 hrs;	x = 21.430 + 1.950 (T)	98.78	12.20ns
	y = 19.492 + 2.113 (T)	92.96	0.57ns
	z = 18.550 + 2.525 (T)	92.52	1.04ns
72 hrs;	x = 23.483 + 2.088 (T)	92.43	1.92ns
	y = 21.167 + 1.612 (T)	8[illegible].45	1.82ns
	z = 20.675 + 1.962 (T)	86.38	2.97 ns

ns = not significant; T = Temperature

The models show similar values of constant and temperature coefficient for each component at both time intervals.

Since the temperature was obviously an important determinant of colour of finished Nham, the rate constant for colour development with temperature was calculated (Appendix 6.3; Table A3.2). The relationship between the rate constants of tristimulus values (x, y, z) and the reciprocal of temperature showed that only x-value fitted well with R^2 = 93.66% whereas R^2 of y and z values were 79.39% and 80.57% respectively. Therefore, only the rate constant of x-value was plotted with the reciprocal of temperature (1/T) (Figure 6.7). As can be seen in Figure 6.7, the higher temperature during Nham fermentation, the greater rate of x-value development.

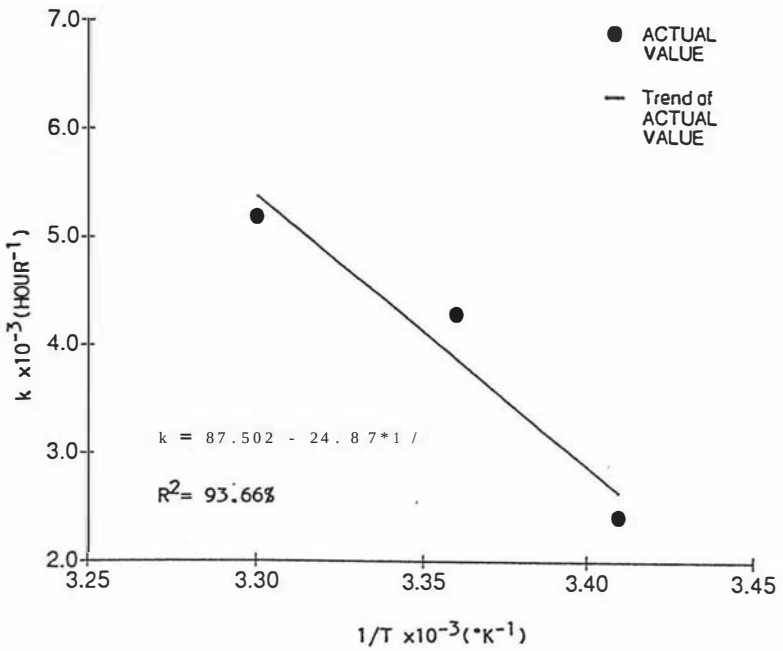


Figure 6.7 The rate of colour development (X-value) during fermentation at 20·c. 25·c and 30·c.

6.5.5 Bacterial Studies

The bacterial counts were determined after 1 and 3 days of fermentation. In all treatments there was a decrease in the log number of aerobic mesophiles of about 0.5 (Table 6.7).

Table 6.7 The bacterial numbers in Nham fermented 1 and 3 days at different temperatures and relative humidities.

Treatments	Log Number											
	Aerobic Mesophiles		L. plantarum		P. cerevisiae		M. varians		Enterobacteriaceae		S. aureus	
	N_1	N_3	N_1	N_3	N_1	N_3	N_1	N_3	N_1	N_3	N_1	N_3
(1)	8.89	8.70	3.21	7.71	6.60	7.26	3.42	7.32	8.16	4.36	7.12	4.13
a	8.73	8.23	3.56	8.11	6.50	7.08	3.36	4.36	8.53	2.70	7.01	3.19
b	8.96	8.70	3.18	7.81	6.65	7.24	3.48	7.43	7.96	4.51	6.36	4.15
ab	9.01	8.29	3.49	8.12	6.53	7.07	3.26	4.54	8.02	2.18	6.94	3.29
cp_1	8.60	8.09	3.35	7.99	6.71	7.33	3.54	5.76	8.26	3.20	6.53	3.88
cp_2	8.50	8.08	3.46	7.91	6.60	7.35	3.71	5.80	8.65	3.16	6.86	3.89

(1) control; a = temperature; b = relative humidity; cp = centrepoint

N_1 = fermented 1 day; N_3 = fermented 3 days

The log numbers of L. plantarum in the high temperature treatments increased more than in the samples at low temperatures (Table 6.7). As can been seen from equation below, temperatures significantly increased the number of L. plantarum at 3 days.

$$L.\ plantarum\ (\log N) = 7.942 + 0.177\ (temperature) \quad R^2 = 93.71\%$$

The log numbers of P. cerevisiae increased slightly during the fermentation time but the increase was not statistically significant. However, the temperature had a marked effect on the behaviour of M. varians. Equation shows that temperature significantly decreased the number of M. varians at 3 days.

$$M.\ varians\ (\log N) = 5.868 - 1.463\ (temperature) \quad R^2 = 99.46\%$$

The potentially pathogenic bacteria in Nham were also enumerated in terms of Enterobacteriaceae and S. aureus. As can be seen in Table 6.7, the log numbers of S. aureus decreased during the fermentation period by approximately 4 log cycles at high temperature (30°C) but only 3 log cycles at low temperature (20°C). Additionally, the log numbers of Enterobacteriaceae declined during fermentation by approximately 6 log cycles at 30°C,4 log cycles at 20°C. This behaviour is reflected in the empirical models for destruction of pathogens as shown below:

$$S.\ aureus\ (\log N) = 3.772 - 0.450\ (temperature) \quad R^2 = 89.87\%$$
$$Enterobacteriaceae\ (\log N) = 3.385 - 0.997\ (temperature) \quad R^2 = 95.39\%$$

Significant correlations between the bacterial counts and pH were found. The L. plantarum correlated with pH ($r = -0.97$; $P<0.01$). The M. varians correlated with pH ($r = 0.96$; $P<0.01$) and Enterobacteriaceae and S. aureus correlated with pH ($r = 0.99$, $r = 0.92$; $P<0.01$ respectively).

6.5.6 Sensory Evaluation

Of the various characteristics of Nham measured in these experiments, the most important were colour, firmness,juiciness, sourness and overall acceptability (Table 6.8). The Nham fermented at high temperature (30°C) gave nearly ideal ratio scores. The overall acceptability of Nham fermented at 30°C was higher than that produced at 20°C, the

mean ideal ratio scores at 30°C being 0.96, while that at 20°C was about 0.7.

Table 6.8 The mean ideal ratio scores of most important attributes and overall acceptability of Nham fermented at different temperatures and relative humidities.

Treatment	Colour	Firmness	Juiciness	Sourness	Overall acceptability
(1)	0.86	0.82	1.08	0.77	0.69
a	1.04	1.02	0.99	0.95	0.96
b	0.88	0.87	1.04	0.82	0.73
ab	1.01	1.05	0.96	0.96	0.96
cp_1	0.98	0.98	0.97	0.91	0.85
cp_2	0.96	0.97	1.00	0.92	0.86

(1) control; a = temperature; b = relative humidity; cp = centrepoint

The models developed for the Nham characteristics on the third day of fermentation are shown in Table 6.9 (developed from Appendix 6.2; Table A 2.6).

Table 6.9 Fitted models for Nham characteristics (Sensory evaluation) fermented at different temperatures for 3 days.

Fitted equations	R^2 (%)	Lack of fit (F-ratio)
Colour = 0.955 + 0.078 (T)	94.03	2.21ns
Visual texture = 0.918 + 0.025 (T)	81.08	3.55ns
Air pockets = 1.035 - 0.025 (T)	90.91	1.33ns
Firmness = 0.952 + 0.095 (T)	91.43	22.22ns
Sourness = 0.888 + 0.080 (T)	88.02	22.89ns
Overall acceptability = 0.842 + 0.125 (T)	97.83	8.89ns

ns = not significant; T = Temperature

It can be seen that colour, visual texture, firmness, sourness and overall acceptability of Nham were significantly increased at the high temperature. The production of air pockets was significantly less at high temperature.

The overall acceptability was regressed with all attributes of Nham. It appears that acceptability can be expressed very precisely in terms of colour, firmness and juiciness:

$$\text{Overall acceptability} = -2.208 + 0.365\,(\text{Colour}) + 1.558\,(\text{Firmness}) + 1.210\,(\text{Juiciness}) \qquad R^2 = 99.96\%$$

Significant correlations between subjective and objective tests were found (Table 6.10). The pH correlated with colour, air pockets, firmness, sourness and overall acceptability ($P<0.01$). In addition, the pH correlated with other objective tests: tristimulus values (x, y, z) and compression force (Table 6.11). The pH also correlated with the overall acceptability ($P<0.01$). Additionally, the overall acceptability also correlated with both firmness and colour of Nham.

Table 6.10 Correlations between subjective and objective tests on Nham fermented for 3 days.

Subjective test	Objective test	Correlation coefficient (r)
Colour	pH	-0.93**
	x-value	0.92**
	y-value	0.89*
	z-value	0.91*
Air pockets	pH	0.99**
	Compression force	-0.91*
Firmness	pH	-0.96**
	Compression force	0.93**
Sourness	pH	-0.92**
	Compression force	0.92**
	x-value	0.92**
	y-value	0.92**
	z-value	0.94**
Overall acceptability	pH	-0.96**
	Compression force	0.98**
	x-value	0.95**
	y-value	0.91*
	z-value	0.94**

* significant at 98% level ($r \geq 0.88$)

** significant at 99% level ($r \geq 0.92$)

Table 6.11 The significant correlations between pH and compression force and tristimulus values of Nham fermented for up to 3 days.

Correlation		Correlation coefficient (r)
24 hours; pH vs	x-value	-0.90*
	y-value	-0.91*
	z-value	-0.89*
pH vs	compression force	-0.83*
48 hours; pH vs	x-value	-0.98**
	y-value	-0.95**
	z-value	-0.96**
pH vs	compression force	-0.98**
72 hours; pH vs	x-value	-0.92**
	y-value	-0.86*
	z-value	-0.90*
pH vs	compression force	-0.93**

$r \geq 0.88$ are significant at $P<0.02$ (*)

$r \geq 0.92$ are significant at $P<0.01$ (**)

6.6 DISCUSSION

6.6.1 Effects of Temperature and Relative Humidity

The metabolic activity of starter cultures present in Nham depended upon the fermentation temperature. The lactobacilli and pediococci employed as starter cultures have optimum growth temperatures of 32°C and 37°C respectively (Bacus, 1984). Their initial performance in lowering pH was dictated by the product temperature. The number of L. plantarum increased approximately 4-5 log cycles during 72 hours fermentation, while P. cerevisiae increased only 0.5 log cycle both at 20°C and 30°C. Deibel et al. (1961 (a); (b)) and Adams (1986) stated that pediococci are found as a minor component of the microflora where a higher fermentation temperature is employed. The higher the

temperature, the higher the rate of the growth of L. plantarum. On the contrary, at the higher temperature, the rate of growth of M. varians was lower.

The fermentation temperature influenced directly the rate of acid development. With constant ripening temperature the carbon sources in Nham were used by lactic acid-producing bacteria, particularly L. plantarum, influencing the rate of pH fall. Thus at the higher fermentation temperature, the pH decreased quickly during the first 24 hours of Nham fermentation leading to a rapid increase in firmness. The higher the fermentation temperature, the greater the rate and extent of firmness development. There was a significant correlation between pH and compression force during the fermentation period. This is not surprising since if the pH value is 5.3 or below, gel formation of the meat protein occurs and this in turn leads to an increase in firmness (Klement et al. 1974; Klettner and Baumgartner, 1980).

Subsequently, the acid development during fermentation inhibited the Enterobacteriaceae and S. aureus at 3 days. Wood (1985) and Adams (1986) stated that increasing the amount of added salt to at least 2.5% and addition of some carbohydrate for acid production in fermented meat product, or small amounts of acidulant, was most effective in the control of Enterobacteriaceae. The higher the temperature during the ripening process, the greater rate of the destruction of Enterobacteriaceae and S. aureus. It was clear that the increased temperature caused rapid pH reduction which destroyed the pathogens. Also, S. aureus shows poor growth in anaerobic conditions and low pH values (Hurst and Collins-Thompson, 1979). A rapid pH drop in the sausages early in fermentation ensures inhibition of S. aureus (Raccach, 1981). It also appears that the suppression of S. aureus by lactic acid bacteria is partially due to the production of peroxide and related compounds (Smith and Palumbo, 1978; Talon et al., 1980).

It is generally accepted that to ensure good colour development and subsequent retention of the colour, the oxygen content within the Nham should be minimal and pH be adequately low. The nitrate used in formulation is reduced to nitrite by micrococci at pH about 5.6 (Klettner and Baumgartner, 1980). In these experiments the higher temperature and lower pH decreased the number of M. varians at 3 days. Therefore, if pH is too low at the beginning, the M. varians was inhibited. The most favourable pH range for the nitrate reduction is 5.9-6.1 (Nurmi, 1966) and the pH of Nham during 18 hours of fermentation was 5.90-5.96 at 30°C. Thus, the M. varians could reduce the nitrate to nitrite during this time. Next, the nitrite reduction to nitric oxide was accelerated with a low pH and then the nitric oxide combined with the meat pigments to give a pink colour development. The higher the fermentation temperature, the greater the rate of colour

development. Thus there is a balance between the desirability of rapid rate of pH reduction at high temperature and the loss of M. varians and hence colour development.

The water loss in Nham depended upon the water vapour gradient between the Nham and the ripening incubator. The weight loss increased quickly in Nham fermented at low relative humidity (43%) and high temperature (30°C) whereas the weight loss was slight in the Nham fermented at high relative humidity both at 20°C and 30°C. Ideally, the relative humidity in the ripening chamber should be 5-10% lower than the RH value (Water activity x 100) within the sausage (Klettner and Baumgartner, 1980). Thus, fermentation is usually carried out at relative humidities around 90-95%. For the Nham produced in these trials the water activity was 0.96-0.97, so the relative humidity of the chamber used should be 96-97%.

Increasing the temperature of fermentation significantly increased many important characteristics of Nham particularly colour, visual texture, firmness and sourness but decreased air pockets. The higher the fermentation temperature, the higher the overall acceptability at a very high score (0.96) with the product fermented at 30°C. This depended upon the firmness, colour development and juiciness of the Nham. The colour, firmness and sourness are the most important attributes in determining a good product. These attributes correlated with the pH of the product which also related to overall acceptability.

6.6.2 Kineticsof Nham Fermentation

From the kineticsof Nham fermentation, the system of Nham at various temperature (20-30°C) can be predicted the final quality of Nham. The relationship between the rate constants of pH reduction and colour development, particularly x-value, and the reciprocal of temperature fitted very well and had a good prediction. However, the rate constants of firmness development did not fit well because R^2 was low as well as y and z values. The future experiment should be studied to get more information between 20°C and 30°C. Two or three points between the range of the temperatures should be concentrated. The fresh meat is usually used in commercial Nham production in Thailand, the rate of pH reduction may be different from this experiment because of the effect of initial pH of Nham. The future study should concentrate at the initial pH of Nham. The pH of frozen meat used for Nham development in New Zealand was higher than the fresh meat using in Thailand. However, the good relationship and prediction of pH reduction in this study can be used to predict the final pH of the product.

Normally the finished Nham is distributed to the Thai market place at ambient temperatures and stored at these temperatures in the market. Therefore, the Nham which was developed at New Zealand will be studied on storage life in order to investigate the Nham quality deterioration in the market place. There may be a possibility to export the product to overseas countries, therefore the lower temperatures (1-10°C) will be also studied (described in detail in Chapter 7).

In Thailand, the fermentation is held at ambient temperature, so the quality of Nham varies from batch to batch. There are fluctuations of temperature and relative humidity in Thailand during a year. A controlled fermentation process was very important to produce consistent quality. An incubator could be used for Nham fermentation in Thailand. For this study, the suitable conditions for Nham fermentation were 30°C and 97% relative humidity. The conditions and suitable formulation developed at New Zealand will be used for production trial in Thailand to investigate the possibility to apply the formulation and process into industrial line in Thailand (described in detail in Chapter 8).

6.7 CONCLUSION

The processing conditions affected the rate of Nham fermentation and particularly pH reduction. The processing conditions appeared to select the type of microorgansims that predominated during fermentation and made the final characteristics of the product. Processing parameters such as time, temperature and relative humidity were used effectively to control the total process and, therefore, prevent the inherent problems of traditionally-produced Nham. The higher fermentation temperature is preferred because of the increasing rate of pH reduction, firmness and colour development. The panelists accepted the Nham fermented at 30°C with very high score (0.96). The relative humidity in the ripening chamber should be high enough to ensure a slow weight loss of Nham to avoid the formation of a dry, hard outer layer could be considered. Additionally, the Nham fermented at high temperature had a low pH and could be considered safe in terms of Enterobacteriaceae and S. aureus.

It was concluded that a satisfactory product can be produced by using a mixed starter culture: 10^3 cfu.g^{-1} both L. plantarum and M. varians; 10^6 cfu.g^{-1} P. cerevisiae with 0.5% glucose and 6% cooked rice. Controlled fermentation conditions are very important and this may involve the use of an incubator rather than the traditional open room. Suitable conditions for a 3-day fermentation are 30°C and 97% relative humidity. The conditions and suitable formulation developed at New Zealand will be used for storage

test study at high temperatures (20-30°C) and low temperatures (1-10°C) in the next experiment (Chapter 7) and also for production trial in Thailand (Chapter 8).

CHAPTER 7
STORAGE TEST ON NHAM

7.1 INTRODUCTION

Nham is sold in Thailand in the markets at ambient temperatures, in supermarkets at chiller temperatures, and it can be exported at low temperatures. The consumer sometimes stores the Nham in the household refrigerator. A study on the storage life of Nham at all these temperatures was necessary to investigate the quality and stability of the product in each of these markets.

The aims of the storage test were to investigate the deterioration of the Nham and to predict storage life for the product at the different temperatures. The Nham formulation developed in New Zealand was used. Chemical, physical, microbiological and sensory evaluation analyses were used to determine the product quality during the storage time, and for optimization of the storage time for the Nham.

7.2 EXPERIMENTAL DESIGN

For the study of product shelf-life, six different temperatures were used for storing the Nham as shown below. 24 kilograms of the Nham were prepared and 4 kilograms of the product was stored at each of the different temperature conditions.

1°C (exporting temperature)
5°C (retail chiller temperature)
10°C (domestic refrigerator temperature)
20°C (mean minimum ambient temperature in Thailand)
25°C (median ambient temperature in Thailand)
30°C (mean maximum ambient temperature in Thailand).

7.3 SAUSAGE PREPARATION, TESTING AND ANALYSIS FOR STORAGE TEST

Nham formulation and process developed in Chapter 6 was used in this study. The sausage mixes were prepared as previously (Section 2.3) and the bacterial cultures were grown again by the same method as the previous experiments (Section 2.2). The Nham was held in an incubator at 30°C and 97% relative humidity for 3 days. After the

ripening process, the Nham sausages were randomly separated into six groups. Each group was stored at one of six temperatures (1, 5, 10, 20, 25 and 30°C). The relative humidity was not controlled. The Nham was stored for 10 weeks and tested at 1, 2, 5, 8 and 10 weeks at 1-10°C and for 1 and 2 weeks at 20-30°C. The Nham stored at 20-30°C was unacceptable after 2 weeks of storage and after 10 weeks at 1-10°C.

Two Nham sausages were randomly sampled from each treatment for chemical, physical, microbiological and sensory evaluation analyses during the storage test.

pH, weight loss, compression force and colour measurement were determined using the methods from Sections 2.4.1 and 2.4.2.

The counts of L. plantarum, P. cerevisiae, and M. varians, Enterobacteriaceae, S. aureus, mesophilic aerobic counts and yeasts and moulds were determined using the methods from Section 2.4.3.

A trained panel of 8 Thai postgraduate students at Massey University was used for sensory evaluation (Section 2.4.4). The Nham was evaluated for appearance, texture, flavour and taste and overall acceptability using the questionnaire shown in Appendix 7.1. The off-flavour attribute was put on the questionnaire. The ideal profile of Nham particularly off-flavour attribute was discussed by the panelists. The panelists agreed on the profile. The fixed ideal points of off-flavours was also put on the scale with colour, firmness and sourness. The definitions of colour, firmness, sourness and overall acceptability used were as the same previous experiments but the off-flavour definition was discussed by the panelists and they agreed on this attribute and definition. The mean ideal ratio scores were calculated at each testing time. The relationship between the overall acceptability with other attributes was determined using multiple regression method as previously.

7.4 DETERMINATION OF THE RATE OF DETERIORATION OF NHAM DURING STORAGE

A recently developed interest in quantitative approaches to analysis of food quality deterioration during storage has been motivated in part by a growing consumer awareness and by mandatory governmental requirements (Saguy and Karel, 1980). Improvements have been made possible by the increase in knowledge of kinetics of food deterioration, and many workers have assumed that food deterioration is a first-order reaction in which log (food quality) is linearly related to storage time (Joslyn and Miller, 1949; Freed et al.,

1949; Lamden and Harris, 1950; Huelin, 1953; Waletzko and Labuza, 1976; Passy and Mannheim, 1979; Saguy et al., 1978(a), (b); Hill and Grieger-Block, 1980; Lenz and Lund, 1980; Labuza and Schmidl, 1985; Wells and Singh, 1988; Rhim et al., 1989).

To investigate the rate of Nham deterioration, the Arrhenius equation for a first-order reaction was applied to calculate the rate of pH reduction, weight loss, firmness and colour deterioration. This method was also used for prediction of the shelf-life of Nham using two selected attributes from the sensory panel: off-flavour and overall acceptability. The reaction constants (k-values) were determined using the same method as Section 6.4.

7.5 RESULTS

7.5.1 Evaluation of Fermented Nham Before Storage

Nham fermented at 30°C and 97% relative humidity for 3 days was evaluated before storage at the different temperatures. The results for chemical, physical, microbiological and sensory evaluation were:

pH	= 4.32 ± 0.01*
Weight loss (%)	= 0.26 ± 0.03
Compression force (Newtons)	= 134.8 ± 1.3
Tristimulus values; x-value	= 25.55 ± 0.05
y-value	= 22.85 ± 0.05
z-value	= 22.60 ± 0.01
L. plantarum (log N)	= 8.31 ± 0.04
P. cerevisiae (log N)	= 7.14 ± 0.07
M. varians (log N)	= 4.75 ± 0.03
S. aureus (log N)	= 3.23 ± 0.05
Enterobacteriaceae (log N)	= 2.30 ± 0.02
Aerobic mesophiles counts (log N)	= 8.38 ± 0.03
Colour mean ideal ratio score	= 1.01 ± 0.01
Firmness mean ideal ratio score	= 1.03 ± 0.01
Sourness mean ideal ratio score	= 0.97 ± 0.01
Off-flavour mean ideal ratio score	= 1.00 ± 0.00
Overall acceptability mean ideal ratio score	= 0.95 ± 0.01

* mean ± standard error

7.5.2 pH Change During Storage

The temperature of storage affected the rate and extent of pH change of Nham during storage. There was a significant change in pH during storage and there was also a significant difference in responses at the various temperatures. The pH of the Nham during 2 weeks of storage at 20, 25 and 30°C were significantly different from the Nham stored at 1, 5 and 10°C. During the first week, the pH of Nham stored at 30°C fell from 4.32 to 3.98, while the pH of Nham stored at 20-25°C showed a slight decrease of pH, i.e. 0.15 unit decrease, and that stored at 1°C remained at practically constant pH up to 10 weeks storage (Figure 7.1 and Appendix 7.2; Table A 2.1).

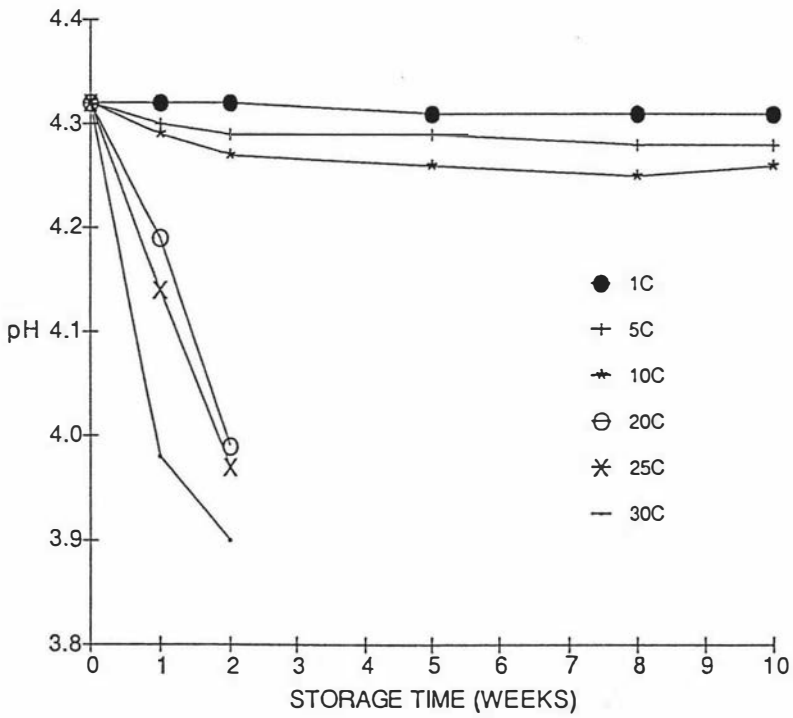


Figure 7.1 pH change during storage at different temperatures (1-30°C).

7.5.3 Weight Loss During Storage

Nham stored at 25°C and 30°C had higher weight loss values than the samples at 1, 5, 10 and 20°C (Figure 7.2 and Appendix 7.2; Table A 2.2). Nham kept at 30°C had the greatest weight loss with 4.49% at 2 weeks of storage. However, the Nham stored at 1-20°C had less than 1% weight loss during storage.

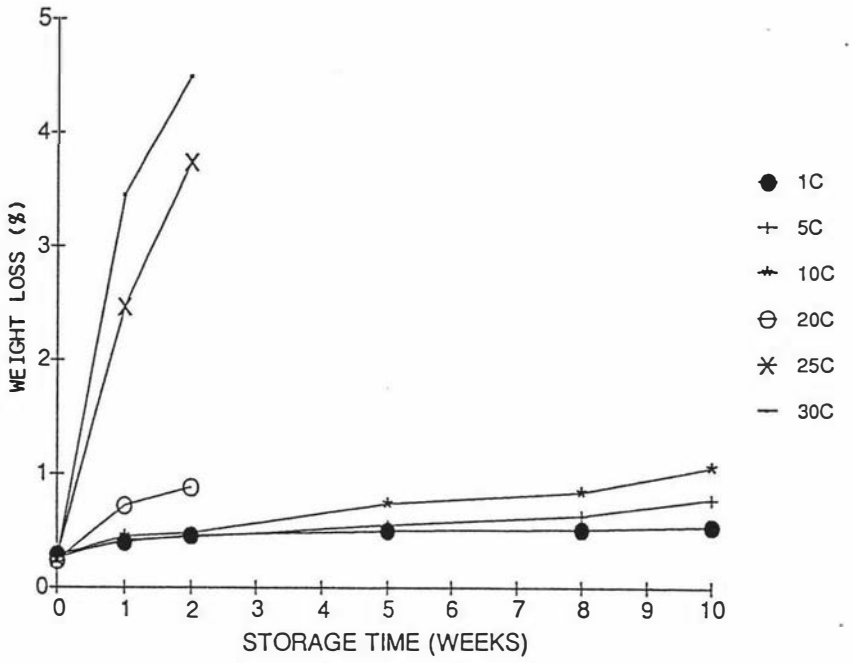


Figure 7.2 Weight loss during storage at different temperatures (1-30°C).

The Nham stored at low temperatures (1-10°C) continued to lose weight slowly during the next 8 weeks of storage with the least weight loss occurring at the lowest storage temperature.

7.5.4 Firmness Change During Storage

Textural quality was evaluated on the basis of physical measurement of compression force. As the temperature was increased, the measured compression force decreased. Samples stored above 20°C had lower compression forces than those stored at chiller

temperatures (Figure 7.3; and Appendix 7.2; Table A2.3). However, the compression forces increased during 2 weeks storage at chiller temperatures particularly at 1 and 5°C and then decreased markedly after 2 weeks storage. The results suggest that firmness retention is greatest at 1°C.

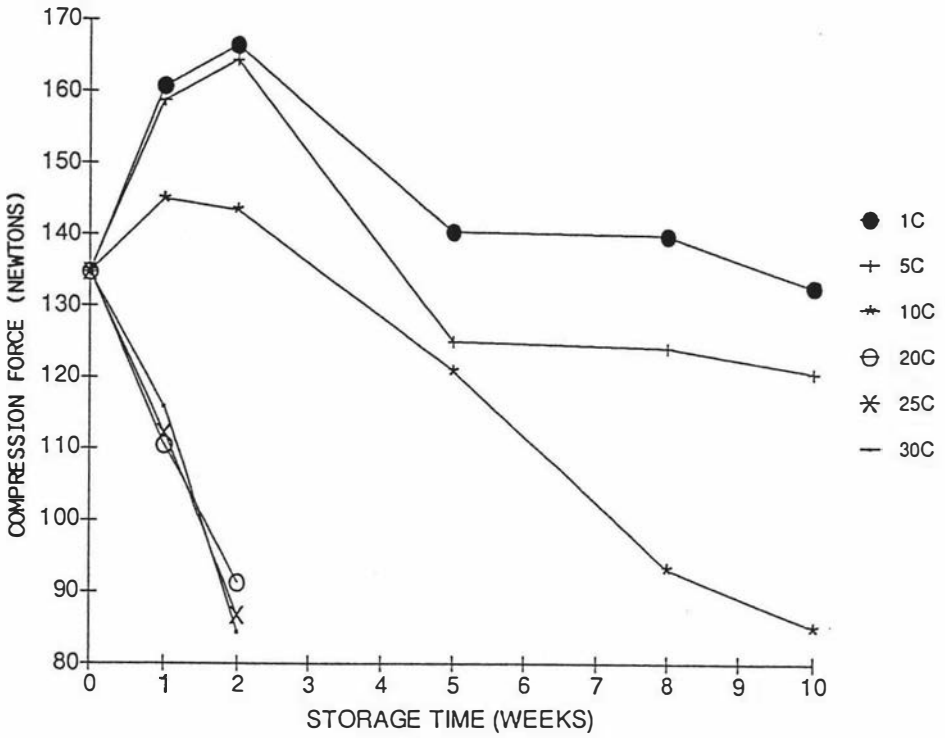


Figure 7.3 Compression force during storage at different temperatures (1-30°C).

7.5.5 Colour Change During Storage

The rate and extent of colour change was dependent on the storage time; with increase in storage time decreasing the colour of Nham. The Nham samples stored at high temperatures (20-30°C) had a greater decrease in the tristimulus values than the others at 1-10°C during 2 weeks of storage (Figure 7.4 and Appendix 7.2; Table A 2.4). However, the Nham samples stored at 1-10°C during 8 weeks storage maintained the tristimulus values particularly at 1°C and then the values decreased quickly after 8 weeks storage. The results suggest that colour retention is greatest at 1°C.

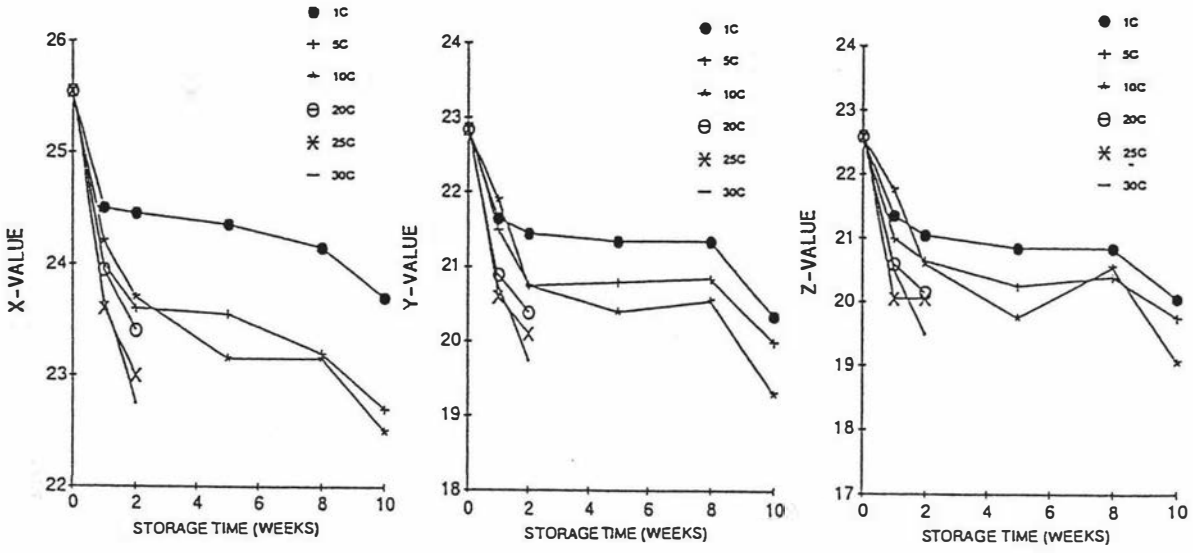


Figure 7.4 Tristimulus values (X Y Z) during storage at different temperatures (1-30°C).

7.5.6 Bacterial Studies During Storage

Aerobic mesophiles counts showed little change during 2 weeks of storage and then decreased approximately 0.5 log cycle at 10 weeks (Figure 7.5 and Appendix 7.2; Table A 2.5).

The numbers of L. plantarum followed a similar pattern (Figure 7.5; Appendix 7.2; Table A 2.6). There were no significant differences between the effect of different storage temperatures on the number of L. plantarum during the first 2 weeks of storage, but survival was greater at low storage temperatures (1-10°C) over the 10 weeks of storage. The Nham kept at 5°C had the lowest decrease in the number of L. plantarum during storage approximately 0.3 log cycle.

The numbers of P. cerevisiae decreased quickly during the first 2 weeks at 20-30°C (Figure 7.5 and Appendix 7.2; Table A2.7). Survival was greatest at 1°C; during 10 weeks storage, the numbers of P.cerevisiae decreased by 0.3 log cycle at 1°C and 1 log cycle at 5 - 10°C.

The numbers of M. varians showed the greatest decreases during storage (Figure 7.5 and Appendix 7.2; Table A 2.8). This was particularly apparent at 20-30°C, where the loss was approximately 2.5 log cycles over the first 2 weeks.

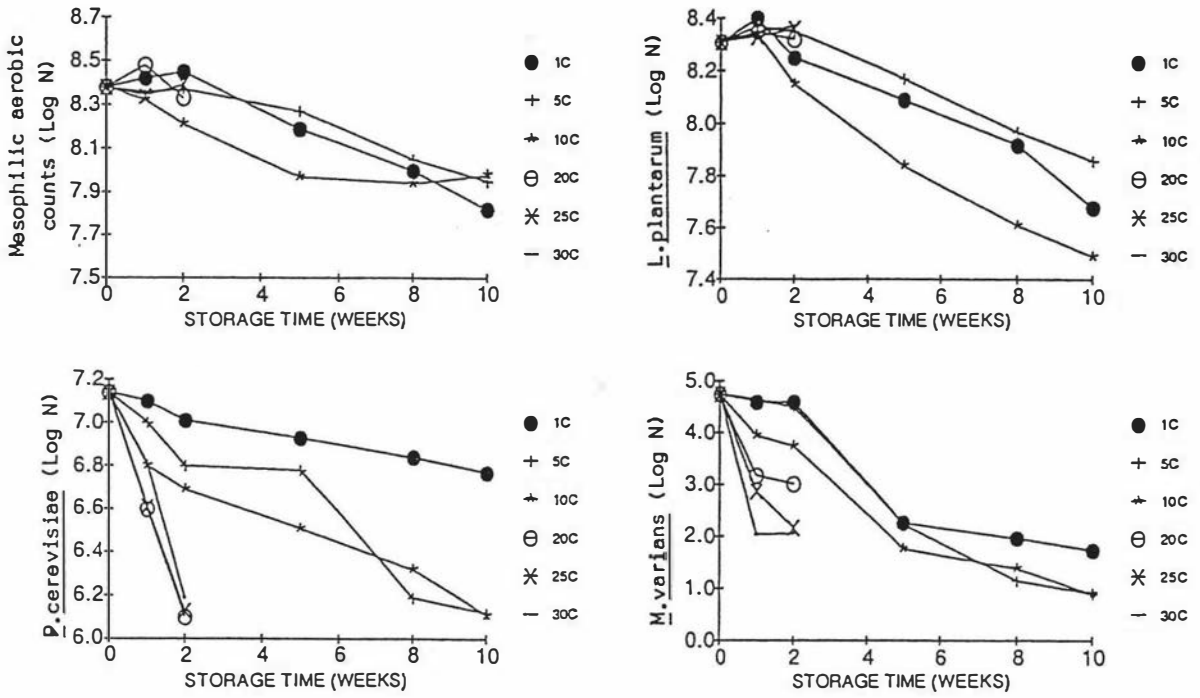


Figure 7.5 Starter cultures (L.plantarum, P.cerevisiae and M.varians) and mesophilic aerobic counts during storage at different temperatures (1-30°C).

Some potentially pathogenic bacteria including Enterobacteriaceae and S. aureus were also investigated during Nham storage (Figure 7.6; Table A2.9, A2.10). It was found that in Nham stored at 20-30°C the Enterobacteriaceae were undetectable at 2 weeks. Survival was greater at lower temperatures particularly at 1 and 5°C, the Enterobacteriaceae were constant at 5°C during 5 weeks storage while at 1°C the numbers decreased approximately 0.7 log cycle during 8 weeks storage, but Enterobacteriaceae were undetectable in all samples at 10 weeks.

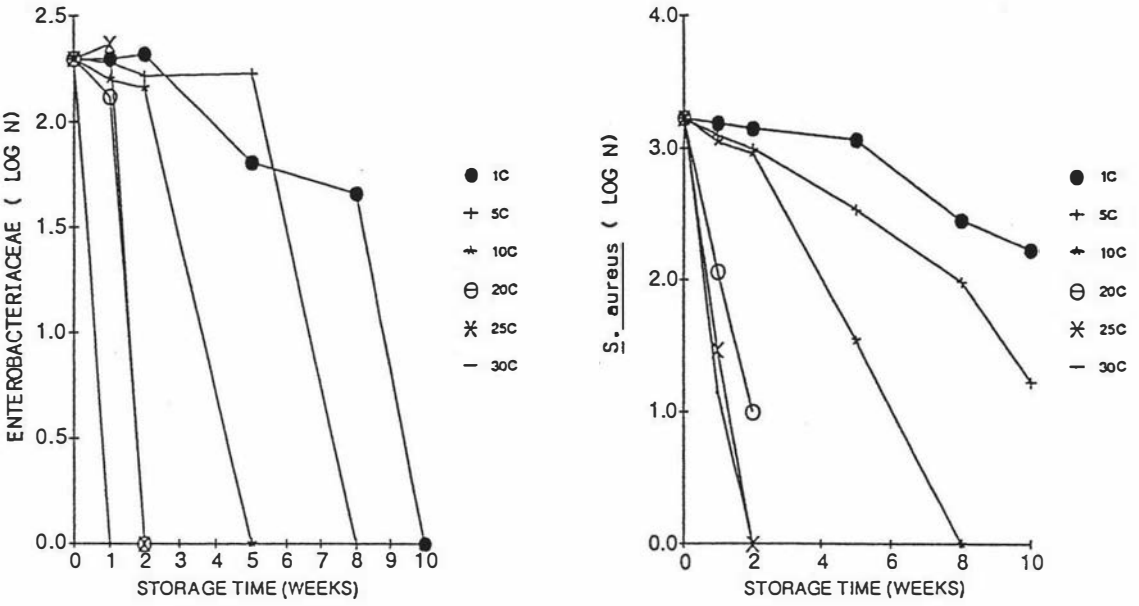


Figure 7.6 Pathogenic bacteria (Enterobacteriaceae and S.aureus) during storage at different temperatures (1-30°C).

Similar patterns in decrease of S. aureus were also found. The higher the temperature of storage, the greater the decrease in the numbers (Figure 7.6 and Appendix 7.2; Table A 2.10). S. aureus was undetectable at 10 weeks in all samples except those stored at 1-5°C, where the decreases were 1 and 2 log cycles respectively.

In this study no moulds or yeasts were detected during storage at any temperatures (Appendix 7.2; Table A 2.11).

7.5.7 Sensory Evaluation During Storage

Sensory ideal ratio scores of Nham products stored for different periods of time are shown graphically in Figures 7.7 (a) and (b). These figures show that the product sensory attributes deteriorated with increasing temperature of storage.

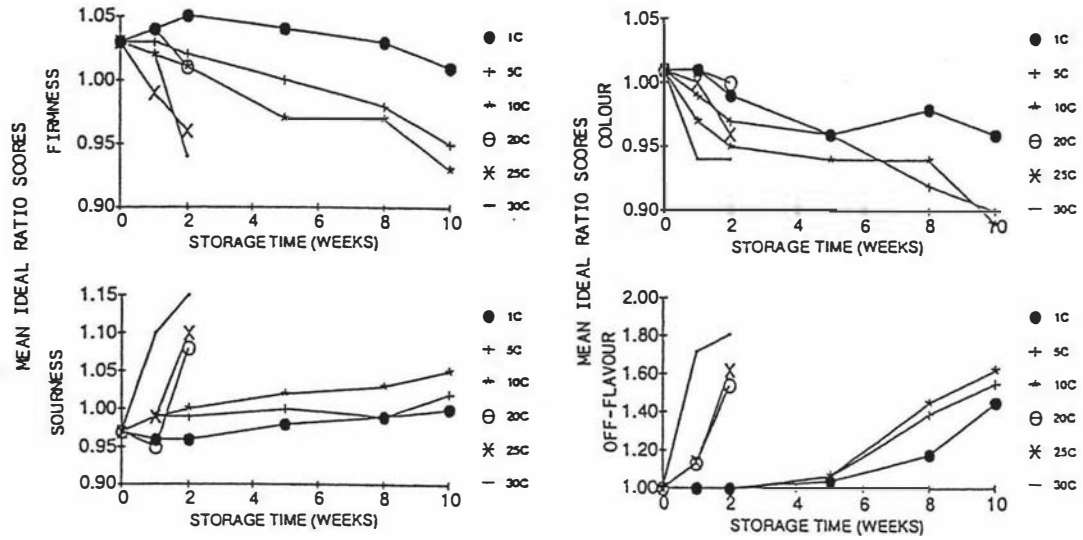


Figure 7.7(a) Sensory attributes (firmness, colour, sourness and off-flavour) during storage at different temperatures (1-30°C).

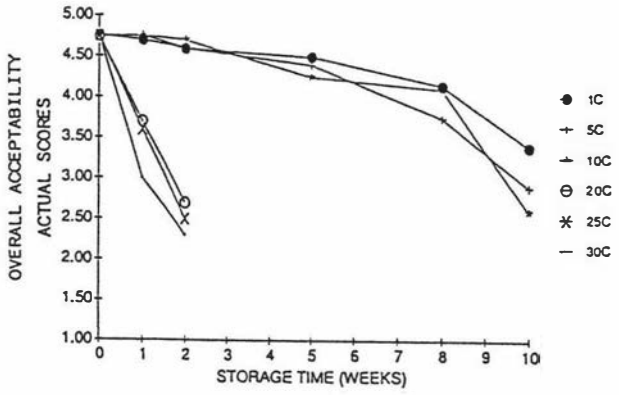


Figure 7.7(b) Overall acceptability during storage at different temperatures (1-30°C).

There was an increase in firmness during 2 weeks storage at 20-30°C. The Nham storage at 1°C had a higher firmness score than the Nham stored at 5°C and 10°C during storage(Figure 7.7 (a) and Appendix 7.2; Table A 2.12). The firmness of Nham at 1°C increased during 2 weeks storage and then decreased slowly during 10 weeks storage, while the Nham stored at 5°C and 10°C decreased continuously during storage.

The perceived colour of Nham decreased slightly during the first 2 weeks of storage, though no significant difference was found between the effects of different temperatures of storage (1-30°C) during this period. However, there was a decrease in colour at 1-10°C during 10 weeks of storage. Generally the lower temperature allowed greater colour retention (cf. tristimulus values).

Similarly, deterioration of other attributes was more rapid at high storage temperatures. The sourness and off-flavour of Nham increased quickly at high temperatures of storage during the first 2 weeks of storage (Figure 7.7 (a) and Appendix 7.2; Table A 2.14 and A

2.15). With increasing time of storage, the higher scores were the sourness and the off-flavour scores particularly the Nham stored at 30°C had a very high sourness score (= 1.15) and off-flavour score (= 1.81) at 2 weeks of storage. Although sourness and off-flavour built up in all samples, their development was slowest at 1°C.

With regard to overall acceptability of Nham, there was a decrease in this score at 20-30°C during 2 weeks of storage. The Nham stored at 1-10°C had a higher acceptability score than the Nham stored at 20-30°C. The Nham at the high temperatures of storage at 2 weeks reached to less than 3.00 on a scale of 5 (Figure 7.7 (b) and Appendix 7.2; Table A 2.16). The Nham stored at low temperatures (1-10°C) was continuously observed during 10 weeks in terms of overall acceptability. With increasing storage time, the overall acceptability score decreased slowly during 8 weeks storage and then the scores dropped quickly after 8 weeks. The overall acceptability scores were less than 3.00 at 5°C and 10°C, while at 1°C the score dropped to 3.50 at 10 weeks storage.

Relationships between overall acceptability and all attributes of Nham - firmness, colour, sourness and off-flavour were determined in this study in an effort to discover the determinants of acceptability. It was found that there was a significant effect of off-flavour on the overall acceptability, both at low and high temperatures. The relationship equation is shown as:

Overall acceptability = 1.507 - 0.580 * off-flavour
(ideal ratio score) R^2 = 88.83%

7.6 THE RATE OF DETERIORATION OF NHAM QUALITY DURING STORAGE

The kinetic approach for predicting quality factor losses in Nham was investigated. The rate of pH change, weight loss, firmness and colour deterioration are presented in Appendix 7.3; Table A 3.1- A3.4 The rate constants were plotted against the reciprocal of the temperatures in °K (1/T) (Figure 7.8). The trend of the actual values was calculated using linear regression.

As can be seen in Figure 7.8, there appeared to be two different relationships between the rate of pH changes, weight loss, colour deterioration and the absolute temperatures. Firmness showed a single continuous trend over the whole temperature range. The plots can be divided into 2 areas - the high temperatures (20-30°C) and the low temperatures (1-10°C).

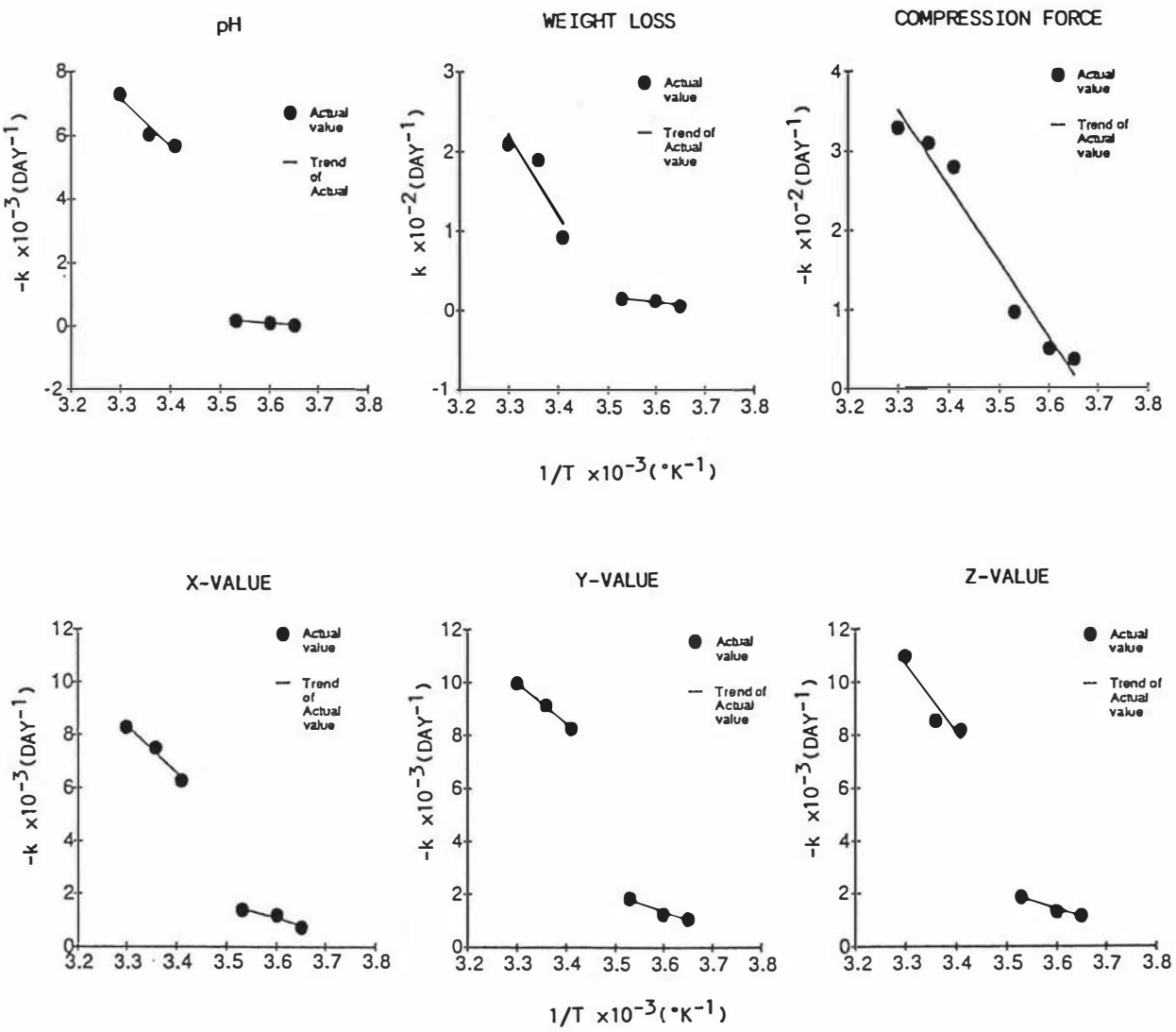


Figure 7.8 The rate of pH change; weight loss; firmness; and colour deterioration during storage at different temperatures (1-30°C).

7.7 ESTIMATION OF SHELF LIFE OF NHAM

The main aim behind the storage test of the Nham developed in this study was to produce a model to estimate the product shelf-life and finally to determine any adjustment of formulation or processing needed to the product to improve the storage life.

Using the Arrhenius relationship, rate constant plots were prepared using selected attribute scores from sensory panels which related to overall acceptability i.e. off-flavour. It was assumed that it was a first order relationship, so the equation used was the same equation as in Section 6.6. The 'k' values were also plotted against the reciprocal of the temperature in ^{o}K (1/T) and the linear relationships determined by linear regression using the MUTAB computer package (Boag, 1988).

From the relationship between the overall acceptability and off-flavour, the "reject points" for the product were estimated as off-flavour actual score = 1.56 and overall acceptability actual score = 3.00. As some of the Nham samples were not off-flavoured or unacceptable at the end of the 10 weeks storage test, the results were extrapolated to predict the time when the reject point was reached. The calculation was made in order to obtain the time necessary to reach the "reject point" values. Therefore, at the reject time 't'

for acceptability: C/Co = 3.00/4.75 = 0.63

for off-flavour: C/Co = 1.56/1.00 = 1.56

'k' value was calculated as in Chapter 6. There were also two different relationships for each attribute. Thus, the calculations were divided into four sets of the 'k' values (Figure 7.9 and Appendix 7.3; Table A 3.5-A 3.6).

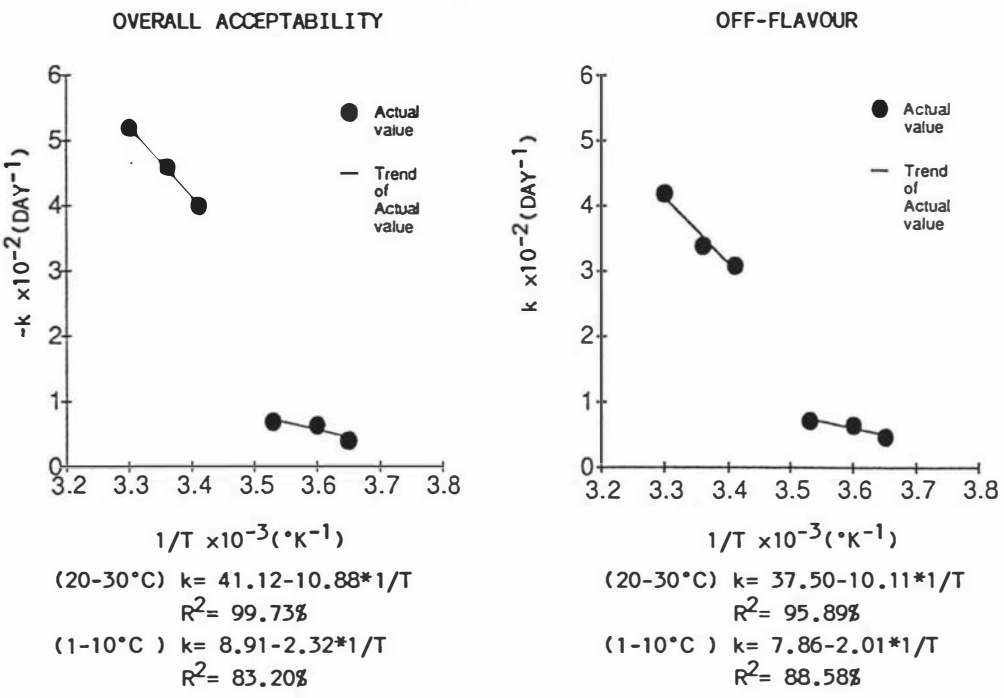


Figure 7.9 The rate of off-flavour; and overall acceptability changes during storage at 1-30°C.

The main finding from Figure 7.9 is that rate of deterioration, as measured by overall acceptability or off-flavour is dependent on temperature. As storage temperature rises, the rate of deterioration also rises.

The shelf-life of Nham at different temperatures (1-30°C) were calculated using the linear plots for off-flavour and acceptability (Appendix 7.4; Table A 4.1) and tabulated for comparison in Table 7.1.

Table 7.1 Prediction the shelf-life of Nham (days) based on overall acceptability and off-flavour.

Temperatures (°C)	Shelf-life based on	
	acceptability	off-flavour
1	103	87
5	82	73
10	63	59
20	11	15
25	10	13
30	9	11

The predicted shelf-life agreed reasonably with the actual time assessed by the sensory panel, except in the 5°C sample. The shelf-life of Nham stored at high temperature was 9-11 days where as at low temperature was 63-103 days based on overall acceptability. Although the off-flavour of Nham was detected at low temperatures, the Nham was still accepted by the panelists in terms of overall acceptability.

7.8 DISCUSSION

7.8.1 The Quality of Nham During Storage

The changes of Nham quality during storage at different temperatures (1-30°C) were investigated. There were two relationships between the Nham quality changes during storage and temperatures except the firmness deterioration. The Nham stored at high temperatures (20-30°C) had a quicker deterioration rate than the Nham stored at low temperatures (1-10°C). It may be possible to combine the two relationships together if a temperature of 15°C is used for the experiment. Thus in future work a more continuous range of temperatures between 10 and 20°C should be studied. The two relationships which were found might be because of the effects of different types of organisms at different temperatures, that is the mesophilic bacteria grow at high temperatures while psychrophilic bacteria grow at low temperatures. Similar patterns for pH change, colour

and firmness degradation were obtained. However, when storage time was increased there was significant weight loss. The pH was constant at low storage temperatures. This was because the activities of starter cultures were retarded under this condition, whereas the high temperature stimulated the activities of the cultures and resulting in a greater pH reduction. The starter cultures used died off at low storage temperatures, particularly in the case of M. varians which decreased approximately 2.5-3.0 log cycles during 10 weeks of storage. In contrast, the number of L. plantarum increased during the first 2 weeks of storage at high temperature, probably causing the pH drop during this time.

With the increasing storage time, the colour and firmness of Nham decreased significantly. Pezacki and Urbaniak (1965), Reuter et al. (1968), Dierick et al. (1974) reported that the rate of peptides destruction in fermented sausage increased by the proteolytic activity of bacteria in the product. Additionally, many reports (Lamanna et al., 1973; Nurmi, 1966; Cerise et al., 1973; Bacus, 1984) have indicated that there was oxidation occurring in the sausage during ripening process. These might decrease the firmness and colour of the product.

The Nham developed was very safe in terms of Enterobacteriaceae and S. aureus. The numbers of these pathogens decreased during the storage, and no moulds or yeasts were detected. Bacus (1984) stated that the shelf-life of fermented meat products is generally not limited by bacterial deterioration, but by chemical and physical spoilage.

In terms of sensory evaluation, the firmness and colour decrease were similar to the objective measurement - compression force and tristimulus values respectively. In addition, the sourness increase was the same pattern as the pH reduction during storage. This indicated that most of the measurement methods used were reasonably suitable for this study.

7.8.2 Prediction of Nham Shelf-Life

Based on the prediction of Nham shelf-life, the Nham using mixed starter cultures had a longer shelf-life than the native Nham, which is usually kept at ambient temperature in Thailand for 3 days storage life (consumer survey result). The predicted time from this study was in reasonable agreement with the actual time from the sensory panel, except for Nham stored at 5°C. The Nham stored at 1-10°C would last for 103-63 days respectively whereas the Nham stored at 20-30°C would last 11-9 days respectively.

This showed that there was a possibility to export the product to overseas countries. The shelf-life was limited by off-flavour (staleness, rancidity, putrid and ammonia flavour) during storage. Many researchers (Stoychev et al., 1972 (a), (b); Coretti, 1965; Mihalyi and Kormendy, 1967; Townsend, et al., 1975; Dobbertin et al., 1975; Acton, 1977) have reported that there were increases in hydrolytic rancidity, oxidative rancidity and ammonia flavour in fermented sausage during storage. In this study, there was a strong relationship between off-flavour and acceptability during the storage of Nham at 1-30°C. However, the panelists accepted the product stored at low temperatures even though off-flavour was detected at the same level as in the product stored at high temperatures. This might be because the other attributes were maintained while the off-flavour occurred. the panelists then perceived the product to be acceptable overall.

7.9 CONCLUSION

In conclusion, the results of this study showed that the prediction of the Nham shelf-life could be achieved reasonably from the changes in Nham attributes which could be obtained from a planned storage test. The shelf-life of Nham was limited by chemical spoilage but not by microbial spoilage. It appears that different rates of degradation were obtained at the low (1-10°C) and high (20-30°C) temperatures. The rate of deterioration of Nham stored at 20-30°C was quicker than the rate of 1-10°C. The Nham stored at low temperatures (1-10°C) remained at practically constant pH during 10 weeks storage. the firmness and colour retention are greatest at 1°C. From the sensory evaluation the off-flavour development controlled the shelf-life of Nham. The Nham was also kept at ambient temperatures for 9-11 days. This showed that it was very successful to produce the Nham by use of mixed starter cultures and keep the Nham quality during storage. There was also a possibility to export the product to overseas countries under the low temperature of 1°C.

The Nham formulation and process developed in New Zealand showed to have a potential to produce the Nham in commercial scale. Thus, the same formulation and process will be used in Thailand for production trial in order to investigate the possibility of semi-commercial production in Thai meat processing plant (Chapter 8).

CHAPTER 8
TESTING OF THE DEVELOPED NHAM IN THAILAND

8.1 INTRODUCTION

The purpose of this study was to see if the product formulation and process developed in New Zealand could be transferred into industrial production in Thailand and if the product was acceptable in the Thai market.

To ensure a positive attitude towards the Nham, an understanding of the current behaviour of the target population was obtained by using consumer discussion panels in Thailand. The product was tested by the Thai consumer panel and improvements suggested. The final product was produced on a semi-commercial scale in a Thai meat processing plant and tested by consumers in some Thai villages and Chiang Mai city. Final consumer testing was an 'in-home use' test among several hundred respondents. A hedonic scaling method was used to determine the degree of liking/disliking towards the developed Nham. Other information related to the product was given by representatives of the households.

Floating ideal was used with trained panel at Chiang Mai University and consumer panel in Thailand. Comparisons were made of the ideal scores for Nham between the panelists in New Zealand and the panelists in Thailand. The acceptability of the Nham by the Thai consumer panel was also linked to the consumer testing in the target market in Thailand.

8.2 EXPERIMENTAL PLANNING

The plan for this study was:

* to develop on a small scale the formulation and process for Nham so that it could fit into an industrial line in Thailand. Nham was made in 5 kilogram lots using the formulation and process which had been developed in New Zealand (see Section 8.3) but replacing the frozen lean pork with fresh lean pork.
* to ensure this product formulation and process was acceptable, a consumer panel tested the product produced.
* to make the Nham acceptable to the consumers on a large production scale (115 kilograms).
* to consumer test the Nham from the production trial.

8.3 LABORATORY DEVELOPMENT

Most of the raw materials and ingredients, bacterial cultures, formulation and process were the same as in New Zealand. Only garlic, white pepper and rice were purchased from a fresh market in Chiang Mai. The fresh lean pork was used instead of the frozen lean pork used in New Zealand.

8.4 PRODUCTION TRIAL

The raw materials, ingredients, bacterial cultures, formulation and process were the same as laboratory development except the fermentation time which was reduced to 2 days.

The production trial was divided into ten batches, each approximately 11.5 kilograms of meat and pork skin, due to the capacity of the mixer.

The meat was trimmed to remove, as much as possible, visible fat and/or connective tissue. The trimmed meat was coarsely ground once through a 5-mm plate. Pork skin was cleaned with water, the hairs and fat removed and the skin boiled in water for 5 minutes. On removal from the water, the skin was dried with a clean cloth and the fat completely stripped and then the skin was cut by cutting machine into shreds approximately 0.1 cm x 2-4 cm.

Sausage mixture were prepared by blending in a Kenwood Mixer equipped with a K-beater. The curing agents and seasonings were blended into the meat for 2 minutes at 40 rpm before adding starter cultures. The cultures were then added into the Nham mass to give the same levels as those used in the laboratory development. The mixture was then mixed for an additional 1 minute at the same speed. The final temperature of the mixture after mixing was 26°C. The mixture was then stuffed in cylindrical plastic bags. After production, the Nham was held in an incubator at 30°C, 97% RH for 2 days.

After 2 days fermentation, 5 Nham sausages were packed into a polyethylene bag giving a total of 112 bags. The bags were immediately placed in a chill room (4°C) until distribution the following day for the in-home use testing. Approximately 1 kg from 115 kg of sample was retained for pH determination.

8.5 CHEMICAL, PHYSICAL AND MICROBIOLOGICAL TESTING

In the laboratory development, two Nham sausages were randomly sampled for the chemical and physical analyses on each day of fermentation. Microbiological studies were made only on the third day of fermentation. pH and weight loss were measured at 0, 1, 2 and 3 days using the methods from Sections 2.4.1 and 2.4.2. Microbiological analysis used the methods from Section 2.4.3. The L. plantarum, P. cerevisiae and M. varians counts, also Enterobacteriaceae, S. aureus and aerobic mesophiles counts were studied.

For the consumer panel and consumer test only pH was determined on the finished product.

8.6 SENSORY TESTING

For the sensory testing in Thailand, a trained taste panel was used in laboratory development. The vital role which sensory evaluation plays in product development has long been recognised (Dixon, 1970; Ellis, 1970; Civille, 1978; Erhardt, 1978; Radtke and Rodriguez, 1981). The other approach for sensory testing of Nham was based on consumer panels who have no special training or knowledge of the product being tested. Earle (1981) stated that various types of people have been used in consumer panels for food product testing. These people can be representative consumers in the target market or they can be randomly sampled. For laboratory development, the floating ideal was used as Lai Pai Wan (1987) advised strongly that once products were close to the ideal they be tested by target market consumers. Consumer at this point should be free to indicate their ideal. The logarithms of ideal ratio scores were suggested to use for floating ideal (Cooper et al., 1989). Use of raw ideal ratio scores can give rise to mathematical difficulty. For example, an absolute score of 4 and an ideal score of 5 leads to an ideal ratio score of 0.8. If the absolute and ideal scores are reversed, the ideal ratio score becomes 1.25. Thus a difference in score of 1 gives rise to percentage changes of -20% or +25%. The ratio scores can be symmetrised by taking logarithms, which also reduced the skewness of the data considerably. That was an absolute score of 4 and ideal score of 5 transforms to a logarithm of ideal ratio score of -0.097. If the absolute and ideal score are reversed the logarithms of ideal ratio score becomes +0.097. Hence a difference in logarithm scores of $\pm$ 1 gives a symmetrical change of $\pm$9.69%. Additionally, further analysis was carried out with greater confidence.

For consumer test, the consumers in Chiang Mai province were selected randomly as a target consumer. They were untrained evaluators who base on their judgment. Consequently, their impressions and judgment might be entirely different from those of trained panels (Klemmer, 1968; Cross et al., 1978; Gatchalian, 1981).

Therefore, for a panel with lower capabilities for sensory judgment, category scaling should be used (Sinthavalai, 1986). For this study, a 5 point just right category scale was used. A 3 point scale results in a loss of discrimination and therefore the 5 point scale was used. Category scales can be transformed into a "ratio mean" using "just right" scores as the ideal. It was "just right ratio". Gatchalian (1981) outlined the in-home use tests which are conducted under uncontrolled conditions. The products are evaluated under natural use or home conditions (Eastlack, 1964; Sinthavalai, 1986; Lai Pai Wan, 1987; Chinprahast, 1988). In-home use testing may require from one week to one month for completion (Girardot, 1952). But in the case of Nham, the products must be tested by the consumer during the week after they receive the products from the experimentators, because the qualities of the product change rapidly with storage time.

8.6.1 Trained Sensory Panel

For sensory testing, a trained taste panel was used. 10 Thai undergraduate students at Chiang Mai University were invited to be the panelists. They were trained using two brands of commercial Nham (Nham brand A and brand B). The commercial samples were held at ambient temperatre after production for 4 days.

Firstly, the panelists discussed the Nham characteristics using the definitions developed in New Zealand but translated into Thai (Section 2.4.4.7; Appendix 2.8). The same definitions were used so that the correlation of the ideals of the Massey University panel and of the Chiang Mai University panel could be determined. The panelists all understood and agreed on the Nham definitions.

Secondly, the panelists tasted the two commercial brands of Nham and evaluated the Nham characteristics by scoring on the profile attributes. The scoring form was also translated into Thai (Appendix 2.8). They were asked to give the sample scores and also the ideal score for each attribute. The mean scores for the ideal product were determined and discussed among the panelists so that they all understood and agreed on the ideal profile.

Finally, the Nham produced in the small batch using the formulation and process developed in New Zealand (Section 8.3.1) was tasted by the 10 Thai trained panelists using the same profile form and definitions as in the training section. The Nham was evaluated using floating ideals in this stage. The logarithms of the ideal ratio scores were calculated.

8.6.2 Consumer Panel

The formulation of Nham was the same as tested by the trained panel except that the fermentation time was reduced to 2 days. Only pH determination and consumer panel testing were used at this stage in order to ensure this product formulation and process were safe and the product acceptable. 20 Thai people from the King's Project at Chiang Mai University were invited to be on consumer panels. They were considered to be representative of the target consumers. They tasted and evaluated the Nham using the same form which the trained panels used as well as the definitions. It was necessary to explain the definitions and the form before evaluating so that all the consumers understood the form and definitions. The floating ideal was used. The logarithms of ideal ratio scores were calculated.

8.6.3 Consumer Test

Consumer testing of the developed Nham was done in an in-home use test in the Chiang Mai area. This method was chosen because:

* the developed Nham needed to be tested under natural home use condition.
* there was only one product sample to be tested. Thus, the time and budget required to complete the test was not the major concern.
* it gave more information related to the product being tested.

8.6.3.1 The Sample

Only one sample was given to each household. The testing of one sample was suitable for a very large number of respondents as less time and a small budget was required for organising it. It was also suitable for general consumers who were not experienced in judging sensory attributes of food products. These consumers would feel at ease when required to assess several sensory attributes of only one product.

The packages were not coded or labelled. There were 5 Nham sausages in each clear polyethylene bag, so it was expected that there would be sufficient for 4-5 members in each household to test.

8.6.3.2 The Respondents

One hundred and twelve families with 503 people in Chiang Mai province were selected for the consumer test. People from two villages - Ban Don Chai, Yangkram district (village 1) and Ban Ma-Kran, Shongkual district (village 2) were selected to test the product as well as the people in Chiang Mai city. Random selection of the respondents was done as gaining access to households in the Chiang Mai area was very easy. Only respondents who normally ate Nham were selected. They were interviewed by six coordinators and were asked if they would be willing to take part in the test.

The samples were given to one hundred and twelve families including 57 families in Chiang Mai city, 29 families in village 1 and 26 families in village 2.

There were two groups of respondents who completed the questionnaires. One group of respondents was asked to assess the product's characteristics. The age for these respondents was specified as "not under 15 years" since it was expected that young children might not follow instructions, might be reluctant in testing the product and also have difficulty in filling in the questionnaires and thus there might be a possibility of obtaining unreliable results. The other group of respondents were housewives. The housewife in each household was asked to answer the supplementary questionnaire.

8.6.3.3 The Questionnaires

Five copies of the product testing questionnaire were given to people in the villages and four copies for people in Chiang Mai city. This was because the family size in the city was smaller than in villages. One copy of the housewives' questionnaire and the sample of Nham were also given to each household.

The product testing questionnaire was for evaluating the sensory characteristics of the developed Nham, namely colour, firmness, sourness, saltiness, spiciness and overall acceptability. A 5 point just right category scale was used. In the questionnaire, an explanation of the sensory terms was provided so that all respondents knew the sensory

characteristics they were assessing and they perceived all these attributes in the same manner. Sex, age group and career were also asked to attain some background for each respondent (see Appendix 8.1). The colour of this questionnaire was white.

A second type of questionnaire was also provided, but only for the housewife, in each household. Information related to buying and keeping of the developed Nham was sought. Details of this questionnaire are given in Appendix 8.2. The colour of this supplementary questionnaire was blue. All questionnaires were in Thai but the English translations are shown in Appendix 8.3.

8.6.3.4 Organisation of the Test

One hundred and twelve bags of the Nham were firstly given to the six coordinators. These six people then distributed the Nham samples from Chiang Mai University to the randomly selected target families at ambient temperatures. No attempt was made to keep the Nham chilled. Every family received, together with a bag of five Nham samples, a set of questionnaires. When testing of the product was finished after one week, the six coordinators collected the questionnaires and returned them to the author. This method of distributing and collecting the questionnaires was expected to give a high percentage of returned responses.

All sets of questionnaires were returned. Four hundred and fifty of the product testing questionnaires were completed, this gave the high return rate of 89%. One hundred and twelve of the housewives' questionnaires were completed and returned (100%).

Fifty three of the product testing questionnaires were not completed and returned. Mostly this was because the family size was smaller than 4 people.

8.6.3.5 Analyses of the Data

Before processing of the data, the questionnaires were firstly checked by the author and the data coded. The data obtained from the consumer test was processed by computer using the SPSS^x package (Norman, 1983). Chi-square analysis was used for this study. The detailed results are tabulated in Appendix 8.4. Correlations were also determined using the MUTAB package (Boag, 1988). Other information relating to the developed Nham was summarised and presented as percentage of the total households.

8.7 RESULTS OF NHAM PRODUCTION IN THAILAND

8.7.1 Comparison of Laboratory Fermentation with Fresh Pork in Thailand and Frozen Pork in New Zealand.

Nham produced using fresh pork had an initial pH lower than the Nham produced with frozen pork (Table 8.1) and the pH dropped more quickly during the fermentation. This might be because the final temperature of the mix using fresh pork (25°C) was higher than that of the mix using frozen pork (15°C). Therefore, starter cultures grew very rapidly at the beginning of fermentation causing the pH to drop quickly. There was a similar weight loss of both Nham samples during fermentation (Table 8.1).

On the third day of fermentation, the aerobic mesophiles counts, starter culture counts, and pathogenic bacterial counts, in the Nham using fresh pork were generally less than in the Nham using frozen pork. This was particularly noticeable in the case of S. aureus (Table 8.1).

Table 8.1 Comparison of Nham produced in the laboratory (using fresh or frozen meat) in terms of pH, weight loss and bacterial counts.

	Time (days)	Fresh pork	Frozen pork
pH:	0	6.04	6.66
	1	4.65	5.02
	2	4.13	4.52
	3	3.98	4.35
Weight loss: (%)	1	0.08	0.03
	2	0.18	0.05
	3	0.30	0.24
Mesophilic aerobic counts (log N)	3	7.71	8.29
L. plantarum (log N)	3	7.57	8.12
P. cerevisiae (log N)	3	6.33	7.07
M. varians (log N)	3	3.43	4.54
S. aureus (log N)	3	1.81	3.29
Enterobacteriaceae (log N)	3	2.25	2.18

8.7.2 Production Trial

According to the laboratory experiment, the pH of Nham reduced quickly on the second day to 4.13 and the third day to 3.98. To give an acceptable pH in the Nham, the fermentation time was reduced from 3 days to 2 days for this experiment. The Nham fermented 2 days with the same formulation and process as in the laboratory experiment gave a pH of approximately 4.10 in the final products both for the consumer panels and the production trial.

8.8 RESULTS OF SENSORY TESTING

8.8.1 Comparison of Experimental and Commercial Nham

The experimental Nham was significantly different from the commercial Nham sausages, both brand A and B, in some attributes - air pockets, firmness, smoothness, sourness and spiciness (Table 8.2). The Nham brand A and brand B were also significantly different from each other in terms of air pockets, juiciness and spiciness.

According to the overall acceptability scores, the experimental Nham had a significantly ($P<0.01$) higher acceptability score than the Nham brand A and brand B. The Nham brand B had also a significantly ($P<0.05$) higher score than the Nham brand A (Table 8.2). The mean ideal ratio scores of the experimental Nham were close to the ideal product (1.0) in most attributes; the sourness, colour and visual texture were significantly different ($P<0.01$) from the ideal product. Additionally, the actual score of acceptability was very high (4.70). This indicated that the experimental Nham was superior to the commercial sample tested.

Table 8.2 Comparison between experimental and commercial Nham (brand A and brand B). Ratios analysed using logarithms of ideal ratio scores.

Nham attributes	Mean ideals ratio scores			T - test		
	Experimental Nham (X)	Commercial A	B	X&A	X&B	A&B
Colour	0.92[a]	0.92	0.95	-0.07	-1.15	-0.71
Visual texture	0.92[a]	0.90	0.86	0.80	1.89	0.80
Air pockets	0.99	1.17	0.99	-3.05**	-0.70	3.41**
Firmness	1.00	0.83	0.88	3.90**	5.14**	-1.40
Juiciness	0.99	0.91	1.06	1.84	-1.74	-2.94**
Smoothness	0.98	0.86	0.86	3.02**	4.41**	-0.15
Sourness	1.08[a]	0.92	0.89	3.56**	8.20**	0.63
Saltiness	1.00	0.95	0.96	1.76	2.04	-0.31
Spiciness	0.98	0.93	1.08	1.79	-3.42**	-5.14**
Pork flavour	1.02	1.03	1.06	-0.12	-1.80	-0.85
Overall acceptability(1)	4.70	3.70	4.10	6.95**	7.55**	-2.52*

X = Nham fermented 3 days; (1) actual scores.

* significant at 95% level ($t_{18} = 2.101$)

** significant at 99% level ($t_{18} = 2.878$)

a = Ratio significantly different from ideal score of 1.0 at 99% level

8.8.2 Consumer Panel Results

According to the previous experiment, the sourness of the experimental product was too high. To reduce the sourness, the fermentation time was reduced from 3 days to 2 days. It was found that there were no significantly differences from the ideal product (Table 8.3).

Table 8.3 The mean ideal ratio scores of Nham fermented 2 days at 30°C, 97% RH for each attribute.

Nham attributes	Nham fermented 2 days
Colour	1.01
Visual texture	0.97
Air pockets	1.04
Firmness	0.98
Juiciness	1.02
Smoothness	0.98
Sourness	0.99
Saltiness	0.98
Spiciness	1.02
Pork flavour	1.03

8.8.3 Comparison of Trained Sensory Panel and Consumer Panel Results

The trained panel and consumer panel had similar ideals for the product. It was found that most of the attribute scores of the Nham attributes were not significantly different between both panels (Table 8.4). However, the sourness, colour and visual texture were significantly different because of the reduction of fermentation time to 2 days for the consumer panel samples. These scores were closer to the ideal product than the Nham fermented for 3 days. The overall acceptability as assessed by the trained panel and consumer panel was not significantly different. The experimental Nham had a very high score of acceptability; i.e. 4.70 for trained panel and 4.75 for consumer panel.

Table 8.4 Comparison of trained sensory panel and consumer panel using raw ideal ratio scores and logarithms of ideal ratio scores.

Nham attributes	Raw ideal ratio score					Logarithms of ideal ratio score				
	Trained panel N=10		Consumer panel N=20		T-test	Trained panel N=10		Consumer panel N=20		T-test
	X	SD	X	SD		X $x10^{-3}$	SD $x10^{-3}$	X $x10^{-3}$	SD $x10^{-3}$	
Colour	0.92	0.05	1.01	0.06	-4.09**	-36.0	25	5.2	28	-4.12**
Visual texture	0.92	0.04	0.97	0.06	-2.38*	-35.0	19	-15.0	18	-2.82**
Air pockets	0.99	0.08	1.04	0.12	-1.19	-3.9	36	16.0	49	-1.17
Firmness	1.00	0.04	0.98	0.04	1.33	-0.7	16	-7.3	16	1.06
Juiciness	0.99	0.06	1.02	0.07	-1.15	-6.5	28	9.4	28	-1.44
Smoothness	0.98	0.05	0.98	0.06	0.00	-10.0	21	-11.0	25	0.11
Sourness	1.08	0.05	0.99	0.03	6.20**	35.0	20	-3.0	14	6.08**
Saltiness	1.00	0.05	0.98	0.06	0.91	1.3	21	-10.0	24	1.26
Spiciness	0.98	0.06	1.02	0.08	-1.38	-9.9	25	7.2	35	-1.42
Pork flavour	1.02	0.05	1.03	0.07	-0.40	6.9	21	13.0	28	-0.61
Overall acceptability(1)	4.70	0.11	4.75	0.21	-0.70					

(1) actual score

* significant at 95% level ($t_{28} = 2.048$)

** significant at 99% level ($t_{28} = 2.763$).

In this section, a comparison between raw ideal ratio scores and the logarithms of ideal ratio scores was studied. The visual texture scores were assessed as significantly different at 95% level when using raw ideal ratio scores, whereas it was significant at 99% when using logarithms of ideal ratio scores. The t-values of most attributes when using logarithms of ideal ratio scores were higher than the t-values of those attributes when using raw ideal ratio scores. It was clear that the raw ideal ratio scores transformed to logarithms of ideal ratio scores gave a greater confidence in analysis.

8.8.4 Consumer Product Test

8.8.4.1 Overall Results for Nham Characteristics and Acceptability

The frequencies of the just right score for each sensory characteristic of the experimental Nham product were tabulated in Table 8.5. Half of the respondents said the Nham's colour was pink while 33% of the respondents thought that it was red pink. However, 52% of the consumers liked the pink colour of Nham and 40% liked the red pink. It was clear that the experimental Nham had an acceptable colour.

On the other hand, approximately half of the respondents thought that the firmness and sourness of the experimental Nham were just right while a minority of the respondents said that these attributes were slightly firm and sour. In terms of saltiness and spiciness of the Nham, the majority of the respondents said just right.

With regard to overall acceptability, the majority of the respondents (approximately 90%) from the total sample liked the experimental Nham from like it slightly (46%) to like it very much (47%) (Table 8.5).

Table 8.5 Nham characteristics and acceptability in consumer product test.

Nham characteristics	Scores										Total sample	
	5		4		3		2		1		No	%
	No	%	No	%	No	%	No	%	No	%		
Colour[a]	72	16.0	230	51.1	148	32.9	0	0	0	0	450	100
Firmness[b]	22	4.9	160	35.5	251	55.8	17	3.8	0	0	450	100
Sourness[b]	34	7.6	138	30.7	250	55.5	28	6.2	0	0	450	100
Saltiness[b]	10	2.2	81	18.0	317	70.4	38	8.5	4	0.9	450	100
Spiciness[b]	17	3.8	82	18.2	290	64.4	58	12.9	3	0.7	450	100
Overall [c] acceptability	213	47.3	206	45.8	26	5.8	5	1.1	0	0	450	100

(a) scores 5 = pale pink, 4 = pink, 3 = red pink, 2 = red, 1 = dark red

(b) scores 5 = very strong, 4 = slightly strong, 3 = just right
2 = slightly lacking, 1 = very lacking

(c) scores 5 = like very much, 4 = like slightly, 3 = neither like nor dislike
2 = dislike slightly, 1 = dislike very much

8.8.4.2 Acceptability of the Nham

The level of acceptability of the experimental Nham did not depend upon the location, sex and age group but depended upon the career of the respondents significantly (P=0.01) (Table 8.6). The housewives and the respondents who were government officers liked the Nham less than the other respondents. However, both these two groups of respondents liked the Nham, approximately 90% of total sample between like it slightly and like it very much (see details in Appendix 8.4).

Table 8.6 Acceptability of the experimental Nham in consumer product test by location, sex, age groups and career.

Demographic information	Scores								Row total		Signficance level
	5		4		3		2				
	NO	%*	No	%	No	%	No	%	No	%	
Locations:											
Village 1	58	50.0	49	42.3	7	6.0	2	1.7	116	25.8	
Village 2	54	51.9	46	44.3	4	3.8	-	-	104	23.1	
City	101	43.9	111	48.3	15	6.5	3	1.3	230	51.1	0.62
Sex:											
Male	96	46.8	95	46.3	13	6.4	1	0.5	205	45.6	
Female	117	47.8	111	45.3	13	5.3	4	1.6	245	54.4	0.67
Age groups:											
15-20	34	57.6	19	32.2	5	8.5	1	1.7	59	13.1	
21-30	54	47.4	54	47.4	6	5.2	-	-	114	25.3	
31-40	68	43.3	79	50.3	9	5.8	1	0.6	157	34.9	
41-50	36	52.2	28	40.6	3	4.3	2	2.9	69	15.4	
>50	21	41.2	26	50.9	3	5.9	1	2.0	51	11.3	0.48
Career:											
Others	169	52.8	129	40.3	19	5.9	3	1.0	320	71.1	
Housewives & government officers	44	33.9	77	59.2	7	5.4	2	1.5	130	28.9	0.01
Column total	213	47.3	206	45.8	26	5.8	5	1.1	450	100.0	

* Row percentage

5 = like it very much, 4 = like it slightly

3 = neither like nor dislike, 2 = dislike it slightly, 1 = dislike it very much

It was apparent that the experimental Nham was acceptable both to people in the villages and city and independent of sex. Additionally, the experimental Nham was liked by

consumers belonging to all age groups. These mean scores for overall acceptability were between like it very much and like it slightly.

When considering the total number of consumers, the results showed that the consumers liked the experimental Nham. The mean score for overall acceptability was 4.39 that was close to the 'like it very much' category.

8.8.4.3 Correlation of Acceptability with Selected Attributes

The scores for overall acceptability given by respondents from the total sample were correlated against the scores for selected attributes. The results obtained are shown in Table 8.7.

Table 8.7 Correlation of selected attributes of Nham with overall acceptability using the data from consumer product test.

	Colour	Firmness	Sourness	Saltiness	Spiciness
Firmness	-0.008				
Sourness	0.064	0.043			
Saltiness	-0.098*	0.123**	0.095*		
Spiciness	0.001	-0.032	-0.047	-0.024	
Overall acceptability	0.081	-0.018	0.401**	0.132*	0.191**

* significant at 95% level ($r \geq 0.085$ df = 448)

** significant at 99% level ($r \geq 0.111$ df = 448)

It was shown, by comparing the correlative values for the variables, that sourness was the sensory characteristic which related more with overall acceptability than any other attributes. Consumers in Chiang Mai province thought that sourness, spiciness and saltiness were the important attributes related to the overall acceptability of Nham.

8.9 COMPARISON OF SENSORY TESTING RESULTS

8.9.1 Comparison of Ideal Profiles from the Trained and Consumer Panels

A comparison was made between the mean ideal scores from the trained panel at Massey University, the trained panel at Chiang Mai University and the consumer panel. The results are shown in Table 8.8.

Table 8.8 Mean ideal scores of Nham for the trained panel at Massey University (MU), trained panel at Chiang Mai University (CMU) and consumer panel (CP), in Thailand.

Attributes	Mean ideal scores of Nham tested			T-tests		
	MU (N=8)	CMU (N=10)	CP (N=20)	MU&CMU	MU&CP	CMU&CP
Colour	2.55	2.99	3.10	-2.02	-3.07**	-0.93
Visual texture	3.90	3.87	3.89	0.13	0.07	-0.15
Air pockets	2.20	2.23	1.98	-0.14	1.94	1.83
Firmness	3.90	4.02	4.00	-1.23	-1.06	0.29
Juiciness	2.90	3.55	3.12	-2.45*	-1.13	2.56*
Smoothness	3.90	4.06	3.91	-1.05	-0.07	1.46
Sourness	3.40	3.27	3.34	0.63	0.40	-0.74
Saltiness	3.10	3.16	3.20	-0.61	-1.53	-0.63
Spiciness	3.60	3.28	3.37	1.99	1.51	-0.72
Pork flavour	3.60	3.20	3.24	2.46*	2.71*	-0.34

Scale used: 1 = very weak to 5 = very strong

* $t_{16} = 2.120$ ($P< 0.05$); ** $t_{16} = 2.921$ ($P< 0.01$)

* $t_{26} = 2.056$ ($P<0.05$); ** $t_{26} = 2.779$ ($P< 0.01$)

* $t_{28} = 2.048$ ($P< 0.05$); ** $t_{28} = 2.763$ ($P< 0.01$)

The results in Table 8.8 show that the mean ideal scores of Nham did not change much between the panels. Major exceptions were for colour, juiciness and pork flavour. The mean ideal scores of the consumer panel were higher than the trained panel for colour; for juiciness particularly, the mean ideal score of the trained panel at Chaing Mai University was higher than ideals of the trained panel at Massey University and the consumer panel. The mean ideal score for pork flavour of the trained panels at Massey University was higher than the ideals of both panel at Chiang Mai University and the consumer panel.

8.9.2 Comparison of Ideals from the Same Panel Using Different Test Products

When the same panels tested different Nham samples separately, most of the ideal scores were similar for all these sensory tests. There were differences in firmness and smoothness ideals between Nham brand A and the experimental Nham; juiciness between Nham brand B and the experimental Nham; saltiness and spiciness between Nham brand A and Nham brand B (Table 8.9).

Table 8.9 Mean ideal scores of two commercial Nham (brand A and brand B) and experimental Nham (X) by trained panel in Chiang Mai.

Attributes	Mean ideal scores of Nham tested			T-tests		
	brand A	brand B	experimental (X)	A&B	A&X	B&X
Colour	3.22	3.02	2.99	2.06	2.03	0.29
Visual texture	3.98	3.83	3.87	1.20	0.64	-0.24
Air pockets	2.59	2.28	2.23	1.78	1.58	0.28
Firmness	3.45	3.80	4.02	-1.76	-3.63**	-1.68
Juiciness	3.25	2.93	3.55	1.53	-1.22	3.35**
Smoothness	3.84	3.90	4.06	-0.57	-2.21*	-1.65
Sourness	3.35	3.44	3.27	-0.78	0.49	1.56
Saltiness	3.06	3.22	3.16	-2.12*	-1.17	0.70
Spiciness	3.20	3.42	3.28	-2.20*	-0.68	1.33
Pork flavour	3.23	3.33	3.20	-0.85	0.23	0.98

Scale used: 1= very weak to 5 = very strong

* $t_{18} = 2.101$ ($P< 0.05$); ** $t_{18} = 2.878$ ($P<0.01$)

8.9.3 Comparison of Ideal Ratio Scores from Consumer Panel and the Consumer Test

In this comparison, the product was judged by the consumer to be 'just right' (score = 3). Therefore, the results from the consumer test were calculated as just right ratio to compare with ideal ratio of the consumer panel. The results are shown in Table 8.10. It was indicated that the consumer panel and consumer test had a similar pattern of ideal ratio

means except the firmness and sourness ratio means; i.e. there were higher ratio means in the consumer test than in the consumer panel ($P< 0.01$).

Table 8.10 Comparison of the ideal ratios for the consumer panel and consumer test of selected attributes.

Attributes	Ideal ratio means Consumer panel (N=20)		Just right ratio means Consumer test (N=450)		T-test
	X	SD	X	SD	
Colour	1.01	0.06	1.04	0.18	-0.75
Firmness	0.98	0.04	1.14	0.22	-3.26**
Sourness	0.99	0.03	1.13	0.24	-2.59**
Saltiness	0.98	0.06	1.03	0.20	-1.11
Spiciness	1.02	0.08	1.03	0.25	-0.18

** t_α = 2.576 ($P< 0.01$)

* t_α = 1.960 ($P< 0.05$)

8.10 CONSUMER SURVEY ON BUYING NHAM

8.10.1 Eating Pattern for Nham

It was found from the survey that all consumers, both in villages and in Chiang Mai city, ate Nham (100%). When asked how long they had eaten Nham, the majority of consumers in the villages had eaten Nham for between 1 and 20 years while more than 58% of the consumers in city had eaten Nham for more than 20 years (Table 8.11).

The consumers in village 1 had a significantly different eating pattern for Nham from the consumers in city and in village 2, half of the consumers (52%) had eaten the product once a week, 17% once a fortnight and 31% uncertain (Table 8.11 and Appendix 8.5 (a)). The majority of consumers in village 2 (62%) had eaten Nham between once a fortnight and once a month whereas the consumers in city (44%) had eaten Nham once a fortnight.

Table 8.11 Eating pattern for Nham

Information	Village 1		Village 2		City	
	No	%	No	%	No	%
• Years eating Nham						
1-10 years	19	65	15	58	9	16
11-20 years	6	21	6	23	15	26
21-30 years	2	7	3	11	13	23
> 30 years	2	7	2	8	20	35
• Frequency of eating Nham						
once a month	0	0	8	31	7	12
once a fortnight	5	17	8	31	25	44
once a week	15	52	6	23	9	16
uncertain	9	31	4	15	16	28
Total	29	100.0	26	100.0	57	100.0

8.10.2 Criteria for Nham Buying Choice

Table 8.12 shows the major influences on the decision to buy Nham. A majority of consumers in both villages agreed that the major reasons for buying Nham were family preference and convenience. City dwellers also liked to eat the product and their family expressed a preference for the product, but convenience was less important to them. City dwellers also bought Nham as a souvenir, presumably for their travels outside the city.

Both in the city and the villages, the most common outlet was the local market, shops and service stations. There are no supermarkets in the villages, so this appeared as a difference between rural and urban populations. It appeared that the consumers in city bought Nham from factory (Table 8.12 and Appendix 8.5 (b)).

Table 8.12 Major influences on buying decisions and retail outlets.

Information	Villages		City	
	No	%	No	%
• Major influence				
Like to eat	16	15.8	33	28.4
Family preference	37	36.6	36	31.0
Convenience	38	37.6	22	19.0
Reasonable price	10	10.0	15	13.0
As a souvenir	0	0	10	8.6
• Retail outlets				
Supermarkets	0	0	12	13.8
Local markets	46	59.7	49	56.3
Service stations or retail shops	31	40.3	18	20.7
Factory	0	0	8	9.2

8.10.3 Packaging Preference for Nham

At present, Nham is packed either in individual cylindrical plastic bags or plastic bags wrapped with banana leaves. The consumers both in villages and city had the same needs in terms of packaging material for Nham. The majority (73%) of the consumer preferred individual cylindrical plastic bags (Table 8.13). Two other methods of packaging were suggested; i.e. the Nham packed in cylindrical plastic bags and put in a paper box. Alternatively, the Nham packed in cylindrical plastic bags and put together in a vacuum bag. It was found that a half of consumers (53%) both in villages and city preferred the Nham packed in cylindrical plastic bags and put in a paper box, whereas approximately 30% preferred the Nham packed in cylindrical bags and put together in a vacuum bag. (See details in Appendix 8.4 (c)).

With regard to the most acceptable weight for buying Nham, the consumers both in villages and city had a similar opinion (Table 8.13). Most agreed that 100-500 g was a suitable weight range for purchase of Nham. Only a very small percentage wanted to buy the product in quantities outside this range.

Consumers both in villages and city had the same opinion on the information to be included on the label (Table 8.13). Most consumers were concerned about the expiry date,the name and address of the factory which produced the Nham. Smaller proportions wanted to know the net weight, the ingredients and method to keep it. Interest in nutritional value was about the same in the city and in the villages.

Table 8.13 Packaging preferences for Nham

Information	Villages		City		Total	
	No	%	No	%	No	%
• Packaging of Nham						
Individual cylindrical plastic bags	40	72.7	42	73.7	82	73.2
Plastic bags, wrapped with banana leaves	15	27.3	15	26.3	30	26.8
• Weight of Nham						
1000 g	1	1.8	1	1.7	2	1.8
500 g	10	18.2	14	24.6	24	21.4
250 g	20	36.4	26	45.6	46	41.1
100 g	24	43.6	14	24.6	38	33.9
50 g	0	0	2	3.5	2	1.8
• Information on labels						
Expiry date	39	27.3	53	22.0	92	24.0
Address, name of factory	40	28.0	46	19.1	86	22.4
Net weight	19	13.3	42	17.4	61	15.9
Ingredients	20	14.0	38	15.8	58	15.1
Method to keep it	11	7.6	32	13.3	43	11.2
Nutritional value	14	9.8	17	7.1	31	8.1
Others, price and date to eat	0	0.0	13	5.3	13	3.3

8.10.4 Predicted Keeping Quality of Nham

In the city, the majority of the consumers (98%) stored their Nham in the refrigerator. This was less common in the villages: 75% in village 1 and 50% in village 2. Otherwise it was kept in the kitchen cabinet. Most likely this was related to the availability of refrigerators in the different households.

In terms of shelf-life, nearly all consumers in the city and village 2 thought that the unrefrigerated shelf-life would be less than 3 days. Few respondents believed that the shelf-life could be extended beyond two weeks by refrigeration. Most consumers (approximately 60-70%) thought that the Nham could be kept in a refrigerator for a week.

Table 8.14 Storage and keeping quality of Nham

Information	Village 1		Village 2		City	
	No	%	No	%	No	%
• Storage of Nham						
Kitchen cabinet	7	24	13	50	1	2
Refrigerator	22	76	13	50	56	98
• Predicted life of Nham						
* Kitchen cabinet						
Less than 3 days	3	43	12	92	1	100
3-7 days	4	57	1	8	0	0
* Refrigerator						
One week	15	68	8	62	36	64
Two weeks	7	32	3	23	16	29
One month	0	0	2	15	4	7

8.10.5 Buying Prediction for Experimental Nham

When comparing the experimental Nham with the Nham at present in the market, there was little difference between the villagers and the city consumers. Approximately half of

the consumers (52%) thought that the experimental Nham was different from the Nham in the market.

There was a difference between the price that the villagers in village 1 and (village 2 + city) would pay. The majority of the consumers (78%) in village 2 and in the city would buy the product at the same price as the commercial Nham, while approximately 59% of the consumers in village 1 would buy at the same price (Table 8.15 and Appendix 8.5 (e)). Nearly a third of the respondents in village 1 were prepared to pay more for the experimental Nham than for the currently available commercial product.

Most of the consumers both in the villages and in the city indicated that they were willing to buy the experimental Nham. Approximately 4% in the city were not willing to buy the product (Table 8.15).

Table 8.15 Buying prediction for experimental Nham.

Information	Village 1		Village 2		City	
	No	%	No	%	No	%
• Difference from commercial Nham						
Differences	16	55	10	38	32	56
No difference	13	45	16	62	25	44
• Price						
* Greater than prices of the commercial Nham	9	31	2	8	4	7
* Equal to the prices of the commercial Nham	17	59	20	77	45	79
* Less than the price of the commercial Nham	3	10	4	15	8	14
• Buying intentions						
Definitely would buy	18	62	18	69	35	61
Probably would buy	11	38	8	31	20	35
Probably would not buy	0	0	0	0	1	2
Definitely would not buy	0	0	0	0	1	2

8.11 DISCUSSION

8.11.1 Comparison of Proposed Production Method with the Present Commercial Methods

Usually, commercial Nham is made using fresh meat in the formulation, no starter cultures are added and the mixture is mixed by hand before stuffing. The ripening process is not controlled as the products are usually held at ambient temperatures. The products can be eaten up to about 4-5 days after production, depending on the native microflora and fermentation temperature (Pakrachpan, 1981; Adams, 1986; Wongkhalaung and Boonyaratanakornkit, 1986). Nham using mixed starter cultures had shorter ripening times; i.e. 2 days fermentation. The Nham made with the mixed starter cultures were superior to the commerical product. The majority of consumers in Chiang Mai province liked it.

The proposed Nham production method used the final formulation developed in New Zealand but the process was changed slightly. Fresh pork was used instead of frozen pork, and no attempt was made to keep the mixing temperature the same as in New Zealand. Many researchers have indicated that fresh lean meat is suitable for raw sausage production particularly the pale, soft and exudative (PSE) meat (Newton and Gill, 1981; Wood, 1985).

The experimental Nham was accepted by panelists for most attributes except sourness and colour. This was because of the temperature of mix (i.e. 25-26°C) or the effect of using fresh meat. It allowed a quick fermentation causing the product to be too sour. The use of fresh or frozen lean meat therefore probably influenced the fermentation in several ways. Since starter cultures have an optimum growth temperature, the initial product temperature was critical to the fermentation time. A formulation using frozen meat took longer time to achieve the desired fermentation temperature, thereby extending the total incubation period. Bacus (1984) and Gilliland (1985) reported that the higher the lean percentage, i.e. lower fat, the greater the moisture resulting in a more rapid pH drop. The contribution of glycogen from lean tissue appears to also have an effect on total acidity (Acton and Dick, 1975). In addition, frozen meats exhibited rapid drip loss during thawing. A reduced moisture level, attributed to previously frozen raw materials, reduced the rate of pH drop (Bacus, 1984). Therefore the use of frozen meat in suasage will retard the inital fermentation rate.

The initial pH of Nham using frozen and fresh meat were 6.66 and 6.04 respectively. The Nham using frozen meat needed 3 days fermentation time to achieve pH to 4.3 whereas the Nham using fresh meat needed only 2 days to achieve pH to 4.1. The initial meat pH is therefore important as to subsequent fermentation time to achieve the desired final product pH. Meat with higher pH values will require more acid production to achieve the same end point (Acton et al., 1977; Bacus, 1984).

Therefore, Nham processing in Thailand was changed by reducing the fermentation time from 3 days to 2 days. The pH of experimental Nham at 30°C, 97% RH for 2 days was 4.10. All attributes of the Nham were then similar to the ideal product profile. However, the Nham using frozen meat can be produced by holding at the same conditions for 3 days.

8.11.2 Possible Marketing of New Nham Against the Present Commercial Products

The experimental Nham was better than the commercial Nham (brand A and B) in most attributes. The majority of consumers in Chiang Mai province liked the experimental Nham and they were willing to buy the Nham at the same price as the commercial Nham. The consumers said the experimental Nham was different from the commercial Nham in general appearance - more meat and no chilli. They suggested the addition of more pork skin, chilli and white pepper in the experimental Nham formulation. The colour, firmness, sourness, spiciness and saltiness of the Nham were accepted by the consumers.

A packaging plan to produce the Nham for the Thai market would include supplying the Nham in quantities of 100-500 g packed in cylindrical plastic bags and labelled with the expiry date and name and address of the factory. The Nham would be sold at the local markets, service stations and retail shops.

The experimental Nham had a longer shelf-life than the commercial Nham. All consumers in Chiang Mai city and village 2 (Ban Ma-Kran) thought that the shelf-life of commercial Nham stored at ambient temperatures would be less than 3 days. However, Techapinyawat (1975) reported that the commercial Nham can be kept at ambient temperatures in Thailand for a week. On the other hand, the Nham developed by using mixed starter cultures can be kept at 20-30°C for 11-9 days respectively. Therefore the experimental product could compete well in the market. It can be also stored at 10°C for

appoximately 2 months. There is a possibility to export the product to overseas countries when the product is stored at low temperature of 1°C. (See results in Chapter 7).

8.11.3 Use of Trained Panel, Consumer Panel and Consumer Test

In product development, it is the consumer panelists who are important in determining acceptability. A prototype product is developed and technically tested by the experienced judges or trained panel. After that, the product developer turns to the consumer panel and the final judge, the consumer (Urban and Hauser, 1980; Gatchalian, 1981; Sinthavalai, 1986; Lai Pai Wan, 1987; Chinprahast, 1988).

The profile test, using line scale for attribute intensity judgment, was suitable for the trained panel. The trained panels both at Massey University and Chiang Mai University worked reasonably with the profile test. There are many reports which used fixed ideals during the product development (Sinthavalai 1986; Lai Pai Wan, 1987; Chinprahast, 1988). However, it was shown that if floating ideals were used when the product was close to the ideal, logarithms of ideal ratio scores could be done with greater confidence. This result agreed with the work of Cooper et al. (1989).

The mean ideal scores of Nham tested did not change much between the panels at Massey University, Chiang Mai University and consumer panels in Thailand. In addition, the trained panel and consumer panel had similar ideal ratio scores. The overall acceptability of Nham using the trained panel and consumer panel was not significant different. It was clear that the acceptability of the Nham by the trained panel can be linked to the consumer panel who were representative consumers in the target market.

The just right ratio scores used by consumer test were compared with the ideal ratio scores used by consumer panel. There were a similar pattern of the ratio scores except the firmness and sourness ratio scores; i.e. there were higher ratio scores in the consumer test than in the consumer panel. This was due to the handling at room ambient temperature during the distribution to the households and perhaps in the households before testing. The households and the consumer panels also tested the product samples at different times. All those reasons might allow change in the product samples because the time and room temperature affected the activities of cultures - causing the sourness and firmness to increase. Additionally, the consumers in Chiang Mai province said that sourness, spiciness and saltiness of Nham were important attributes related to the overall

acceptability of Nham. However, the sourness was the sensory characteristic which related more with overall acceptability than the other attributes.

8.12 CONCLUSION

The developed Nham product was acceptable to the target Thai consumers, both in the villages and in the Chiang Mai city. Additionally, a high percentage of the consumers both in the villages and in the city accepted the Nham as just right in terms of firmness, sourness, saltiness and spiciness. The most important attributes affecting the acceptability to the consumers in Chiang Mai province were sourness, spiciness and saltiness. The majority of the consumers were willing to buy the developed Nham and would only buy it at the same price as the existing commercial Nham.

The consumer test on the Nham demonstrated that the systematic technique used during the product development process was effective for determining the consumers needs in the Nham product.

The Nham formulation and process developed from this project had a potential to fit into the industrial line if the starter cultures preparation room, mixing machine, and incubator were added into the processing line of Nham. When the fresh meat was used in the same formulation developed at New Zealand, the fermentation time should be reduced to 2 days at 30^{o}C and relative humidity of 97%. However, the frozen meat can be also used in the same formulation but the fermentation time should be extended to 3 days at the same conditions in order to achieve the desired final product pH.

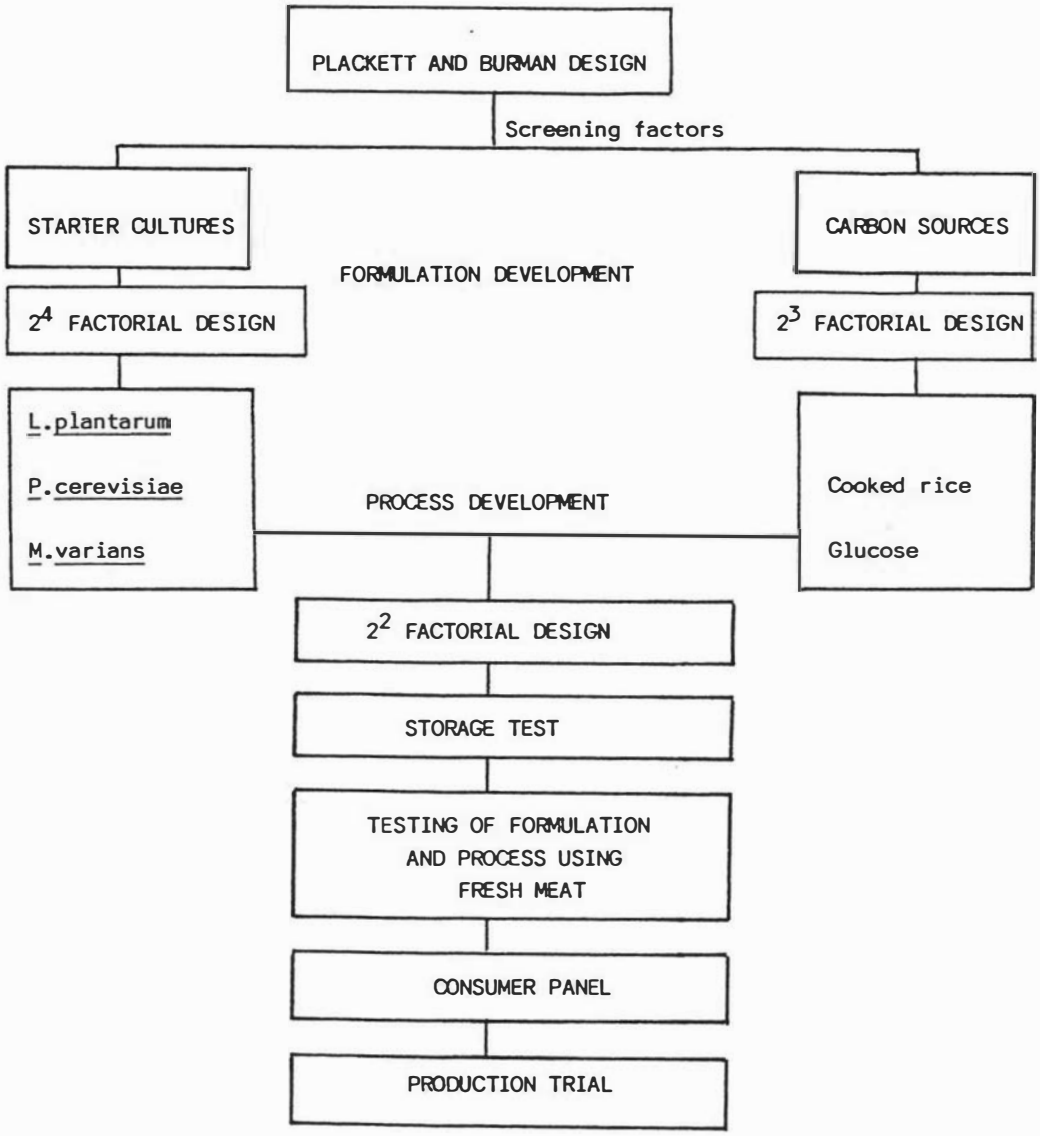
CHAPTER 9
DISCUSSION AND CONCLUSION

9.1 INTRODUCTION

The work described in this thesis aimed at improving the quality of a Thai fermented sausage, Nham, by using systematic product development techniques that have been successfully used for many products (Anderson, 1975; Chittaporn, 1977; Ngarmask, 1983; Earle and Anderson, 1985; Sinthavalia, 1986; Lai Pai Wan, 1987; Chinprahast, 1988). In addition, the detailed behaviour of the flora and the contribution of the individual species in the starter culture together with objective measurements on the product, were studied in a systematic manner, to discover the determinants of product quality and safety. Previous studies on fermented meat products do not appear to have used such a rigorous approach. Experimental designs were used for optimization of the formulation and process. Consumer studies were needed for this systematic development. Trained panels were used for laboratory evaluation of the product during formulation and process development. The acceptability of final product was tested by consumer testing in Chiang Mai province.

9.2 USE OF EXPERIMENTAL DESIGNS IN NHAM DEVELOPMENT

The systematic approach employed in this work is shown diagrammatically in Figure 9.1. The important factors which affected quality were first screened using a Plackett and Burman design. The most important factors were found to be the use of starter cultures and the inclusion of suitable carbon sources in the formulation. The formulation and process were further developed, and shelf-life was determined. The same formulation and process developed in New Zealand were then made in Thailand by using fresh pork instead of frozen pork. The final phase was testing of production and consumer product acceptability in three locations in Northern Thailand.



Fifure 9.1 Systematic product development system and experimental designs used for improvement of Nham quality.

Experimental designs have long been used as essential tools for research in various disciplines. With the appropriate choice of designs, the results obtained from any experiment can be conclusive and the inference can be correctly drawn for that experiment. In this work, several experimental designs were used for the formulation and process development of the Nham. Table 9.1 outlines the experimental designs, the stages at which they were used, and the objectives during the formulation and process development.

Table 9.1 Experimental designs, stages, and objectives during the formulation and process development.

Experimental design	Stage	Objective
Plackett and Burman design	Selection of factors in Nham system	To screen the important factors affecting the Nham quality
2^4 Factorial design	Formulation development	To study the effects of starter cultures in Nham formulation
2^3 Factorial design	Formulation development	To study the effects of carbon sources and a starter culture in Nham fermentation
2^2 Factorial design	Process development	To study the effects of temperature and relative humidity in Nham fermentation

The selection of each experimental design for use in this research was based on specific purposes and conditions at various stages of the development process.

The Plackett and Burman design was shown to be appropriate for the preliminary stage of formulation development. It was very effective for rapidly screening the important factors affecting quality. The important factors screened were starter cultures and carbon sources which needed to be studied in detail by using factorial designs. Although some factors such as salt, sodium tripolyphosphate and garlic also affected the Nham quality,

but they were set at suitable levels throughout this project from many researchers' recommendations (Techapinyawat, 1975; Zaika et al., 1978; Bacus, 1984); i.e. 3% salt, 0.3% sodium tripolyphosphate and 7% garlic were set. Sodium nitrate and white pepper had no effect were also set at 200 ppm sodium nitrate and 0.05% white pepper.

The full 2^2, 2^3, and 2^4 factorial designs were useful for formulation and process development of Nham. Empirical equations were obtained relating the sensory mean ideal ratio scores or the objective test values to the independent variables studied. These equations related product attributes to compositional or processing parameters and could be solved to indicate the formulation and processing conditions for the ideal Nham. There have been a few published research articles concerning the use of the empirical equations (Beausire and Earle, 1986; Joglekar and May, 1987; Floros and Chinnan, 1988), but none relating subjective and objective response variables to the type and quantity of starter cultures in fermented food formulations. The present research has emphasized the usefulness of the appropriate experimental designs and the exploitation of the data for guiding formulation and process development of fermented foods. Sensory response variables were also very important and needed to be studied during the systematic development. The acceptability of Nham was also related to other attributes of Nham and was used for guiding the suitable formulation and process of the product.

9.3 USEFULNESS OF CONSUMER INPUTS AND SENSORY EVALUATION

In this work, sensory panels were used in practically all stages of the product development process. The selection of the basic product profile for Nham was considered very important because there was no published identification of Nham characteristics. The final profile for Nham was established. Colour, firmness and sourness were important attributes. These attributes also related to overall acceptability of the product. Table 9.2 presents types of panel and their sizes, sensory evaluation techniques and stages at which they were used in this project.

Table 9.2 Types of sensory panels and evaluation techniques used in the project.

Type of panel	Size	Sensory evaluation technique	Stage
Laboratory panel (Massey University)	10	Ideal profile test	To identify the Nham characteristics and generate their ideal points in New Zealand.
Laboratory panel (Massey University)	8	Ideal profile test	Formulation and process development in New Zealand.
Laboratory panel (Chiang Mai University)	10	Ideal profile test	Formulation development in Thailand.
Consumer panel	20	Ideal profile test	Optimization of prototype formulation and process development in Thailand.
Home-use test panel	450	Just right score	Final testing for acceptance in Thailand.

In this project, three types of taste panels were used to develop the Nham - a laboratory panel at Massey University, a laboratory panel at Chiang Mai University and a consumer panel. For the laboratory panel, those with current food habits similar to the target consumer were preferred, because they were to work with the profile of the product desired by the consumer. The laboratory panel was also trained before testing the prototype product during development. This was necessary for this project particularly, since the product changed its characteristics during fermentation. The panelists had to identify exactly the intensity of the characteristics. Therefore, the trained panel was a very important part of this project.

It was important that the consumer panelists truly represented the target consumers. They were used to test the prototype before the production trial and for assessing acceptability of the product.

For this work, linear scaling and ideal ratio profile analysis were recommended for using during the formulation and process development steps by the laboratory panel. The trained panel worked reasonably well and they were very useful in guiding the development of the product. Multiple regression between the Nham attributes and overall acceptability was recommended to investigation of the attributes which affected acceptance. This method has been also recommended to use during formulation and process development by Beausire and Earle (1986) and Chinprahast (1988). The analysis of variance between the mean ideal ratio scores of different formulae or treatments was also recommended for the selection of formulae for this study.

The ideal ratio scores proved their value in guiding long term Nham product development. It was very useful for making decision on how the product could be designed. The panelists felt more comfortable using fixed ideals over a long period. This was also found by Lai Pai Wan (1987). The final formulation developed by the laboratory panel in New Zealand needed only a slight development by the laboratory panel in Thailand. The panels at Massey and Chiang Mai Universities and consumer panel in Thailand agreed closely on the ideal points for most attributes.

The ideal ratio scores were also found to be suitable for use in regression analysis to develop empirical models relating independent variables with the Nham attributes. There was no need for use logarithms with fixed ideals, because the ideal ratio scores were divided by a constant value; i.e. ideal points. However, floating ideals were used by the laboratory panel at Chiang Mai University and the consumer panel in Thailand, because the product was already close to ideal. Lai Pai Wan (1987) advised strongly that consumers at this point should be free to indicate their own ideals. Where ideal ratio scores were calculated from floating ideals, the analysis could be done with greater confidence if logarithms of ideal ratio scores were used. However, the mean ideal scores of Nham tested did not change much between the panels at Massey University, Chiang Mai University and consumer panels in Thailand. The trained panel and consumer panel had similar ideal ratio scores. The overall acceptability of Nham using the trained panel and consumer panel was not significantly different. It was clear that the acceptability of the Nham by trained panel can be linked to consumer panel.

The category scaling profile test was an effective method recommended for use after a positive result was obtained from the final consumer (Sinthavalai, 1986; Cooper et al., 1989). The five-category 'just right' scale was suitable for general scaling. The Nham was finally tested by a home-use test with consumers using category scaling;the results were analysed by SPSS^{x} programme and Chi-square analysis was used. Sensory

evaluation by one hundred and twelve families in Chiang Mai city and two northern Thailand villages provided the final test by target consumers. Chiang Mai province was selected as a target area, as it was an original Nham production centre and many consumers have eaten Nham for a long time. The majority of the consumers accepted the experimental Nham. This showed that the optimisation process, using a consumer panel, was successful.

The relative results from the consumer panel and the in-home consumer test show that handling and distribution of the product are important phases in the product life. The in-home results have greater firmness and sourness scores. The probable explanation is that the product was distributed at room temperature, which would have allowed continued activity of the microbial population and hence change in quality. The Nham should therefore be transported to the point of sale at a controlled low temperature.

The information obtained from housewives was very useful for guiding Nham production and also for the marketing aspects.

9.4 FORMULATION AND PROCESS DEVELOPMENT

The type and activity of microbial starter cultures was a key element in structuring a consistent fermentation process, so it was justifiable to attempt to develop mixed starter cultures which would include strong producers of lactic acid and, on the other hand, nitrate-reducing micrococci. Lactobacilli, pediococci and micrococci were therefore selected for the bacterial cultures to be studied. These cultures were grown separately and mixed together in appropriate proportions before use.

A modified formulation of Nham was used. In the investigation using Plackett and Burman designs, mixed starter cultures and the carbon sources used in the formulation were found to be important in determining product quality. When starter cultures were used the Nham quality, in terms of firmness and colour development, was greater than in the Nham without starter cultures. Cooked rice apparently provided a carbon source for lactic acid production by the starter cultures.

The key points in the Nham system were therefore the starter cultures, carbon sources and processing conditions. The rate of fermentation and the ultimate pH of Nham were directly influenced by the specific formulation and processing conditions, as well as the type and activity of cultures employed. Since the safety and quality of the Nham were

dependent on the rate and extent of acid production, a thorough understanding of formulation was essential to the total control of the product.

Processing parameters such as time and temperature were utilized effectively to control the total process of Nham. Temperature in particular increased the rate of fermentation (as reflected by the pH reduction) the firmness and colour development of Nham. The processing conditions also selected the type of microorganisms that predominated and their metabolic activity which, in turn determined the characteristics of the fermentation and the final product. The lactobacilli, pediococci and micrococci employed as starter cultures have optimum growth temperatures of 32°C, 37°C and 22-37°C respectively (Bacus, 1984; Gilliland, 1985; Gibbs, 1987). Their performance in lowering pH was dictated by the mix temperature and the time held at that temperature. For the experiments in New Zealand, the temperature of the Nham mixtures before stuffing was 15°C, whereas in the experiment in Thailand, the temperature of the mix before stuffing was 26°C. This led to a more rapid reduction in the pH of the Nham produced in Thailand. It was clear that the initial temperature of Nham was very important in determining the quality of the final product. It was found that Nham formulated using frozen meat and fermented at 30°C, 97% relative humidity needed 3 days to achieve a pH reduction to 4.3-4.4, whereas Nham using fresh meat required only 2 days to reduce the pH to 4.10. However, the relative humidity did not influence colour and firmness development, but weight loss during fermentation was affected. However, the overall acceptability scores of products from both processes were high; i.e. 4.75 for 2 days fermentation using fresh meat and 4.80 for 3 days fermentation using frozen meat. The final Nham formulation and process are presented in Table 9.3 and Figure 9.2 respectively.

Table 9.3 Final formulation of Nham

Ingredients	Quantity
Meat system:	
Ground lean pork (Fresh or Frozen)	80%
Sliced pork skin	20%
	% of meat system
Curing agents:	
Sodium chloride (NaCl)	3
Sodium nitrate ($NaNO_3$)	0.02
Sodium tripolyphosphate ($Na_5P_3O_{10}$)	0.3
Seasonings and carbon sources:	
Minced raw garlic	7
White pepper powder	0.05
Carbon sources:	
Glucose	0.5
Cooked rice	6
	cfu/g of meat system
Starter cultures:	
Lactobacillus plantarum	10^3
Pediococcus cerevisiae	10^6
Micrococcus varians	10^3

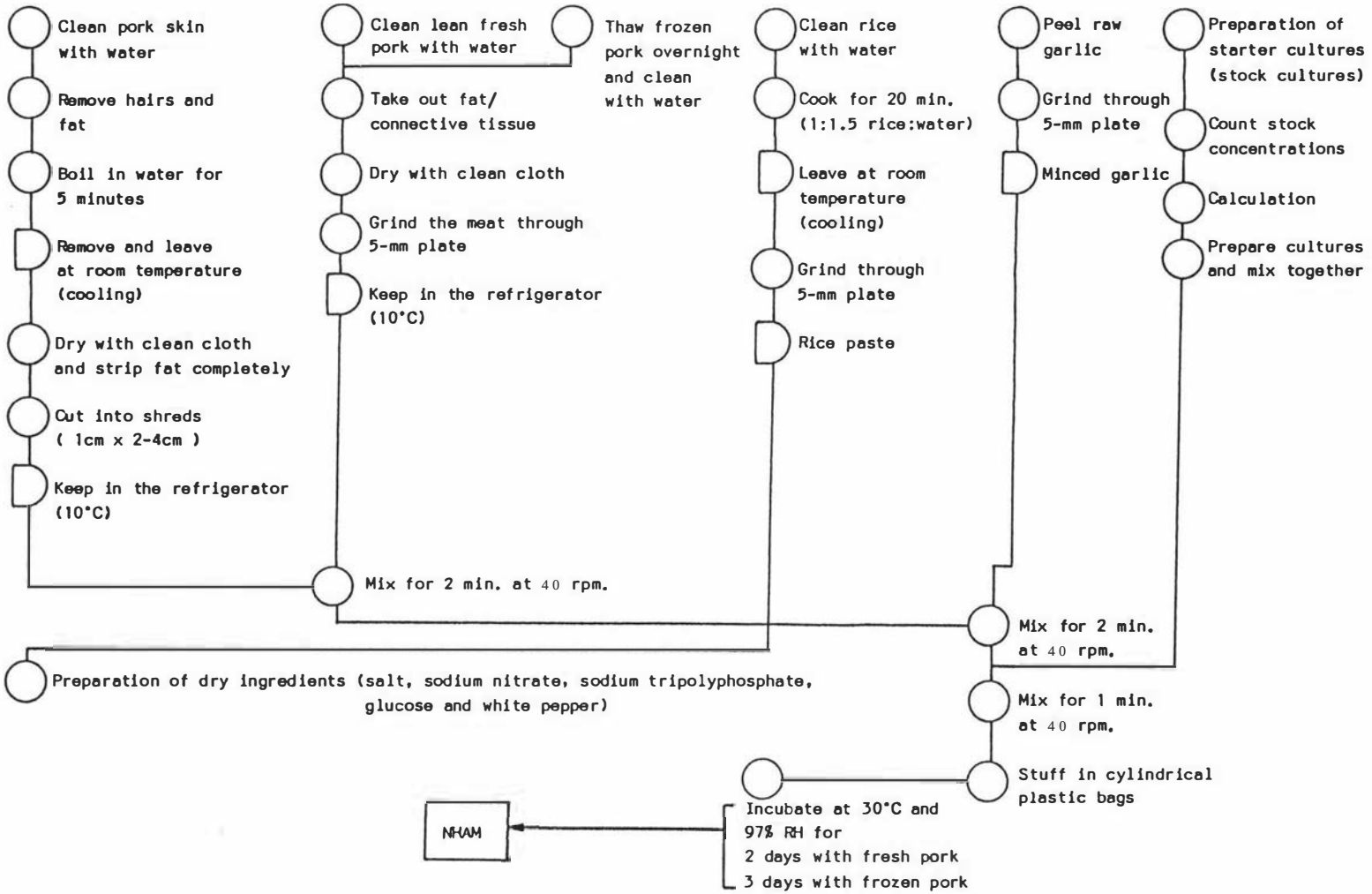


Figure 9.2 Process chart of Nham production using mixed starter cultures

9.4.1 The Necessity of Using Starter Cultures in Nham

At present, the Nham fermentation depends upon a random bacterial flora, the process varies in the time required for ripening and the final products can be very different in quality and flavour. Faulty product, too, can occur rather often. The initial flora varies, of course, in different manufacturing lots. This, on the other hand, can bring about problems in the manufacture of Nham because the initial flora may then be insufficient to bring about the normal ripening process. Failure of the flora to reduce the pH allows pathogens such as Salmonella to survive and multiply, possibly causing illness. Since the majority of the Thai people eat the Nham in a raw form, particularly in the Northern part of Thailand - Chiang Mai, Chiang Rai, Lampang and Lamphun provinces - the safety of the product is vital. Thus, there is a necessity for starter cultures to be used in the Nham system in order to improve the Nham quality. The utilization of starter cultures in fermented meat products has been recommended by numerous researchers (Liepe, 1983; Bacus, 1984; Gilliland, 1985; Gibbs, 1987). Interest in starter cultures arose parallel to the trend towards industrial production of fermented meat products, requiring shorter ripening times and standardization of the product properties.

9.4.2 Effect of Starter Cultures on Characteristics of Nham

There have been several attempts to develop bacterial cultures for the meat industry. In the USA the Pediococcus cerevisiae pure culture, suggested by Deibel and Niven (1957); Deibel et al. (1961 (a) and (b)), has gained considerable ground. Besides pediococci, lactobacilli are also used in the USA as pure cultures or as mixed cultures of two species (Bacus, 1984). In Europe the micrococcus culture, developed by Niinivaara (1955), is employed for fermented sausage production. In the present investigation, the possibilities of developing mixed cultures of pediococci, lactobacilli and micrococci were investigated.

The mixed starter cultures were initially composed of Lactobacillus plantarum, Lactobacillus brevis, Pediococcus cerevisiae and Micrococcus varians. Those cultures had a very marked effect on the Nham characteristics. The addition of L. plantarum to the Nham mass accelerated very distinctly the decrease in the pH of the Nham; the pH was about 4.0 at 3 days. Consequently, the firmness and colour developed, influenced directly by the acid production. The findings were in agreement with the work of many workers (Klement et al., 1973; 1974; 1975; Palumbo et al., 1976; Acton, 1977; Klettner and Rodel, 1978; Klettner and Baumgartner, 1980; Liepe, 1983). P. cerevisiae increased the firmness during 7-10 days of fermentation. The optimum growth of the P. cerevisiae occurs at pH 5.0 (Buchanan and Gibbons, 1974); the conditions during the later period of

fermentation allowed good growth and acid production, causing the increase in firmness. Therefore, the L. plantarum inoculation had a very distinct effect in terms of firmness development when it was used together with P. cerevisiae.

One of the most important changes occurring during the ripening process of Nham was the development of the normal pink colour. When nitrate was used in Nham formulation, the formation of the colour required the presence of the nitrate-reducing M. varians in the Nham system which reduced nitrate to nitrite during the initial period of fermentation. This finding agreed with the work of Deibel et al. (1961 (a)); they reported that nitrate reducing activity generally occurred during the first 2-16 hours of the fermentation period, while acid production was initiated after 8-16 hours. It was important to ensure that the nitrate reducing activity of M. varians took place prior to its inhibition by the growth of lactic acid bacteria with the consequent pH reduction. Nurmi (1966) noted that the most favourable pH range for the reduction of nitrate is 5.9-6.1.

The probably mechanism of nitrate reduction is that the micrococci produce nitrate reductase which transfers protons from nicotinamide adenine dinucleotide (NADH) to the substrate (Liepe, 1983):

$$NO_3^- + NADH + H^+ \xrightarrow[\text{reductase}]{\text{nitrate}} NO_2^- + NAD^- + H_2O$$

Very low concentration of residual nitrite was obtained by the use of starter cultures. 35-87 ppm of residual nitrite was found at the first day and then reduced to 0.3-2.0 ppm at 14 days. The nitrite formed decomposes spontaneously in acid surroundings into nitric oxide (Pfeil and Liepe, 1973; 1974; Klettner and Baumgartner, 1980) which subsequently reacts with myoglobin to form a pink compound, nitrosomyoglobin. So, the residual nitrite is reduced quickly when acid is produced. The L. plantarum accelerated colour development in the Nham system. The rate of nitrosomyoglobin formation increased with falling pH and this reaction takes place best in the pH range 5.0-5.5 (Niinivaara, 1955; Fox and Thomson, 1963; Liepe, 1983).

M. varians increased the tristimulus values (x, y, z) at the beginning of fermentation and then the L. plantarum continuously increased the values later. It was clear that in terms of colour development the inoculation of micrococci had a very distinct effect when used together with L. plantarum. Nurmi (1966) indicated that when only lactobacilli were added, grey and greenish discoloration was seen; this evidently resulted from formation of hydrogen peroxide by lactobacilli, which then oxidized the myoglobin and nitrosomyoglobin. There have been many reports supporting this finding (Niven et al., 1949; 1954; Cerise et al., 1973; Bacus, 1984; Gilliland, 1985; Gibbs, 1987). However,

catalase has been found to counteract peroxide formation (Liepe, 1983; Bacus, 1984). M. varians also generates catalase activity (Bacus, 1984) which may retard hydrogen peroxide accumulation.

During ripening of Nham, two basic microbiological reactions occurred simultaneously which influenced each other and were directly dependent upon each other:

* The production of nitric oxide by nitrate reducing bacteria.
* A decrease of the pH of the Nham by lactic acid bacteria.

These reactions were interconnected by two factors: competition of microorganisms for available carbohydrates and the pH dependency of nitrate and nitrite reduction. Nitric oxide formation and pH decrease were thus the two crucial points of the Nham fermentation. A simplified diagram of these interconnections is presented in Figure 9.3.

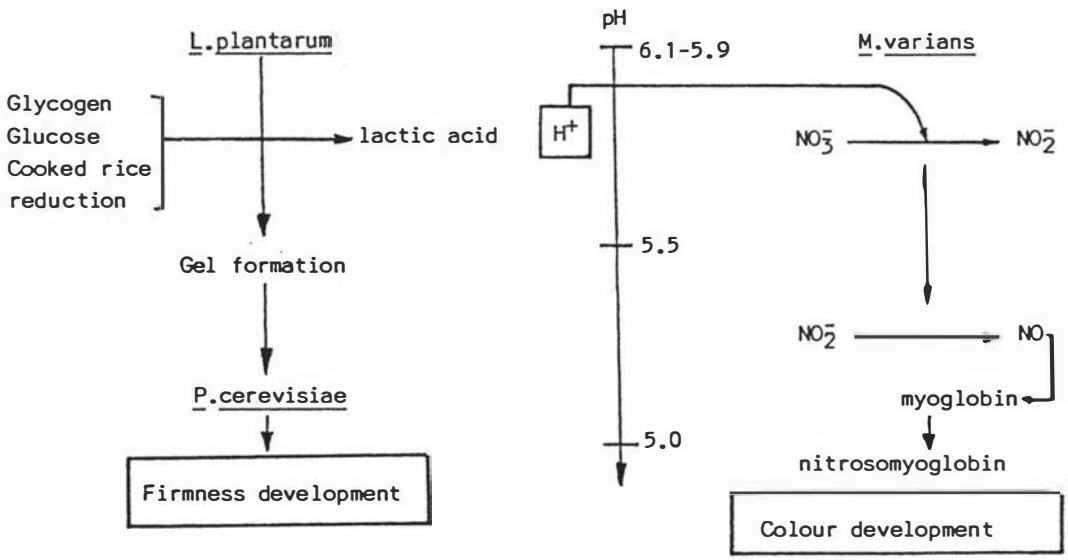


Figure 9.3 Effect of mixed starter cultures on Nham system.

As can be seen from Figure 9.3, L. plantarum used available carbohydrates to produce lactic acid and consequently to develop firmness. P. cerevisiae could grow at pH about 5.0 and produced acid - affecting the firmness development later. On the other hand, M. varians produces nitrate reductase and reduced nitrate to nitrite under acid condition. Then nitrite decomposes to nitric oxide and subsequently reacts with myoglobin to form nitrosomyoglobin.

The level of nitrate should be reduced in Nham formulation because nitrosamines could be formed in the product. In addition, nitrate had no significant effect in this study. Therefore, it was set a low level (200 ppm) which the Food Regulations in Thailand permitted 500 ppm (Food and Drug Administration, Ministry of Public Health, 1984). The necessity of nitrite in Nham system is to give colour and also protect against Clostridum botulinum (Christiansen et al., 1975). However, the lower pH dissipated residual nitrate in Nham system. This could reduce potential nitrosamine formation.

On the other hand, L. brevis seemed to be a poor lactic acid producer in these conditions. Its presence resulted in decreased colour and decreased the firmness of the product as a result of its tendency to produce carbon dioxide because it is heterofermentative lactic acid bacteria.

When Nham base was inoculated with a high level of P. cerevisiae (10^6 cfu.g^{-1}) with the other cultures at low levels (10^3 cfu.g^{-1}) the product had the highest acceptability. Acid production, firmness and colour development were satisfactory and the product was microbiologically safe. Nham with high levels of L. plantarum or M. varians were judged to be of lower quality in terms of firmness development and overall acceptability.

The use of starter cultures in the Nham mix reduced the number of pathogenic bacteria, including Enterobacteriaceae and S. aureus. L. plantarum strongly inhibited the Enterobacteriaceae probably because of the rapid acid production, which then inhibited directly the Enterobacteriaceae. Several workers have reported the inhibition of meat spoilage by lactic acid bacteria (Reddy et al., 1970, 1975; Gilliland and Speck, 1977; Roth and Clark, 1975; Raccach et al., 1979; Bacus, 1984; Gilliland 1985; Gibbs, 1987). Nham products are often held at high temperatures during processing to ensure rapid fermentation, but these temperatures can also accentuate the growth of the pathogenic bacteria. In addition, Nham is usually eaten without further cooking by the consumer. These considerations make strict control of the product essential. Although proper sanitation, employee hygiene, and the control of raw materials definitely reduces contamination, the ultimate control of product safety must be inherent in the formulation and process. The use of mixed starter cultures provided sufficient microbial numbers to ensure numerical dominance of acid producing bacteria over the natural flora, including pathogenic bacteria, and combination with the proper processing controls, guaranteed the safety and quality of the final Nham.

9.4.3 Change in Carbon Sources During Fermentation

During post-mortem glycolysis, a small portion of the glycogen is converted to glucose rather than lactic acid (Lucke, 1985). This glucose is utilized preferentially by all microorganisms of importance for meat and meat products. The glucose content of fresh, post-rigor pork is 7 μmol/g fresh weight (Kastenschmidt et al., 1968), and does not allow a significant reduction in pH. Therefore, fermentable carbohydrate added to the system is crucial because this determines the rate and extent of lactic acid formation and the composition of the product microflora. In commercial Nham formulation, addition of carbohydrate in the form of cooked rice (polysaccharides), which is degraded only slowly, may result in growth of undesired organisms during fermentation, particularly at high ripening temperature. When the rapidly metabolisable carbohydrate, glucose, was added, the pH reduction could be so rapid that micrococci, which contribute to the desired properties of the Nham, were suppressed.

For this research, therefore, a combination of rapidly and slowly metabolisable carbohydrates was most favourable. This ensured a sufficiently rapid initial growth of micrococci and nitrate reduction by micrococci and a rapid later pH drop, without inhibiting microbial, chemical reactions necessary for the development of the firmness and desired colour.

The beginning of cooked rice reduction coincided with the increase of reducing sugars at 12 hours of fermentation; the decline in reducing sugars after 12 hours coincided with the decrease in pH. The longer the fermentation time, the greater the pH reduction. The pH had fallen significantly by 18 hours of fermentation at 30°C. If both cooked rice and glucose were used at high levels (10% and 1% respectively), the pH dropped more slowly than when the low levels were used (8% cooked rice and 0.5% glucose).

In Thailand, where regulations permit such additives, quite large amounts of cooked rice are sometimes added to raw Nham mixtures. This, however, is obviously based on economical rather than microbiological considerations.

On increasing the level of cooked rice, the firmness of Nham decreased. There was an increase in weight loss at high level of glucose and the glucose also decreased the colour development during the last period of fermentation. There were also 1.0-1.3% reducing sugars and 2.0-3.0% cooked rice in the Nham after being fermented for 3 days. This residual carbohydrate could be used by undesirable organisms such as heterofermentative lactic acid bacteria, yeasts and moulds during storage. Rice (1971) recommended that the

range of carbohydrate content for sausage products be generally 1.2-1.7% in the finished state. Therefore, the carbon source levels in the Nham base should be low. When the glucose was maintained at 0.5% but the level of cooked rice was reduced because when the higher molecular weight of the carbohydrate substrate increase, a longer period was required to attain adequate fermentation end products (Pycrz and Pezacki, 1974; Pezacki, 1978; Acton, 1977) - showing that it was difficult to use higher molecular weight carbohydrates, such as cooked rice, which are not so readily utilized by the starter cultures. In the present work it was found that 6% cooked rice with 0.5% glucose in the formulation, using mixed cultures - 10^3 cfu.g^{-1} both L. plantarum and M. varians and 10^6 cfu.g^{-1} P. cerevisiae fermented at 30°C and 97% RH gave a rapid pH reduction by 18 hours, produced desirable increases in firmness and colour and also resulted in a product which was microbiologically safe.

9.4.4 Effects of Frozen and Fresh Meat

Normally, the meat used in Nham formulation is fresh, but the Nham developed at New Zealand used a frozen form. In Nham made with fresh pork, the pH dropped more rapidly than when frozen pork was used. The most likely explanation is that the initial temperature of the mix affected the growth of the starter cultures and hence acid production in the initial period of fermentation. The Nham formulation using frozen lean pork exhibited rapid drip loss during thawing. A reduced moisture level, attributed to previously frozen raw lean pork, would reduce the rate of pH reduction. Bacus (1984) indicated that the use of frozen meat as an ingredient in dry sausage will retard the initial fermentation rate due to the reduced moisture. Bacus (1984) also reported that in general, the higher the lean percentage, i.e. low fat, the greater the moisture, resulting in more rapid pH drop. The contribution of glycogen from lean tissue appears to also have an effect on total acidity (Acton and Dick, 1975). The initial meat pH was also important in determining subsequent fermentation time and final product pH. Meats with higher pH values would require more acid production to achieve the same end point.

9.5 THE SHELF LIFE OF NHAM

Nham is usually sold in Thai markets at ambient temperatures (20-30°C) and in supermarkets at chiller temperature (5°C), and it can also be exported at low temperature (1°C). Additionally, consumers usually store the Nham in the household refrigerator (10°C). The pH reduction continued when the product was stored at 20-30°C, though

the change was slower at 5-10°C. The pH remained constant at 1°C. The same patterns of colour and firmness deterioration were also found. The weight loss continued during storage. Thus the higher the storage temperatures, the greater the changes observed in Nham quality. This study showed two rates of Nham quality deterioration, one at 20-30°C and the other at 1-10°C. However, if temperatures between 10 and 20°C are studied more closely, it is likely that a curved relationship between spoilage and temperature will be found. Acceptability of the Nham directly correlated with off-flavour attribute when the product was stored at 1-30°C. However, the consumers accepted the product stored at low temperature even though the off-flavour was detected at the same level as in the product stored at high temperatures. This might be because the other attributes, such as sourness, firmness, and colour of Nham stored at low temperatures were maintained while the off-flavour occurred. The consumers then percieved the product to be acceptable overall.

The counts of Enterobacteriaceae and S. aureus decreased during storage and no yeasts or moulds growth were observed. The Nham product was therefore not limited by microbial deterioration, but by chemical and physical spoilage.

It was found that Nham stored at 20-30°C would have a shelf-life of 9-11 days. From the consumer survey it was clear that shelf-life of Nham using mixed cultures had a longer shelf-life than the native Nham, which had only 3 days shelf-life. This finding was in agreement with the work of many researchers using starter cultures in the area of fermented meat products (Herborg and Johansen, 1977; Schubring and Kuhlmann, 1978) and in the area of nonfermented meat products (Reddy et al., 1970; Tezcan and Yuecel, 1975; Petaja, 1977; Raccach and Baker, 1978).

These results will enable product to be sent from Chiang Mai to all provinces in Thailand, particularly in Bangkok. It is possible that the product could be sent to overseas countries if refrigeration were applied. To be acceptable to importing countries, the product would have to demonstrate a consistently high standard of quality and safety. This would be assisted by the use of defined starter cultures.

9.6 SUBJECTIVE TESTS AND OBJECTIVE TESTS

In this project, subjective tests and objective tests were used in order to relate the sensory to the instrumental evaluations of the product. It was found that the Instron compression force was highly correlated with the ideal ratio scores of the sensory firmness

characteristics of Nham. This finding was in agreement with the work of Chinprahast (1988). Additionally, the tristimulus values (x, y, z) and pH were also highly correlated with the ideal ratio scores of the sensory colour and sourness characteristics of Nham respectively. These results showed that the data obtained by using subjective tests were in agreement with the data obtained by using objective tests.

Many researchers have also investigated correlations between sensory and instrumental evaluation in meat and meat products particularly between the sensory colour visual score and reflective colour; i.e. for pork meat r = 0.77 to 0.87 (Ockerman and Cahill, 1969); for beef meat r = 0.40 to 0.65 (Eagerman et al., 1977) and r = 0.80 (Jeremiah et al., 1972). Additionally, correlations between sensory and instrumental texture parameters were also found; i.e. Montejano et al. (1985) stated that sensory cohesiveness and hardness of meat protein gels correlated well with torsion shear strain (r = 0.87) and torsion shear stress (r = 0.72) respectively. However, the sensory attributes scores were normally obtained from the unstructured linear scales (Voisey et al., 1975; Brady and Hunecke, 1985; Brady et al., 1985), the structured linear scales (Bouton et al., 1975). This study showed that the ideal ratio scores obtained from sensory panel could be correlated with the Instron parameters, tristimulus values and pH value. Thus, pH of the product could be used for process control and final product testing because it correlated with firmness, colour and overall acceptability of the product.

9.7 RECOMMENDATION FOR FUTURE WORK

Although the final Nham was very successfully improved, there were some aspects which would benefit from further study

* Modify the meat system of Nham by varying the lean pork and pork skin and also adding Chilli to make a commercial product as indicated by the consumer.
* Using nitrite without nitrate reducing micrococci instead of nitrate in Nham formulation because nitrite could be reacted directly with myoglobin.
* Rate analysis of Nham quality in terms of firmness, off-flavour, sourness and colour during storage at 1-30°C particularly at 10-20°C, i.e. study the missing area approximately at 15°C.
* Effect of diameter of Nham casing on fermentation.
* Study of packaging methods suitable for export the product.

9.8 CONCLUSION

Nham produced by using a mixed starter culture consisting of 10^3 cfu.g^{-1} Micrococcus varians, 10^3 cfu.g^{-1} Lactobacillus plantarum and 10^6 cfu.g^{-1} Pediococcus cerevisiae was superior to the naturally fermented product and was of very high quality in terms of consistency and microbiological safety. The addition of 6% cooked rice and 0.5% glucose as carbon sources for lactic acid production was very important in achieving the desired pH in the final product. The M. varians reduced nitrate to nitrite at the beginning of fermentation. L. plantarum and P. cerevisiae used the cooked rice and glucose to produce lactic acid, to reduce the pH by 18 hours of fermentation at 30°C and 97% relative humidity. The Nham using fresh meat required only 2 days to reach a pH of 4.10. This ripening process was shorter than the current commercial production time of 4-5 days before the product is ready for consumption.

The major process control factor in Nham production with starter cultures was shown to be the control of ripening room temperature as it affected directly the acid production in the product. However, relative humidity of ripening is also economically important in reducing weight loss.

The firmness and colour development depended upon two factors: a decrease of pH of the Nham by L. plantarum and P. cerevisiae which influenced directly the firmness development and the reduction of nitrate to nitrite by M. varians with acid condition which influenced the colour development in Nham system. Acid production by lactic acid bacteria, particularly L. plantarum inhibited growth of Enterobacteriaceae and Staphylococcus aureus in the product. No yeasts and moulds were detected in the final product.

The Nham could be processed in a simple plant with existing equipment, the only increase in technology required being for culture preparation and control of incubator temperature.

The majority of consumers in Chiang Mai province accepted the experimental Nham and the product had also a longer shelf-life than the current commercial Nham. The product has the potential for markets in other provinces in Thailand and export to overseas countries. This could therefore be an important source of income for the northern Thai villages, which could help to alleviate some of the poverty and malnutrition.

REFERENCES

Acton, J. C. 1977. The chemistry of dry sausages. p. 49-62. In Proceedings 30th Annual Reciprocal Meat Conference of American Meat Science Association. Auburn, Alabama.

Acton, J. C., and Dick, R. L. 1975. Improved characteristics of dry fermented turkey sausage. Food Product Development, 9(8): 91-94.

Acton, J. C., Dick, R. L., and Norris, E. L. 1977. Utilization of various carbohydrates in fermented sausage. J. Food Sci., 42: 174-178.

Acton, J. C., and Keller, J. E. 1974. Effect of fermented meat pH on summer sausage properties. J. Milk Food Technol., 37: 570-576.

Adams, M. R. 1986. Progress in Industrial Microbiology. Volume 23. Micro-organisms in the Production of Food. Elsevier Science Publishers, B. V., The Netherlands.

Amerine, M. A., Pangborn, R. M., and Roessler, E. B. 1965. Principles of Sensory Evaluation of Foods. Academic Press, Inc., New York.

Anderson, A. M. 1975. A Quantitative Model for the Design of Nutritious and Acceptable Foods. Ph.D. Thesis in Product Development, Massey University, New Zealand.

Anderson, A. M. 1981 (a). Process Improvement for Small Food Companies in Developing Countries. A Workshop Manual, Massey University, New Zealand.

Anderson, A. M. 1981(b). Attitude measurement using consumer panels. Food Technol. in New Zealand, 16(5): 7-16.

Andersen, G., and Ten Cate, L. 1965. Addition of sugar and drop in pH-value during manufacture of salami-type sausages. Fleischwirtschaft, 45: 606.

AOAC. 1984. Official Methods of Analysis (13 rd ed.). Association of Official Analytical Chemists, Washington, D. C.

Bacus, J. 1984. Utilization of Microorganisms in Meat Processing. Research Studies Press, Ltd., England.

Bacus, J. N., and Brown, W. L. 1981. Use of microbial cultures: meat product. Food Technol., 35 (1): 74-78, 83.

Bacus, J. N., and Brown, W. L. 1985(a). The pediococci: meat products. p. 85-95. In Bacterial Starter Cultures for Foods. Edited by Gilliland, S. E., CRC Press, Inc., Boca Raton, Florida.

Bacus, J. N., and Brown, W. L. 1985(b). The lactobacilli: meat products. p. 57-71. In Bacterial Starter Cultures for Foods. Edited by Gilliland, S. E., CRC Press, Inc., Boca Raton, Florida.

Baran, W. L., and Stevenson, K. E. 1975. Survival of selected pathogens during processing of a fermented turkey sausage. J. Food Sci., 40: 618-620.

Barber, L. E., and Deibel, R. H. 1972. The effect of pH and oxygen tension on staphylococcal growth and enterotoxin production in fermented sausage. Appl. Microbiol., 24: 891-896.

Beausire, R. L. W., and Earle, M. D. 1986. Optimum location profiles: a profile technique used in product design. Food Technol. in Australia, 38(7): 298-300.

Blair, J. R. 1978. Interface of marketing and sensory evaluation in product development. Food Technol., 32(11): 61-62.

Boag, I. F. 1985 Fitting Equations to Data and the Design of Factorial Experiments. Production Technology Department, Massey University, New Zealand.

Boag, I. F. 1988. MUTAB/PC Computer Package. Massey University, New Zealand.

Booz-Allan and Hamilton Inc. 1980. The Management of New Products. Booz-Allan and Hamilton, Inc. New York.

Box, G. E. P., Hunter, W. G., and Hunter, J. S. 1978. Statistics for Experiments. John Wiley and Sons, Inc., New York.

Box, G. E. P., and Draper, N. R. 1987. Empirical Model-Building and Response Surfaces. John Wiley and Sons, Inc. New York.

Bouton, P. E., Ford, A. L., Harris, P. V., and Ratcliff, D. 1975. Objective-subjective assessment of meat tenderness. J. Texture Studies, 6: 315-328.

Brady, P. L., and Hunecke, M. E. 1985. Correlations of sensory and instrumental evaluations of roast beef texture. J. Food Sci., 50: 300-303.

Brady, P. L., McKeith, F. K., and Hunecke, M. E. 1985. Comparison of sensory and instrumental texture profile techniques for the evaluation of beef and beef-soy loaves. J. Food Sci., 50: 1537-1539.

Breed, R. S., and Dotterrer, W. D. 1916. The Number of Colonies Allowable on Satisfactory Agar Plates. Technical Bulletin 53. New York Agricultural Experiment Station, Geneva, New York.

Buchanan, R. E., and Gibbons, N. E. 1974. Bergey's Manual of Determinative Bacteriology. The Williams and Wilkins Company, Baltimore.

Cerise, L., Bracco, U., Horman, I., Sozzi, T., and Wuhrmann, J. J. 1973. Changes in the lipid fraction during ripening of a pure pork salami. Fleischwirtschaft, 53: 223-225.

Chinprahast, N. 1988. Development of a Mutton-Based Processed Meat Product for Export from New Zealand to Thailand. Ph.D. Thesis in Product Development, Massey University, New Zealand.

Chittaporn, P. 1977. A Quantitative Model for the Design of a Processed Infant Food Product for Thailand. Ph.D. Thesis in Product Development, Massey University, New Zealand.

Christiansen, L. N., Tompkin, R. B., Shaparis, A. B., Johnston, R. W., and Kautter, D. A. 1975. Effect of sodium nitrite and nitrate on C. botulinum growth and toxin production in summer style sausage. J. Food Sci., 40: 488-490.

Civille, G. V. 1978. Case studies demonstrating the role of sensory evaluation in product development. Food Technol. 32 (11): 59-60.

Cole, D. J. A., and Lawrie, R. A. 1975. Meat. Butterworth & Co (Publishers) Ltd., London.

Comenuanta, J. 1966. Thai Fermented Pork I. Microbiology of the Thai Fermented Pork. B. Sc. Thesis, Kasetsart University, Thailand.

Cooper, H. R. 1981. Sensory Evaluation of New Zealand Commercial Whole Milk Powders. Ph.D. Thesis in Product Development, Massey University, New Zealand.

Cooper, H. R., Earle, M. D., and Anderson, A. M. 1989. Use of Consumer and Market Research in Product Development. Product Development Sub-Department, Massey University, New Zealand.

Coretti, K. 1965. The occurrence and significance of lipolytic microorganisms in salami-type sausages intended for long storage. Fleischwirtschaft, 45: 23.

Coretti, K. 1977. Starter cultures in the meat industry. Fleischwirtschaft, 3: 386-388.

Corry, J. E. L., Roberts, D., and Skinner, F. A. 1982. Isolation and Identification Methods for Food Poisoning Organisms. Academic Press, New York.

Cross, H. R., Bernholdt, H. F., Dikleman, M. E., Greene, B. E., Moody, W. G., and West, R. L. 1978. Guidelines for Cookery and Sensory Evaluation of Meat. American Meat Science Association, Ad Hoc Committee, Chicago, Illinois.

Daly, C., Chance, M. L., Sandine, W. E., and Elliker, P. R. 1973. Control of Staphylococcus aureus in sausage by starter culture and chemical acidulation. J. Food Sci., 38: 426-430.

Deibel, R. H. 1974. Technology of fermented, semi-dried and dry sausages. p. 57-60. In Proceedings of the Meat Industry Research Conference. American Meat Institute Foundation, Chicago, IL.

Deibel, R. H., and Niven, C. F. Jr. 1957. Pediococcus cerevisiae: a starter culture for summer sausage. Bacteriol. Proc., 14-15.

Deibel, R. H., Niven, C. F. Jr., and Wilson, G. D. 1961(a) Microbiology of meat curing. III. Some microbiological and related technological aspects in the manufacture of fermented sausages. Appl. Microbiol., 9:156-165.

Deibel, R. H., Wilson, G. D., and Niven, C. F. Jr. 1961(b) Microbiology of meat curing. IV. A lyophilized Pediococcus cerevisiae starter culture for fermented sausage. Appl. Microbiol., 9: 239-243.

De Ketelaere, A., Demeyer, D., Vandekerckhove, P., and Vervaeke, J. 1974. Stoichiometry of carbohydrate fermentation during dry sausage ripening. J. Food Sci., 39: 297-300.

Dierick, N., Vandekerckhove, P., and Demeyer, D. 1974. Changes in nonprotein nitrogen compounds during dry sausage ripening. J. Food Sci., 39: 301-304.

Dixon, M. P. 1970. Sensory descriptive analysis: the flavour profile. The Flavour Industry, 1(1): 45-46.

Dobbertin, S., Siems, H., and Sinell, H. 1975. The bacteriology of fresh Mettwurst. II. Lipolytic activity and its dependency on the bacterial dynamics of fresh Mettwurst. Fleischwirtschaft, 55: 237-242.

Eagerman, B. A., Clydesdale, F. M., and Francis, F. J. 1977. Determination of fresh meat colour by objective methods. J. Food Sci., 42: 707-710, 724.

Earle, M. D. 1981. Selection and organisation of consumer panels. Food Technol. in New Zealand, 16(5): 16,18, 19.

Earle, M. D., and Anderson, A. M. 1985. Product and Process Development in the Food Industry. Harwood Academic Publishers, OPA Ltd., Amsterdam.

Eastlack, J. O. 1964. Consumer flavour preference factors in food product design. J. Marketing Research, 1(1): 38-42.

Ellis, B. H. 1970. Sensory methodology for product development. Food Product Development, 4(5): 86-87, 90-91.

Erhardt, J. P. 1978. The role of the sensory analyst in product development. Food Technol., 32(11): 57-58.

Everson, C. W., Danner, W. E., and Hammes, P. A. 1970(a). Bacterial starter cultures in sausage products. J. Arg. Food Chem., 18(4): 570-571.

Everson, C. W., Danner, W. E., and Hammes, P. A. 1970(b). Improved starter culture for semi-dry sausage. Food Technol., 24(1): 42, 44.

Fisher, R. A. 1941. Statistical Methods for Research Workers. Oliver and Boyd., London.

Fisher, R. A. 1960. The Design of Experiments. Oliver and Boyd., London.

Fiszer, W. 1970. The dynamics of dry sausage fermentation. Fleischwirtschaft, 50: 325.

Fleming, H. P., McFeeters, R. F., and Daeschel, M. A. 1985. The lactobacilli, pediococci, and leuconostocs in vegetable products. p. 97-118. In Bacterial Starter Cultures for Foods. Edited by Gilliland, S. E., CRC Pres, Inc., Boca Raton, Florida.

Floros, J. D., and Chinnan, M. S. 1988. Seven factor response surface optimization of a double-stage lye (NaOH) peeling process of pimiento peppers. J. Food Sci., 53: 631-644.

Freed, M., Brenner, S., and Wodicka, V. O., 1949. Prediction of thiamine and ascorbic acid stability in stored canned foods. Food Technol., 3(5): 148-151.

Food and Drug Administration, Ministry of Public Health. 1984. Food Additives (No. 84). The Food and Drug Administration, Ministry of Public Health, Thailand.

Fox, J. B. Jr., and Thomson, J. S. 1963. Formation of bovine nitrosylmyoglobin. Biochemistry, 2: 465-470.

Gatchalian, M. M. 1981. Consumer preference or acceptance tests. p. 230-273. In Sensory Evaluation Methods with Statistical Analysis. Edited by Gatchalian, M. M., College of Home Economics, University of the Philippines, Philippines.

Genigeorgis, C. A. 1976. Quality Control for fermented meats. J. Vet. Med. Assoc., 169(11): 1220.

Genigeorgis, C. A., Foda, M. S., Mantis, A., and Sadler, W. W. 1971. Effect of sodium chloride and pH on enterotoxin C production. Appl. Microbiol., 21: 862-866.

Gibbs, P. A. 1987. Novel uses for lactic acid fermentation in food preservation. J. Appl. Bacteriol. Symposium Supplement, 51s-58s.

Gilliland, S. E. 1985. Bacterial Starter Cultures for Foods. CRC Press, Inc., Boca Raton, Florida.

Gilliland, S. E., and Speck, M. L. 1977. Antagonistic action of Lactobacillus acidophilus towards intestinal and food-borne pathogens in associative culture. J. Food Protect., 40: 820-823.

Girardot, N. F. 1952. Some requirements for consumer preference testing of foods. Chief Psychometrician, The Coca-Cola Co.

Goepfert, J. M., and Chung, K. C. 1970. Behavior of salmonella during the manufacture and storage of a fermented sausage product. J. Milk Food Technol., 33: 185-191.

Haines, W. C., and Harmon, L. C. 1973 (a). Effect of variations in conditions of inoculation upon inhibition of S. aureus by Pediococcus cerevisiae and Streptococcus lactics. Appl. Microbiol., 25: 169-172.

Haines, W. C., and Harmon, L. C. 1973(b). Effect of selected lactic acid bacteria growth on S. aureus and production of enterotoxin. Appl. Microbiol., 25: 436-441.

Herborg, L., and Johansen, S. 1977. Fish cheese: the preservation of minced fish by fermentation. p. 253. In Proceedings Conference on Handling, Processing and Marketing of Tropical Fish. Ministry of Fisheries, Denmark.

Hill, C. G., and Grieger-Block, R. A. 1980. Kinetic data: generation, interpretation and use. Food Technol., 34(2): 56-66.

H-kittikun, A., Wiriyacharee, P., and Rujanakraikarn, L. 1988. Nham (Thai fermented pork) making with starter cultures. p. 427-429. In Proceedings 34th International Congress of Meat Science and Technology. Brisbane, Australia.

Huelin, F. E. 1953. Studies on the anaerobic decomposition of ascorbic acid. Food Res., 18: 633.

Hurst, A., and Collins-Thompson, D. L. 1979. Food as a bacterial habitat. Adv. Microb. Ecol., 3: 79-134.

Ingolf, F. N., and Skjelkvale, R. 1982. Effect of natural spices and oleoresins on Lactobacillus plantarum in fermentation of dry sausage. J. Food Sci., 47: 1618-1621, 1625.

Institute of Food Technologists. 1981. Sensory evaluation guide for testing food and beverage products. Food Technol., 35(11): 50-59.

Instron Limited. 1974. Instron Food Testing Instrument Manual; Table Model 1140. Serial No. H. 0125 O/No S 88816.

Ivey, F. J., and Robach, M. C. 1978. Effect of sorbic acid and sodium nitrate on Clostridium botulinum outgrowth and toxin production in canned comminuted pork. J. Food Sci., 43: 1782-1785.

Jeremiah, L. E., Carpenter, Z. L., and Smith, G. C. 1972. Beef colour as related to consumer acceptance and palatability. J. Food Sci., 37: 476-479.

Joglekar, A. M., and May, A. T. 1987. Product excellence through design of experiments. Cereal Foods World, 32(12): 857-868.

Joslyn, M. A., and Miller, J. 1949. Effect of sugars on oxidation of ascorbic acid. Food Res., 14: 325.

Karel, M. 1979. Prediction of nutrient losses and optimization of processing conditions. p. 233-263. In Nutritional and Safety Aspects of Food Processing. Edited by Tannenbaum, S. R., Marcel Dekker, New York.

Kastenschmidt, L. L., Hoekstra, W. G., and Briskey, E. J. 1968. Glycolytic intermediates and co-factors in 'fast' and 'slow' glycolyzing muscle of the pig. J. Food Sci., 33: 151-158.

Kiss, I. 1984. Testing Methods in Food Microbiology. Elsevier, Amsterdam-Oxford.

Kissell, L. T. 1967. Optimization of white layer cake formulation by a multiple-factor experimental design. Cereal Chem., 44: 253-268.

Klemmer, E. T. 1968. Psychological principles of subjective evaluation. p. 51-57. In Basic Principles of Sensory Evaluation. ASTM STP 433, American Society for Testing and Materials.

Klettner, P. G., and Baumgartner, P. A. 1980. The technology of raw dry sausage manufacture. Food Technol. in Australia, 32(8): 380-384.

Klettner, P. G., and Rodel, W. 1978. Testing and controlling parameters important to dry sausage ripening. Fleischwirtschaft, 58: 57-60, 63-64, 66.

Klement, J. T., Cassens, R. G., and Fennema, O. R. 1973. The association of protein solubility with physical properties in a fermented sausage. J. Food Sci., 38: 1128-1131.

Klement, J. T., Cassens, R. G., and Fennema, O. R. 1974. The effect of bacterial fermentation on protein solubility in a sausage model system. J. Food Sci., 39: 833-835.

Klement, J. T., Cassens, R. G., Fennema, O. R., and Greaser, M. L. 1975. Effect of direct acidification and heat on the solubility of protein extracts from a fermented sausage mix. J. Animal Sci., 41(2): 554-559.

Labuza, T. P., and Schmidl, M. K. 1985. Accelerated shelf-life testing of foods. Food Technol., 39(9): 57-62.

Lai Pai Wan. 1987. Development of a Bakery Snack for Export from New Zealand to Malaysia. Ph.D. Thesis in Product Development, Massey University, New Zealand.

Lamanna, C., Mallette, M. F., and Zimmerman, L. N. 1973. Basic Bacteriology: Its Biological and Chemical Background; Chapter 10 Nutrition of Bacteria. The Williams and Wilkins Company, Baltimore.

Lamden, M. P., and Harris, R. S. 1950. Browning of ascorbic acid in pure solutions. Food Res., 15:79.

Lee, I. C., Harmon, L. G., and Price, J. F. 1977. Growth and enterotoxin production by staphylococci in Genoa salami. J. Food Protect., 40: 325-329.

Lenz, M. K., and Lund, D. B. 1980. Experimental procedures for determining destruction kinetics of food components. Food Technol., 34(2): 51-55.

Liepe, H.-U. 1983. Starter cultures in meat production. p. 401-424. In Biotechnology A Comprehensive Treatise in 8 Volumes; Volume 5 Food and Feed Production with Microorganisms. Edited by Rehm, H.-J., and Reed, G., Verlag Chemie, Weinheim, Florida.

Lowry, P. D. ca 1984. Microbiology Methods for the Meat Industry. Meat Industry Research Institute of New Zealand; MIRINZ 757.

Lucke, F.-K. 1985. Fermented sausages. p. 41-83. In Microbiology of Fermented Foods (Volume 2). Edited by Wood, B. J. B., Elsevier Applied Science Publishers Ltd. London.

Masters, B. A. 1979. Fate of Salmonella inoculated into fermented sausage. M. S. Thesis, University of Florida, Gainesville, Fl.

Mihalyi, V., and Kormendy, L. 1967. Changes in protein solubility and associated properties during the ripening of Hungarian dry sausages. Food Technol., 21(10): 1398-1402.

Miller, J. C., and Miller, J. N. 1984. Statistics for Analytical Chemistry. Ellis Horwood Limited, Chichester, England.

Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, 31: 426-428.

Molins, R. A., Kraft, A. A., Olson, D. G., and Hotchkiss, D. K. 1984. Recovery of selected bacteria in media containing 0.5% food grade poly-and pyrophosphates. J. Food Sci., 49: 948-949.

Montejano, J. G., Hamann, D. D, and Lanier, T. C. 1985. Comparison of two instrumental methods with sensory texture of protein gels. J. Texture Studies, 16: 403-424.

Moskowitz, H. R. 1984. Relative importance of sensory factors to acceptance: theoretical and empirical analyses. J. Food Quality, 7: 75-90.

Moskowitz, H. R. 1985. New Directions for Product Testing and Sensory Analysis of Foods. Food and Nutrition Press, Inc., Westport, Connecticut.

Moskowitz, H. R., and Chandler, J. 1979. Notes on consumer oriented sensory evaluation. J. Food Quality, 2: 269-276.

Moskowitz, H. R., and Rabino, S. 1983. Alternative strategies for product optimization. Advances in Strategic Management, 2: 99-123.

Myers, R. H. 1971. Response Surface Methodology. Allyn and Bacon, Boston.

Newton, K. G., and Gill, C. O. 1981. The microbiology of DFD fresh meats: a review. Meat Sci., 5: 223-232.

Ngarmsak, T. 1983. A System of Meal Planning for Self-Improvement of the Diet of Villagers in Northeastern Thailand. Ph.D. Thesis in Product Development, Massey University, New Zealand.

Niinivaara, F. P. 1955. The influence of pure bacterial cultures on aging and changes of the red colour of dry sausage. Thesis, University of Helsinki, Finland, Acta Agralia Finnica No. 84.

Niinivaara, F. P., Pohja, M. S., and Komulainen, S. E. 1964. Some aspects about using bacterial pure cultures in the manufacture of fermented sausages. Food Technol., 18(2): 25-31.

Niskanen, A., and Nurmi, E. 1976. Effect of starter culture on staphylococcal enterotoxin and thermonuclease production in dry sausage. Appl. Microbiol., 31: 11-20.

Niven, C. F. Jr., Brettner, L. G., and Evans, J. B. 1954. Thermal tolerance studies on the heterofermentative lactobacilli that cause greening of cured meat products. Appl. Microbiol., 2: 26-29.

Niven, C. F. Jr., Castellani, A. G, and Allanson, V. 1949. A study of the lactic acid bacteria that cause surface discolorations of sausages. J. Bact., 58: 633-641.

Nordal, J., and Slinde, E. 1980. Characteristics of some lactic acid bacteria used as starter cultures in dry sausage production. Appl. and Environ. Microbiol., 43(3): 472-475.

Norman, H. N. 1983. SPSS^x Programme and SPSS^x User's Guide. SPSS Inc., Chicago, Illinois.

Nurmi, E. 1966. Effect of bacterial inoculations on characteristics and microbial flora of dry sausage. Thesis, University of Helsinki, Finland, Acta Agralia Finnica No. 108.

Ockerman, H. W., and Cahill, V. R. 1969. Reflectance as a measure of pork and beef tissue colour. J. Animal Sci., 28(6): 750-754.

Pakrachpan, L. 1981. Fermented Food Industry (in Thai). Biotechnology Department, Faculty of Agro-Industry, Kasetsart University, Thailand.

Palumbo, S. A., Zaika, L. L., Kissinger, J. C., and Smith, J. L. 1976. Microbiology and technology of the pepperoni process. J. Food Sci., 41: 12-17.

Pangborn, R. M. 1980. Sensory science today. Cereal Foods World, 25(10): 637-640.

Park, H. S., and Marth, E. H. 1972. Behaviour of Salmonella typhimurium in skim milk during fermentation by lactic acid bacteria. J. Milk Food Technol., 35: 482.

Parry, R. T., Haysom, L., Thomas, N. L., and Davis, R. 1982. A Manual of Recommended Methods for the Microbiological Examination of Poultry and Poultry Products. British Poultry Meat Association Ltd., High Holborn, London.

Passy, N., and Mannheim, C. H. 1979. The effect of deaeration on quality of concentrated grapefruit juice. p. 141-153. In Tropical Foods: Chemistry and Nutrition (Volume 1). Edited by Inglett, G. E., and Charalambous, G., Academic Press, New York.

Pearson, A. M., and Dutson, T. R. 1986. Advances in Meat Research Volume 2: Meat and Poultry Microbiology. AVI Publishing Company, Inc., Westport, Connecticut.

Pederson, C. S. 1979. Microbiology of Food Fermentations. 2nd edition. AVI Publishing Company, Inc., Westport, Connecticut.

Petaja, E. 1977. Investigations on the use of starter cultures in frankfurter-type sausages. Fleischwirtschaft, 57: 109-112.

Peters, J. W. 1980. Fermentation may answer needs for natural foods, low-energy processes. Food Product Development. 14(9): 48-49.

Pezacki, W. 1978. Technological control of dry sausage ripening. VIII. Effect of pre-drying on the dynamics of carbohydrate changes taking place at the beginning of ripening. Fleischwirtschaft, 58: 124-126, 129-132, 135.

Pezacki, W., and Fiszer, W. 1966. The dynamics of dry sausage fermentation. VI. Quantity relationship of the main biochemical changes when adding 1, 6 ^{14}C glucose. Fleischwirtschaft, 46: 1339.

Pezacki, W., and Jaroszewski, Z. 1963. The dynamics of the fermentation of salami-type sausages. Fleischwirtschaft, 43: 1029.

Pezacki, W., and Szostak, D. 1962. The dynamics of dry sausage fermentation. I. Homo-and hetero-lactic fermentation. Fleischwirtschaft, 42: 180.

Pezacki, W., and Urbaniak, L. 1965. Physiological properties of the aerobic microflora of salami-type sausages. Fleischwirtschaft, 45: 115.

Pfeil, E., and Liepe, H.-U. 1973. The influence of the nitrate reductase system on the residual nitrite content in dry sausage and its dependency on external condition. Fleischwirtschaft, 53: 1745-1747.

Pfeil, E., and Liepe, H.-U. 1974. Nitrate, nitrite and bacteria in the manufacture of raw cured meats. Fleischwirtschaft, 54: 1717-1718.

Pycrz, J., and Pezacki, W. 1974. Technological control of dry sausage ripening. III. The effects of different kinds of carbohydrates on the dynamics of process in a collection of acid fermentation products. Fleischwirtschaft, 54: 203-211.

Raccach, M. 1981. Control of Staphylococcus aureus in dry sausage by newly developed meat starter culture and phenolic-type antioxidants. J. Food Protect., 44: 665-669.

Raccach, M. 1984. Method for selection of lactic acid bacteria and determination of minimum temperature of meat fermentations. J. Food Protect., 47: 670-671.

Raccach, M. 1986. Lactic acid fermentation using high levels of culture and the fate of Staphylococcus aureus in meat. J. Food Sci., 51: 520-521, 523.

Raccach, M., and Baker, R. C. 1978. Lactic acid bacteria as an anti-spoilage and safety factor in cooked, mechanically deboned poultry meat. J. Food Protect., 41: 703-705.

Raccach, M., Baker, R. C., Regenstein, J. M., and Mulnix, E. J. 1979. Potential application of microbial antagonism to extended storage ability of a flesh type food. J. Food Sci., 44: 43-46.

Radtke, K. H., and Rodriguez, N. C. 1981. In-house preference testing: an R & D resource. Food Technol., 35(11): 67-69.

Ramsay, G. A. 1981. Solvent Fractionation of New Zealand Mutton Tallow. Ph.D. Thesis in Biotechnology, Massey University, New Zealand.

Reddy, S. G., Chen, M. L, and Patel, P. J. 1975. Influence of lactic cultures on the biochemical, bacterial and organoleptic changes in beef. J. Food Sci., 40: 314-318.

Reddy, S. G., Henrickson, R. L., and Oleson, H. C. 1970. The influence of lactic cultures on ground beef quality. J. Food Sci., 35: 787-791.

Reuter, G., Langner, H. J., and Sinell, H. J. 1968. Development of microflora in quick-ripening German dry sausage and analagous quantitative amino acid analysis in a salami. Fleischwirtschaft, 48: 170.

Rhim, J. W., Nunes, R. V., Jones, V. A., and Swartzel, K. R. 1989. Kinetics of colour change of grape juice generated using linearly increasing temperature. J. Food Sci., 54: 776-777.

Rice, E. E. 1971. The nutritional content and value of meat and meat products. p. 287-327. In The Science of Meat and Meat Products. Edited by Price, J. F., and Schweigert, B. S., 2nd edition, W. H. Freeman and Co., San Francisco.

Rojanasaroj, C., Khuntaphanit, C., and Sitthichanthasen, S. 1980. The relationship between the bacterial count and pH in Nham bought from the market. Khon Kaen: Northeastern Agricultural Centre, Ministry of Agriculture.

Roth, L. A., and Clark, D. S. 1975. Effect of lactobacilli and carbon dioxide on the growth of Microbacterium thermosphactum on fresh beef. Canadian J. Microbiol., 21: 629-632.

Saguy, I., and Karel, M. 1980. Modelling of quality deterioration during food processing and storage. Food Technol., 34(2): 78-85.

Saguy, I., Kopelman, I. J., and Mizrahi, S. 1978(a). Simulation of ascorbic acid stability during heat processing and concentration of grapefruit juice. J. Food Proc. Eng., 2: 213-225.

Saguy, I., Kopelman, I. J., and Mizrahi, S. 1978(b). Extent of nonenzymic browning in grapefruit juice during thermal and concentration processes: kinetics and prediction. J. Food Proc. Preservation, 2: 175.

Schubring, R., and Kuhlmann, W. 1978. Preliminary studies on application of starter cultures in the manufacture of fish products. Lebensmittel Industrie, 25(10): 455.

Sharpe, A. N., and Jackson, A. K.. 1972. Stomaching: a new concept in bacteriological sample preparation. Appl. Microbiol., 24: 175-178.

Sinthavalai, S. 1986. Development of Nutritionally-Balanced Snack Product for Urban School-Age Thais. Ph.D. Thesis in Product Development, Massey University, New Zealand.

Smith, J. L, Huhtanen, C. N., Kissinger, J. C., and Palumbo, S. A. 1975(a). Survival of salmonellae during pepperoni manufacture. Appl. Micrbiol., 30: 759-763.

Smith, J. L., Palumbo, S. A., Kissinger, J. C. and Huhtanen, C. N. 1975(b). Survival of Salmonella dublin and Salmonella typhimurium in Lebanon bologna. J. Milk Food Technol., 38: 150-154.

Smith, J. L., and Palumbo, S. A 1978. Injury to Staphylococcus aureus during sausage fermentation. Appl. Environ. Microbiol., 36: 857-860.

Somathiti, S. 1982. A Survey of Some Enteropathogenic Bacteria in Thai Fermented Pork. M.S. Thesis, Kasetsart University, Thailand.

Speck, M. L. 1976. Compendium of Methods for the Microbiological Examination of Foods. 2nd edition, American Public Health Association, Washington, D. C.

Srisomwong, P. 1985. The study of lactic acid bacteria in Nham. M. Appl. Sci. Thesis, University of New South Wales, Australia.

Steinkraus, K. H., Roger, E. C., Carl, S. P., Lois, F. N., and Ben, K. G. 1983. Handbook of Indigenous Fermented Foods. Marcel Dekker Inc., New York.

Stoychev, M., Djejeva, G., Brankova, R., and Niinivaara, F. P. 1972(a). Impact of pH and different NaCl concentrations on the lipase activity of starter cultures used in connection with the production of raw-dried meat products. p. 113-118. In Proceedings 18th Annual Meeting of European Meat Research Workers. Guelph, Ontario, Canada.

Stoychev, M., Djejeva, G., and Brankova, R. 1972(b). Studies on lipase activity of some starter cultures at temperatures used in the manufacture and storage of raw-dried meat products. p. 105-109. In Proceedings 18th Annual Meeting of European Meat Research Workers. Guelph, Ontario, Canada.

Stowe, R. A., and Mayer, R. P. 1966. Efficient screening of process variables. Industrial and Engineering, 58(2): 36-40.

Taguchi, G., and Wu, Y. 1980. Introduction to off-line quality control. Central Japan Quality Control Association, Japan.

Talon, R., Labadie, J., and Larpent, J.-P. 1980. Characterization of the inhibitory power of Lactobacillus of meat origin. Zentralblatt fur Bakteriologie und Hygiene, I. Abteilung Originale B 170, 133-142.

Tanaka, N. E., Traisman, M. H., Cassens, R. G., and Foster, E. M. 1980. Inhibition of botulinum toxin formation in bacon by acid development. J. Food Protect., 43: 450-457.

Tanasupawat, S., and Daengsubha, W. 1983. Pediococcus species and related bacteria found in fermented foods and related materials in Thailand. J. Gen. Appl. Microbiol., 29: 487-506.

Tandler, K. 1963. The use of sugar substances in manufacture of salami-type sausage. Fleischwirtschaft, 43: 804.

Techapinyawat, S. 1975. Microbial Study During Fermentation of Thai Fermented Pork. M. S. Thesis, Kasetsart University, Thailand.

Terlizzi, F. M., Acton, J. C., and Skelley, G. C. 1980. Comparison of non-fermented and fermented hams formed with muscle strips, cubes and ground meat. Tech. Contribution No. 1722, South Carolina Ag. Exp. Station, Clemson.

Tezcan, I., and Yuecel, A. 1975. Effect of a Lactobacillus starter culture in vacuum packaged meat on Salmonella and Pseudomonas organisms. Veteriner Hekimler Dernegi Dergisi, 45(4): 7-10.

Townsend, W. E., Davis, C. E., and Mercuri, A. J. 1975. Effect of Chemically adjusted meat pH and drying air velocity on some properties of dry sausage. J. Milk Food Technol., 38: 764-768,

Twiss, B. C. 1980. Managing Technological Innovation. 2nd edition, Longman Inc., New York.

Urban, G. L., and Hauser, J. R. 1980. Design and Marketing of New Products. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Van Tel, H. 1988. Massey Plackett-Burman Screening Package. Food Technology Department, Massey University, New Zealand.

Vichiensanth, P. 1982. The Design of a Shelf-Stable Sausage for Thailand. M. S. Thesis, Massey University, New Zealand.

Voisey, P. W., Randall, C. J., and Larmond, E. 1975. Selection of an objective test of weiner texture by sensory analysis. Canadian Institute of Food Science and Technology Journal, 8(1): 23-29.

Waletzko, P., and Labuza, T. P. 1976. Accelerated shelf-life testing of an intermediate moisture food in air and in an oxygen-free atmosphere. J. Food Sci., 41: 1338-1344.

Walonick, D. S. 1987. Stat-Packets. Walonick Associates Inc., Minneapoils, MN.

Weerasooriya, C. 1987. The Effect of Processing Variables on Fish Smoking. Postgraduate Diploma Thesis in Product Development, Massey University, New Zealand.

Wells, J. H., and Singh, R. P. 1988. A kinetic approach to food quality prediction using full-history time-temperature indicators. J. Food Sci., 53: 1866-1871, 1893.

Wiriyacharee, P., and H-kittikun, A. 1984. Industrial Fermentation Laboratory Manual (in Thai). Food Science and Technology Department, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand.

Wongkhalaung, C. and Boonyaratanakornkit, M. 1986. Fermented Foods in Thailand and Similar Products in Asean and Elsewhere. Institute of Food Research and Product Development, Kasetsart University, Thailand.

Wood, B. J. B. 1985. Microbiology of Fermented Foods. Volume 2. Elsevier Applied Science Publishers, Ltd. London.

Zaika, L. L., and Kissinger, J. C. 1979. Effect of some spices and acid production by starter cultures. J. Food Protect., 42: 572-576.

Zaika, L. L., and Kissinger, J. C. 1984. Fermentation enhancement by spices: Identification of active component. J. Food Sci., 49: 5-9.

Zaika, L. L., Zell, T. E., Palumbo, S. A., and Smith, J. L. 1978. Effect of spices and salt on fermentation of Lebanon bologna-type sausage. J. Food Sci., 43: 186-189.

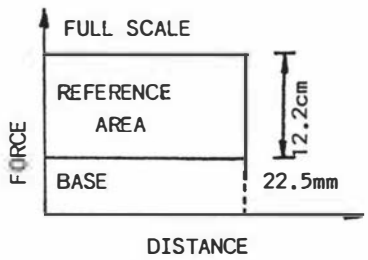
APPENDIX 2.1

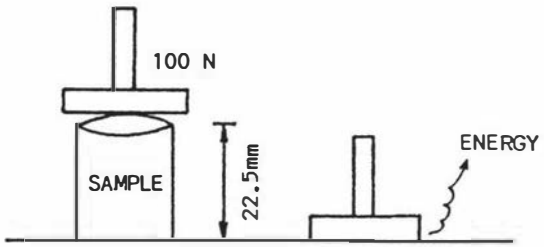
The calculation of compression and shear energy.

The area of peak in each case from chart recorder was measured by planimeter before converting to energy. The calculation

$$\frac{\text{Sample area (A2)}}{\text{Reference area (A1)}} = \frac{\text{Unknown energy}}{\text{Reference energy}}$$

An example is presented as below:





At a chart speed of 200 mm/min (3.3 mm./sec) a sample of 22.5 mm diameter being cut at a crosshead speed of 200 mm/min (3.33 mm/sec) will take (22.5/3.33) sec to cut through that is 6.76 seconds. This will be represented on the chart by a distance of 3.33 x 6.76 equals to 22.5 mm or 22.5 x 10^{-3} m. As a reference area equals force x distance, therefore Newtons x Meter equals energy (joules). The applied force (100 Newtons) that is equivalent to 12.2 cm or 12.2 x 10^{-2} m is used to break down the sample with chart distance of 22.5 x 10^{-3}m.

Therefore, operation of reference energy = (100 N) (0.0225 m)
= 2.25 Nm or Joules

chart of reference area (A_1) = (0.122 m) (0.0225 m)
= 2.74 x10^{-3} m^2

If measured sample area (A_2) = 4.2 cm^2 = 4.20 x 10^{-4} m^2.

$$\text{Unknown energy (Joules)} = \frac{\left(4.2 \times 10^{-4}\right)(2.25)}{\left(2.74 \times 10^{-3}\right)}$$

= 0.345 Joules

APPENDIX 2.2

The gas formation calculation

According to gas law; $V_1P_1/T_1 = V_2P_2/T_2$

that is $$V_1 = V_2 \frac{P_2}{P_1} \frac{T_1}{T_2} \qquad [1]$$

where V_1 = Volume of gas at 1 atm and 0°C
V_2 = Measured volume of gas
P_1 = Reference pressure = 1 atm
P_2 = Pressure of gas on measuring volume (V_2)
T_1 = Reference temperature (0°C or 273°K)
T_2 = Measured temperature in °K

The pressure on measuring the volume is below that of atmospheric pressure (Pa) and because of the column of ethanol (EtOH), therefore the actual pressure drop is give by

$$\Delta P = pq\,(d_2 - d_1)$$

where ΔP = actual pressure drop; $kgm^{-1}s^{-1}$
p = density of EtOH = 789.3 kgm^{-3}
q = specific gravity of EtOH = 9.81 ms^{-1}
d_2 = The height of EtOH in burette (m)
d_1 = The height of EtOH in beaker (m)

Therefore, from [1];

$$V_1 = V_2 \frac{T_1}{T_2} \frac{(Pa - \Delta P)}{P_1}$$

$$V_1 = V_2 \frac{T_1}{T_2} \frac{[Pa - pq\,(d_2 - d_1)]}{P_1} \qquad [2]$$

or if Pa/P_1 are in m of EtOH then

$$V_1 = V_2 \frac{T_1}{T_2} \frac{[Ha - (d_2 - d_1)]}{H_1} \qquad [3]$$

The height of EtOH equivalent to an atmospheric pressure Pa ($kg\ m^{-1}s^{-1}$) is related to height of liquid (H), therefore

$Pa = p\,EtOH \,.\, q.\, H = kg\ m^{-1}s^{-1} = Newtons$

As $1kN/m^2 = 0.2953$ in of Hg = 7.50062 mm of Hg
if 1 atm (760 mm of Hg), the pressure is $101.33 \times 10^3\ N/m^2$

Therefore, $H = Pressure/pEtOH.q$
$H = 13.085$ m of EtOH

So if $T_1 = 273°K$
$H_a = 13.141$ m (≡ 1 atm using EtOH as fluid at 20°C)
$H_1 = 13.085$ m (≡ 1 atm using EtOH as fluid at 0°C)
and $d_2 = 360$ mm = 0.36 m
$d_1 = 60$ mm = 0.06 m
$V_2 = 30$ ml = $3 \times 10^{-5} m^3$
$T_2 = 20°C = 293°K$

Therefore,

$$V_1 = 3 \times 10^{-5} \cdot \frac{273}{293} \frac{[13.141 - (0.3)]}{13.085}$$

$= 2.74 \times 10^{-5}\ m^3$
$= 27.4$ ml [0°C/1 atm = NTP]

APPENDIX 2.3

Standard curve of residual nitrite

Reagents:

a) NED reagent - Dissolve 0.2g N -[1 -naphthyl] ethyleneamine . 2 HCl in 150 ml 15 % v/v HOAC. Filter, if necessary, and store in g-s brown glass bottle.

b) Sulfanilamide reagent - Dissolve 0.5g sulfanilamide in 150ml 15% HOAC. Filter, if necessary, and store in g-s brown glass bottle.

c) Nitrite standard solution
   (1) Stock solution 1000 ppm $NaNO_2$ - Dissolve 1.000g $NaNO_2$ in water and dilute to 1 litre.
   (2) Intermediate solution 100ppm $NaNO_2$ - Dilute 100ml stock solution to 1 litre with water.
   (3) Working solution 1 ppm $NaNO_2$ - Dilute 10 ml intermediate solution to 1 litre with water.

Method:

Add 5, 10, 15, 20, 25, 30, 35, 40, and 45 ml nitrite working solution to 50 ml volumetric flasks and add 2.5 ml sulfanilamide reagent (b) mix, and after 5 min, add 2.5 ml NED reagent (a), mix, dilute to volume, mix and let colour develop 15 minutes. Transfer portion of solution to photometer cell and determine Absorbance at 540 nm. against blank of 45 ml H_2O, 2.5 ml sulfanilamide reagent and 2.5 ml NED reagent.

The standard curve:

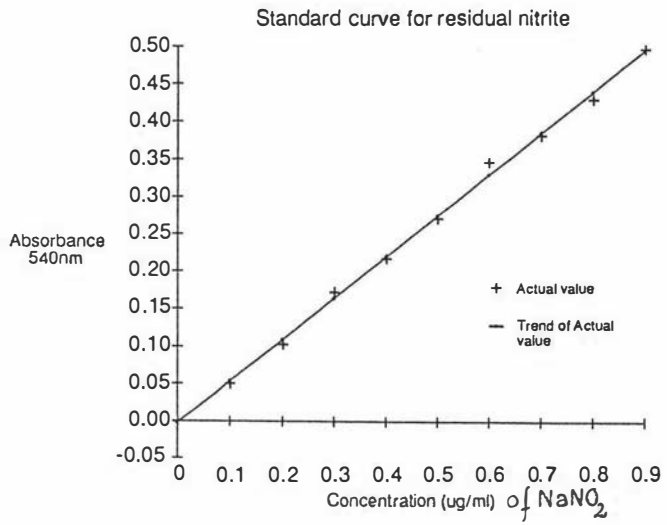
The fitted equation was:

concentration = 0.004 + 1.809 (OD)

As the constant coefficient was not significant (t - value = 0.43) whereas the optical density coefficient was strongly significant at 99.9% confidence level (t-value = 62.80). Therefore,

concentration = 1.809 (OD)

R^2 = 99.80%



APPENDIX 2.4

Reducing sugars and cooked rice determination

Solutions:

Zinc acetate solution -	Dissolve 12 g Zn $(OAC)_2$. 2 H_2O in water and dilute to 100 ml.
Potassium ferrocyanide solution -	Dissolve 6 g $K_4Fe(CN)_6$. $2H_2O$ in water and dilute to 100 ml.
Phosphotungstic acid solution -	Dissolve 20 g phosphotungstic acid in water, dilute to 100 ml and filter.
Dinitro Salicylic acid reagent -	Sodium hydroxide 10 g/l, Potassium sodium tartrate 182 g/l, Dinitro salicylic acid 10 g/l, phenol 2 g/l and sodium sulphite 0.5 g/l of water.

Extraction and glucose measurement

- 10 g homogenated sample were weighed into 250 ml heat-resistant centrifuge bottle. 100 ml water,5 ml of Zn $(OAC)_2$ and 5 ml of $K_4Fe(CN)_6$ solution were then added and the bottle closed tightly and let stand 15 minutes, shaking vigorously several times during this period.

- The sample was centrifuged 15 minutes at 1500 rpm and decanted supernatant into 12.5 cm Whatman No. 3 filter paper in filter funnel. Added 25 ml freshly prepared solution containing 1 ml Zn $(OAC)_2$ plus 1 ml $K_4Fe(CN)_6$ solution/200 ml solution in the bottle to extract residue and let stand 10 minutes, shaking several times during this period; then centrifuged 10 minutes at 1500 rpm and decanted through same paper. Repeated last extraction with addition 25 ml $Zn(OAC)_2$- $K_4Fe(CN)_6$ solution. Rinsed stopper with water.

- The supernatant (Filtrate) was used to determine the reducing sugars as glucose. 1 ml mixed filtrate with 4 ml DNS reagent (Dinitro salicylic acid reagent) and boiled in water bath (90°C) for 30 minutes. Added 5 ml cold water and cooled immediately under running water adjusted to room temperature. Cooling to ambient temperature was necessary because of the effect of temperature on the absorbance of the coloured reaction product. The colour intensities were measured in Beckman Model DU. spectrophotometer at 575 nm with a slit width 0.06 mm. The results were calculated with a standard curve for glucose which was 0 mg/ml - 1.0 mg/ml at different absorbances (Appendix 2.5). If absorbance of sample read more than 0.5, the solution was diluted before colour development.

Therefore, % Reducing sugars as glucose = (3.052) (O.D.).

Starch hydrolysis

- Placed funnel containing the filter paper on top of a centrifuge bottle. 90 ml hot (ca 70°C) 1.5 HCl, poured 40 ml into the filter paper and let acid run into centrifuge bottle. Washed paper with remainder of acid solution (50 ml). Suspended bottle in open boiling water bath so that level of water in bath was at approximately the level of solution within bottle. Did not reflux. Hydrolysed exactly 1.5 hours, keeping water level of bath at original position, stirring contents of bottle occasionally. Did not transfer paper to centrifuge bottle, as it was hydrolyzed and gave high values.

- Cooled immediately (if necessary, sample might stand overnight at this point). Made first alkaline to litmus with 20% NaOH (ca 27ml) and then added 10 ml HCl (1+2). Transferred to 200 ml erlenmeyer flask marked at 200 ml. Rinsed centrifuge bottle with 15 ml phosphotungstic acid solution, followed by several 10 ml portions water. Diluted to volume, if any, just above mark. Closed and mixed, let stand 30 minutes and filtered solution through Whatman NO 1 paper. And then determined reducing sugars by DNS method and converted to percentage of cooked rice.

- In order to calculate a factor for converting from reducing sugars to cooked rice, the 1.000 g homogenized cooked rice (10 replications) was used in the above method and the reducing sugars determined. The factor obtained was

1 g of cooked rice = 0.172 g reducing sugars

Therefore, % starch (as cooked rice) = (12.674) (O.D.).

APPENDIX 2.5

Standard curve for reducing sugars as glucose:

Reagents:

a) Dinitro Salicylic acid reagent - the same as Appendix 2.4.

b) Standard glucose solution - Dissolve 1.00 g AR. glucose in water and dilute to 1 litre.

Methods:

Pipet 0.1 - 0.9 ml of standard glucose solution and dilute up to 1 ml with water and add 4 ml DNS reagent (a) and heat at 90°C water bath for 30 mintues. And then add water up to 10 ml and cool quickly under running water. Eventually, the colour intensities were measured in Beckman Model DU. Spectrophotometer at 575 nm against blank of 1 ml water, 4 ml DNS reagent.

The standard curve:

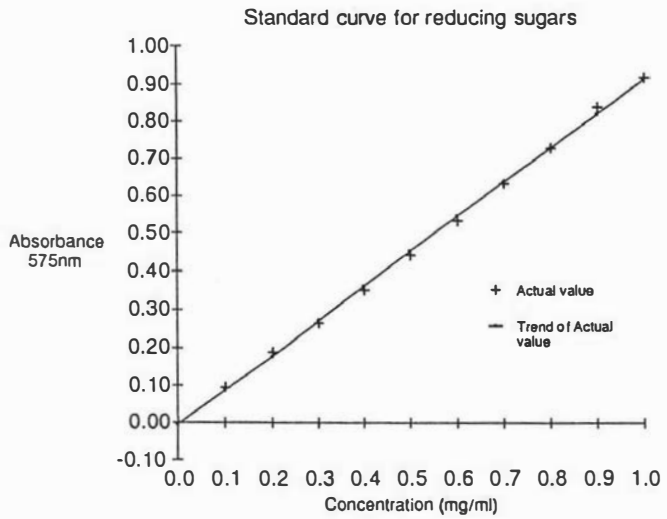
The fitted equation was:

concentration = 0.006 + 1.090 (OD)

As the constant coefficient was not significant (t-value = 0.85) whereas the optical density coefficient was strongly significant at 99.9% confidence level (t-value = 90.72).

Therefore concentration = 1.090 (OD)

R^2 = 99.89%.



APPENDIX 2.6

The important attributes of Nham identified by panelists.

Nham characteristics	Important attributes										Total	Average Number	Order(s)
	Panelists												
	1	2	3	4	5	6	7	8	9	10			
A: Appearance													
1 Colour	1	1	1	4	1	1	5	1	1	1	17	1.7	1
2 Visual texture	2	2	2	7	2	7	6	2	2	2	34	3.4	2
3 Air pockets	6	3	7	7	7	7	7	7	7	7	65	6.5	9
B: Texture													
4 Firmness	5	6	5	2	7	3	1	6	3	7	45	4.5	4
5 Juiciness	7	7	7	7	7	7	7	5	5	7	66	6.6	10
6 Fattiness	7	7	7	7	7	7	7	7	7	7	70	7.0	11
7 Stickiness	7	7	7	7	7	7	7	7	7	7	70	7.0	12
8 Smoothness	7	7	6	7	4	6	7	7	7	6	64	6.4	8
C: Flavour and Taste													
9 Sourness	3	7	3	5	5	5	3	3	4	3	41	4.1	3
10 Saltiness	4	7	7	6	7	7	2	4	7	4	55	5.5	7
11 Spiciness	7	4	4	3	6	4	7	7	6	5	53	5.3	6
12 Pork flavour	7	5	7	1	3	2	4	7	7	7	50	5.0	5

The data presented is the order of important attributes that is 1 = most important, 7 = least important.

APPENDIX 2.7

Formulation of Nham for first trained panel to identify and discuss Nham characteristics

Ingredients	A (g)	B (g)	C (g)
minced lean pork	800	800	800
sliced pork skin	200	200	200
cooked rice	80	80	50
glucose	-	10	5
sodium chloride	20	30	40
sodium tripolyphosphate	-	3	3
sodium nitrate	0.1	0.2	0.2
minced garlic	30	70	50
white pepper; powder	0.2	0.5	0.3
cultures; (cfu/g)			
M. varians	-	10^6	10^6
L. plantarum	-	10^6	10^6
L. brevis	-	10^6	-
P. cerevisiae	-	10^6	-

A: Natural fermentation

B: Four starter cultures fermentation

C: Two starter cultures fermentation

APPENDIX 2.8

The questionnaire and definitions used to train the panelists in Thailand

แบบการทดสอบทางประสาทสัมผัส

ลักษณะที่สำคัญของแหนม

ภาควิชาวิทยาศาสตร์และเทคโนโลยีการอาหาร มหาวิทยาลัยเชียงใหม่

ชื่อผู้ทดสอบชิม ______________________ วันที่ ______________

สวัสดีครับท่านผู้ทดสอบชิม

ขณะนี้ผมกำลังศึกษาลักษณะที่สำคัญของแหนมโดยใช้ดัชนีทางด้านประสาทสัมผัสของผู้ทดสอบชิมในจังหวัดเชียงใหม่

โปรดทดสอบชิมตัวอย่างแหนม และกากบาท (X) ทับสเกลที่ท่านคิดว่าตัวอย่างแหนมที่ท่านกำลังชิมควรจะมีลักษณะนั้น ๆ มากน้อยเพียงใด และขีดอักษรรูปตัว (I) ลงบริเวณที่ท่านคิดว่าลักษณะของตัวอย่างแหนมที่ท่านกำลังชิมควรมีลักษณะนั้น ๆ ที่ดีที่สุดตรงจุดใด

ตัวอย่าง

1. ความเหนียวแน่น

เมื่อ : 001 = ตัวอย่างแหนมที่กำลังทดสอบชิม

I = จุดที่ดีที่สุดของตัวอย่างแหนมที่กำลังทดสอบชิม

หลังจากท่านเขียนลักษณะต่าง ๆ ของตัวอย่างแหนมเป็นที่เรียบร้อยแล้ว ถ้าท่านคิดว่าควรเพิ่มเติมลักษณะอื่น ๆ อีก โปรดระบุลงในส่วนท้ายในคำเสนอแนะ

ส่งคืนแบบทดสอบชิมนี้หลังเสร็จสิ้นการชิมพร้อมตัวอย่างแหนมที่เหลือ

โปรดรอตัวอย่างแหนม ตัวอย่างต่อไป

ขอขอบคุณทุกท่านที่สละเวลาและให้ความร่วมมือในการทดสอบชิมครั้งนี้ ข้อมูลที่ได้รับจากท่านนับว่ามีประโยชน์ต่อการพัฒนาผลิตภัณฑ์ดังกล่าวอย่างมาก..

ไพโรจน์ วิริยจารี

คำนิยามของคำที่อธิบายลักษณะของผลิตภัณฑ์แหนม

ก. ลักษณะปรากฏต่อสายตา

1. สี	ดูความเข้มของสีแดงทั้งภายนอกและภายในตัวผลิตภัณฑ์แหนม
2. ลักษณะเนื้อแหนม	ดูการกระจายตัวของเนื้อหมูและหนังหมูในผลิตภัณฑ์แหนมที่ปรากฏต่อสายตา
3. รูพรุนอากาศ	ดูจำนวนรูพรุนของอากาศในตัวผลิตภัณฑ์แหนม

ข. ลักษณะเนื้อสัมผัส

4. ความเหนียวแน่น	ความรู้สึกของฟันที่ต้องใช้แรงมากน้อยเพียงใดในการขบเคี้ยวแหนมให้แตกละเอียดก่อนกลืนกิน
5. ความชุ่มฉ่ำน้ำ	ความรู้สึกว่าแหนมมีความฉ่ำน้ำในปากขณะเคี้ยว รวมถึงน้ำเนื้อน้ำมันจากตัวแหนม และน้ำลายด้วย
6. ความเนียน	ลักษณะเนื้อสัมผัสของแหนมขณะเคี้ยวที่บ่งบอกถึงสัดส่วนของเนื้อหมูและหนังหมูว่ามีสัดส่วนที่สม่ำเสมอมากน้อยเพียงใด

ค. กลิ่นและรสชาติ

7. ความเปรี้ยว	ระดับของความเปรี้ยวที่สามารถรับได้ในระหว่างการชิมแหนม
8. ความเค็ม	ระดับของความเค็มที่สามารถรับได้ในระหว่างการชิมแหนม
9. กลิ่นเครื่องเทศ	ระดับความแรงของกลิ่นกระเทียมและพริกไทยที่สามารถรับได้ในระหว่างการชิมแหนม
10. กลิ่นหมู	ระดับความแรงของกลิ่นเนื้อหมูที่สามารถรับได้ในระหว่างการชิมแหนม

ง. การยอมรับรวม

11. การยอมรับรวม	จากลักษณะต่าง ๆ ข้างต้น ทั้ง 10 ข้อของแหนม สามารถให้ระดับการยอมรับรวมของผลิตภัณฑ์นี้มากน้อยเพียงใด

Appendix 2.8 (continued)

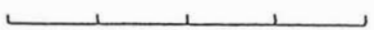
ลักษณะของแหนม

ก. ลักษณะปรากฏต่อสายตา

1. สี

แดงคล้ำ ชมพูอ่อน

2. ลักษณะเนื้อแหนม



ไม่สม่ำเสมอ สม่ำเสมอมาก

3. รูหรูนอากาศ

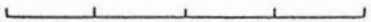
ไม่มีรูหรูนอากาศ มีรูหรูนอากาศมาก

ข. ลักษณะเนื้อสัมผัส

4. ความเหนียวแน่น

ไม่เหนียวแน่น เหนียวแน่นมาก

5. ความชุ่มฉ่ำน้ำ



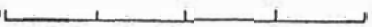
ไม่ฉ่ำน้ำ ฉ่ำน้ำมาก

6. กวามเนียน

ไม่เนียน เนียนมาก

ค. กลิ่นและรสชาติ

7. ความเปรี้ยว



ไม่เปรี้ยว เปรี้ยวมาก

8. ความเค็ม

ไม่เค็ม เค็มมาก

9. กลิ่นเครื่องเทศ

ไม่มีกลิ่น กลิ่นแรงมาก

10. กลิ่นหมู

ไม่มีกลิ่น กลิ่นแรงมาก

ง. กวามยอมรับรวม

11. กวามยอมรับตัวอย่างแหนมนี้

ไม่ยอมรับเลย ยอมรับมากที่สุด

คำแนะนำ

ถ้าท่านต้องการเพิ่มเติมลักษณะอื่น ๆ อีกของตัวอย่างแหนมนี้ โปรดระบุและให้ระดับของลักษณะนั้นด้วย เว้นจุดที่ดีที่สุดของลักษณะนั้น

ลักษณะแหนมที่ต้องการเพิ่มเติม	ระดับ		
	ต่ำ	ปานกลาง	มาก
1.			
2.			
3.			
4.			
5.			

APPENDIX 4.1

TRAINED PANEL FOR NHAM TESTING

NHAM CHARACTERISTICS

PRODUCT DEVELOPMENT SUB DEPARTMENT

MASSEY UNIVERSITY

NAME:_____ DATE:_____

GOOD MORNING / GOOD AFTERNOON

Now, I am carrying out a research into the effects of mixed starter cultures on Nham characteristics. I would like to test the Nham characteristics. You have been presented with_____ samples of Nham coded_____.

I would like you to evaluate it according to your ideal which is fixed on scale. Please mark (X) where you think each property of Nham fits on the scale and write the coded number of each sample as well.

NHAM CHARACTERISTICS:

A: APPERANCE:

1. COLOUR — Dark red — Pale pink

2. VISUAL TEXTURE — Not smooth — Very smooth

3. AIR P ETS — No air holes in sample — Plenty of air holes in sample

B: TEXTURE:

4. FI R NSS — Not firm — Very firm

5. JUICI NSS — Not juicy — Very juicy

6. S t T NESS — Not smooth — Very smooth

C: FLAVOUR AND TASTE:

7. S O R SS — Not sour — Very sour

8. SALTI N SS — Not salty — Very salty

9. SPICINESS — Not spicy — Very spicy

10. P R FLAVOR — Absence of pork flavour — Very strong pork flavour

D: OVERALL ACCEPTABILITY:

11. A C PTABILI T OF N

Dislike extremely — Like extremely

Many thanks for your time and cooperation. Your answers have been very useful.

Pairote Wiriyacharee

Results for 2^4 factorial design used for multiple regression.

Table A2.1 The matrix for 2^4 factorial design used for multiple regression of dependent variables (e.g. L. plantarum).

Run	Treatments	MV	PC	LP	LB	MVPC	MVLP	MVLB	PCLP	PCLB	LPLB	MVPCLP	MVPCLB	MVLPLB	PCLPLB	MVPCLP LB	BLOCK	DEPENDENT VARIABLES LP1	LP3	LP7	LP10	LP14
1	a	+	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	3.29	9.10	8.71	8.10	8.34
2	b	-	+	-	-	-	+	+	-	-	+	+	+	-	+	-	-	5.84	7.75	8.29	8.51	8.36
3	c	-	-	+	-	+	-	+	-	+	-	+	-	+	+	-	-	6.52	8.01	8.04	8.09	7.78
4	d	-	-	-	+	+	+	-	+	-	-	-	+	+	+	-	-	3.84	8.35	7.90	8.33	7.44
5	ab	+	+	-	-	+	-	-	-	-	+	-	-	+	+	+	+	3.81	7.64	8.39	8.21	8.12
6	ac	+	-	+	-	-	+	-	-	+	-	-	+	-	+	+	+	6.90	8.84	8.24	8.04	8.24
7	ad	+	-	-	+	-	-	+	+	-	-	+	-	-	+	+	+	3.24	8.25	8.49	8.02	8.13
8	bc	-	+	+	-	-	-	+	+	-	-	-	+	+	-	+	+	6.56	8.96	8.39	8.01	8.08
9	bd	-	+	-	+	-	+	-	-	+	-	+	-	+	-	+	+	4.32	8.49	8.63	8.38	8.38
10	cd	-	-	+	+	+	-	-	-	-	+	+	+	-	-	+	+	6.49	8.60	8.04	8.18	8.13
11	abc	+	+	+	-	+	+	-	+	-	-	+	-	-	-	-	-	7.12	8.62	7.79	8.08	7.28
12	abd	+	+	-	+	+	-	+	-	+	-	-	+	-	-	-	-	5.02	8.05	7.62	8.38	8.22
13	acd	+	-	+	+	-	+	+	-	-	+	-	-	+	-	-	-	6.10	8.22	7.27	7.61	7.40
14	bcd	-	+	+	+	-	-	-	+	+	+	-	-	-	+	-	-	6.07	8.08	7.99	7.84	7.76
15	abcd	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	7.00	9.00	8.33	8.39	8.03
16	(1)	-	-	-	-	+	+	+	+	+	+	-	-	-	-	+	+	4.99	8.16	8.24	8.49	8.40
17	CP1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	5.57	9.05	8.29	8.17	7.53
18	CP2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	5.01	9.29	8.59	8.08	7.50
19	CP3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	4.95	8.14	8.45	8.17	7.67
20	CP4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	4.94	8.35	8.40	8.20	7.93

MV = M.varians (a); PC = P.cerevisiae (b); LP = L.plantarum (c); LB = L.brevis (d); cp = centrepoint; (1) = control

Appendix 4.2 (continued)

Table A2.2 The mean ideal ratio scores of colour (COL) during 14 days of Nham fermentation affected by starter cultures.

	COL3	COL7	COL10	COL14
1	0.88	0.98	1.01	1.05
2	0.99	1.02	1.02	1.10
3	1.04	1.02	1.05	1.12
4	0.83	0.89	0.93	0.97
5	0.98	1.03	0.98	0.92
6	1.09	1.19	1.12	0.99
7	0.95	0.90	0.94	1.04
8	0.90	1.04	0.96	0.96
9	0.94	1.00	0.96	1.05
10	1.05	0.99	1.05	0.91
11	0.94	0.97	0.98	1.05
12	0.93	0.83	0.82	0.87
13	1.10	1.09	1.01	0.98
14	0.88	0.93	0.83	0.84
15	1.00	1.17	1.11	1.03
16	0.92	0.96	0.98	1.02
17	0.94	1.00	1.06	1.15
18	1.01	1.05	1.09	1.21
19	1.16	1.09	1.03	1.05
20	1.04	0.98	1.03	0.96

Appendix 4.2 (continued)

Table A2.3 The mean ideal ratio scores of visual texutre (VIS) during 14 days of Nham fermentation affected by starter cultures.

	VIS3	VIS7	VIS10	VIS14
1	0.79	0.91	0.91	0.94
2	0.92	0.90	0.94	0.94
3	0.94	0.94	0.90	0.85
4	0.82	0.93	0.91	0.90
5	0.90	0.92	0.91	0.93
6	0.92	0.91	0.92	0.90
7	0.92	0.87	0.88	0.90
8	0.82	0.88	0.94	0.87
9	0.89	0.91	0.88	0.88
10	0.79	0.97	0.88	0.89
11	0.89	0.91	0.92	0.92
12	0.88	0.88	0.95	0.94
13	0.92	0.90	0.83	0.84
14	0.86	0.93	0.90	0.85
15	0.85	0.90	0.98	0.91
16	0.84	0.77	0.90	0.87
17	0.91	0.86	0.92	0.86
18	0.92	0.91	0.96	0.93
19	0.95	0.95	0.86	0.87
20	0.94	0.90	0.85	0.92

Table A2.4 The mean ideal ratio scores of air pockets (AIR) during 14 days of Nham fermentation affected by starter cultures.

	AIR3	AIR7	AIR10	AIR14
1	1.35	1.10	1.20	1.11
2	1.13	1.09	1.06	1.04
3	1.14	1.06	1.18	1.22
4	1.31	1.25	1.19	1.26
5	1.14	1.16	1.17	1.06
6	1.21	1.21	1.00	1.13
7	1.31	1.44	1.32	1.38
8	1.18	1.32	1.06	1.15
9	1.18	1.28	1.15	1.26
10	1.25	1.16	1.16	1.14
11	1.14	1.09	1.12	1.08
12	1.19	1.18	1.03	1.16
13	1.12	1.17	1.22	1.27
14	1.29	1.29	1.06	1.23
15	1.31	1.19	1.11	1.28
16	1.44	1.47	1.15	1.32
17	1.28	1.22	1.25	1.26
18	1.23	1.18	1.21	1.17
19	1.11	1.13	1.04	1.08
20	1.12	1.12	1.18	1.08

Table A2.5 The mean ideal ratio scores of firmness (FIRM) during 14 days of Nham fermentation affected by starter cultures.

	FIRM3	FIRM7	FIRM10	FIRM14
1	0.79	0.98	0.88	0.83
2	1.00	1.00	0.97	0.93
3	1.03	0.97	0.86	0.73
4	0.89	0.89	0.91	0.86
5	0.76	0.90	0.88	0.90
6	0.91	0.94	0.88	0.95
7	0.76	0.85	0.84	0.78
8	0.99	1.00	0.98	0.97
9	0.80	0.92	0.91	0.86
10	0.99	1.01	1.01	1.00
11	1.00	0.94	0.93	0.88
12	0.99	1.00	1.02	0.87
13	1.03	0.97	0.95	0.85
14	0.93	1.01	1.00	0.89
15	0.74	0.86	0.87	0.91
16	0.82	0.90	0.90	0.84
17	1.00	0.95	0.96	0.95
18	0.93	0.98	0.89	0.83
19	0.97	0.91	0.92	0.92
20	0.96	1.00	0.89	0.91

Appendix 4.2 (continued)

Table A2.6 The mean ideal ratio scores of juiciness (JUICY) during 14 days of Nham fermentation affected by starter cultures.

	JUICY3	JUICY7	JUICY10	JUICY14
1	1.06	0.97	1.09	1.14
2	0.98	0.98	1.04	1.04
3	1.01	1.04	1.11	1.16
4	1.09	1.06	1.02	1.04
5	1.04	1.01	0.98	1.01
6	1.02	0.97	1.06	1.09
7	1.22	0.92	1.05	1.02
8	1.13	1.00	1.00	0.98
9	1.10	1.05	1.03	1.09
10	0.98	0.95	0.97	1.01
11	0.96	0.98	1.08	1.10
12	0.95	0.97	0.97	1.02
13	0.93	0.97	0.99	1.05
14	0.93	1.05	0.96	1.02
15	1.10	1.08	1.03	0.98
16	1.11	0.98	1.05	0.93
17	1.00	1.04	1.08	0.97
18	1.01	1.01	0.98	1.03
19	1.00	1.05	1.06	1.06
20	0.92	1.00	1.00	1.09

Appendix 4.2 (continued)

Table A2.7 The mean ideal ratio scores of smoothness (SMOOTH) during 14 days of Nham fermentation affected by starter culture.

	SMOOTH3	SMOOTH7	SMOOTH10	SMOOTH14
1	0.82	0.90	0.97	0.93
2	0.92	0.90	0.98	0.94
3	0.93	0.96	0.92	0.89
4	0.82	0.92	0.94	0.91
5	0.94	0.97	0.82	0.98
6	0.90	0.92	0.96	0.95
7	0.86	0.86	0.88	0.96
8	0.88	0.89	0.92	0.90
9	0.96	0.91	0.87	0.91
10	0.73	0.87	0.85	0.89
11	0.92	0.90	0.93	0.93
12	0.87	0.86	0.88	0.94
13	0.86	0.92	0.89	0.92
14	0.88	0.90	0.87	0.92
15	0.90	0.92	0.93	0.89
16	0.89	0.90	0.97	0.94
17	0.79	0.92	0.90	0.86
18	0.93	0.95	0.96	0.86
19	0.90	0.91	0.93	0.93
20	0.92	0.88	0.86	0.90

Appendix 4.2 (continued)

Table A2.8 The mean ideal ratio scores of sourness (SOUR) during 14 days of Nham fermentation affected by starter cultures.

	SOUR3	SOUR7	SOUR10	SOUR14
1	0.78	1.05	1.15	1.10
2	0.95	1.01	1.08	1.11
3	1.05	1.06	1.12	1.24
4	0.75	0.95	0.96	0.99
5	0.88	0.95	1.01	1.16
6	0.95	1.06	1.11	1.21
7	0.83	0.99	0.92	1.10
8	1.00	1.10	1.08	1.19
9	0.89	1.02	0.96	1.10
10	0.85	1.02	1.10	1.09
11	0.99	1.06	1.10	1.17
12	0.84	0.99	0.97	1.06
13	0.85	1.03	1.04	1.06
14	0.86	1.00	1.01	1.00
15	0.82	0.96	0.88	0.94
16	1.02	1.15	1.06	1.13
17	0.97	1.03	1.06	1.15
18	0.91	0.95	1.02	1.16
19	0.84	1.06	1.09	1.13
20	0.96	1.02	1.06	1.12

Appendix 4.2 (continued)

Table A2.9 The mean ideal ratio scores of saltiness (SALT) during 14 days of Nham fermentation affected by starter cultures.

	SALT3	SALT7	SALT10	SALT14
1	0.89	1.02	1.02	1.05
2	0.98	0.98	0.98	0.97
3	1.02	1.01	1.06	1.05
4	0.94	1.02	0.98	0.99
5	1.03	0.95	1.02	1.06
6	1.02	0.98	1.03	0.99
7	1.16	1.12	1.04	1.09
8	0.98	1.00	1.05	1.11
9	1.10	1.00	1.07	1.00
10	1.02	0.98	1.00	1.02
11	1.03	1.02	1.02	0.99
12	0.96	1.01	1.06	1.01
13	0.99	1.02	1.05	1.01
14	1.04	1.00	1.04	1.01
15	1.01	1.03	1.06	1.02
16	1.05	1.00	1.05	1.02
17	1.01	1.09	1.02	1.06
18	1.12	1.10	1.04	0.94
19	1.00	0.99	1.02	1.09
20	0.98	0.98	1.01	1.10

Appendix 4.2 (continued)

Table A2.10 The mean ideal ratio scores of spiciness (SPICY) during 14 days of Nham fermentation affected by starter cultures.

	SPICY3	SPICY7	SPICY10	SPICY14
1	0.84	0.95	0.96	0.98
2	0.94	0.93	0.96	0.96
3	0.96	0.93	0.97	0.95
4	0.82	0.89	0.93	0.93
5	0.94	1.02	0.98	0.96
6	1.00	0.96	1.00	1.00
7	1.02	1.01	0.98	0.98
8	1.01	0.97	0.96	0.95
9	1.06	0.92	0.92	0.95
10	0.99	0.96	0.84	0.98
11	0.95	0.93	0.96	0.96
12	0.92	0.96	0.98	0.94
13	0.91	0.97	0.96	0.97
14	0.97	0.97	0.96	0.95
15	0.95	0.95	0.95	0.93
16	0.94	0.91	0.95	0.98
17	0.96	0.98	0.97	0.90
18	0.95	0.96	0.97	0.90
19	0.90	1.00	0.92	0.99
20	0.96	0.99	0.93	0.98

Appendix 4.2 (continued)

Table A2.11 The mean ideal ratio scores of pork flavour (PORK) during 14 days of Nham fermentation affected by starter cultures.

	PORK3	PORK7	PORK10	PORK14
1	0.99	0.97	0.96	0.94
2	0.97	0.98	0.95	0.94
3	0.96	0.95	0.90	0.90
4	0.97	0.93	0.91	0.90
5	0.96	0.97	0.99	0.95
6	0.99	1.01	1.04	0.96
7	0.83	1.01	0.95	0.98
8	0.98	0.92	0.96	0.92
9	0.94	0.98	0.98	0.96
10	1.00	0.97	0.99	0.97
11	0.95	0.94	0.94	0.95
12	0.93	0.95	0.93	0.93
13	0.99	0.99	0.92	0.97
14	0.92	0.96	0.94	0.94
15	0.86	0.98	0.96	0.95
16	0.96	0.96	1.00	0.96
17	0.97	0.96	0.94	0.93
18	0.95	0.98	1.01	1.01
19	1.02	0.95	0.92	0.96
20	0.99	0.94	0.93	0.96

Table A2.12 The mean ideal ratio scores of overall acceptability (OA) during 14 days of Nham fermentation affected by starter cultures.

	OA3	OA7	OA10	OA14
1	0.61	0.78	0.71	0.78
2	0.79	0.85	0.84	0.80
3	0.86	0.85	0.68	0.62
4	0.61	0.61	0.63	0.68
5	0.68	0.84	0.77	0.74
6	0.78	0.80	0.76	0.77
7	0.71	0.72	0.58	0.56
8	0.71	0.81	0.80	0.60
9	0.71	0.65	0.59	0.53
10	0.62	0.82	0.67	0.75
11	0.86	0.80	0.78	0.74
12	0.66	0.65	0.66	0.66
13	0.67	0.77	0.67	0.66
14	0.76	0.76	0.66	0.66
15	0.70	0.66	0.53	0.60
16	0.80	0.75	0.71	0.70
17	0.76	0.78	0.56	0.55
18	0.74	0.72	0.62	0.56
19	0.76	0.75	0.68	0.71
20	0.71	0.74	0.67	0.69

Table A2.13 The log number of L. plantarum (LP) during 14 days of Nham fermentation affected by starter cultures.

	LP1	LP3	LP7	LP10	LP14
1	3.29	9.10	8.71	8.10	8.54
2	5.84	7.75	8.29	8.51	8.36
3	6.52	8.01	8.04	8.09	7.78
4	3.84	8.35	7.90	8.33	7.44
5	3.81	7.64	8.39	8.21	8.12
6	6.90	8.84	8.24	8.04	8.24
7	3.24	8.25	8.49	8.02	8.13
8	6.54	8.96	8.39	8.01	8.08
9	4.32	8.49	8.63	8.38	8.38
10	6.49	8.60	8.04	8.18	8.13
11	7.12	8.62	7.79	8.08	7.28
12	5.02	8.05	7.62	8.38	8.22
13	6.10	8.22	7.27	7.61	7.40
14	6.07	8.08	7.99	7.84	7.76
15	7.00	9.00	8.33	8.39	8.03
16	4.99	8.16	8.24	8.49	8.40
17	5.57	9.05	8.29	8.17	7.53
18	5.01	9.29	8.59	8.08	7.50
19	4.95	8.14	8.45	8.17	7.67
20	4.94	8.35	8.40	8.20	7.93

Appendix 4.2 (continued)

Table A2.14 The log number of L. brevis (LB) during 14 days of Nham fermentation affected by starter cultures.

	LB1	LB3	LB7	LB10	LB14
1	2.93	8.58	7.85	7.98	7.73
2	5.79	7.71	7.08	7.64	7.44
3	3.72	6.92	7.44	7.92	7.50
4	6.28	8.60	8.53	7.78	7.04
5	3.90	7.98	8.20	7.86	8.04
6	3.49	7.61	7.97	8.18	8.04
7	6.51	8.12	8.50	8.01	8.03
8	4.80	7.78	8.06	7.78	7.78
9	6.30	8.42	8.56	8.22	8.02
10	6.50	8.30	7.81	7.82	7.76
11	5.46	7.52	7.19	7.42	6.65
12	6.41	7.78	8.22	7.58	7.37
13	6.15	7.72	7.79	7.25	7.48
14	6.18	7.66	7.64	7.53	7.52
15	6.63	8.14	8.39	8.03	8.04
16	5.01	8.53	8.30	8.32	8.07
17	5.41	8.73	8.37	7.77	7.97
18	5.22	8.73	8.22	7.60	8.21
19	5.35	8.76	8.41	8.16	8.08
20	5.39	8.81	8.40	8.15	8.06

Appendix 4.2 (continued)

Table A2.15 The log number of P. cerevisiae (PC) during 14 days of Nham fermentation affected by starter cultures.

	PC1	PC3	PC7	PC10	PC14
1	2.62	7.79	7.79	7.61	6.72
2	6.19	6.52	6.55	6.98	6.71
3	3.93	6.78	6.09	6.37	6.35
4	4.73	7.13	7.27	7.67	7.16
5	8.37	8.60	7.96	7.71	6.77
6	4.16	6.55	4.63	7.86	6.22
7	3.40	8.29	6.75	6.62	6.39
8	7.97	8.20	7.35	6.22	6.20
9	6.13	8.24	7.32	7.41	7.37
10	3.98	8.09	8.01	7.16	7.08
11	6.43	6.94	6.07	6.61	6.50
12	6.07	7.71	7.13	7.81	7.29
13	4.50	7.43	7.76	7.27	7.47
14	6.02	7.58	6.45	6.97	7.51
15	6.49	8.14	7.21	7.48	7.27
16	4.69	8.77	7.88	7.63	7.65
17	4.38	8.34	6.84	6.37	6.41
18	4.57	8.47	7.66	6.76	6.45
19	4.23	8.15	7.75	6.27	6.36
20	4.44	8.13	7.83	6.34	6.49

Appendix 4.2 (continued)

Table A 2.16 The log number of M. varians (MV) during 14 days of Nham fermentation affected by starter cultures.

	MV1	MV3	MV7	MV10	MV14
1	6.60	6.42	2.31	2.50	1.90
2	6.29	5.53	4.76	2.26	1.00
3	6.41	5.49	4.15	2.78	2.32
4	6.49	6.03	4.10	2.62	1.81
5	8.37	6.87	4.76	2.33	2.07
6	8.69	7.11	3.40	2.99	2.56
7	8.85	6.95	3.40	3.39	3.26
8	7.88	6.38	2.81	2.98	2.43
9	7.83	6.53	4.06	3.49	3.45
10	6.90	4.42	2.11	2.38	1.30
11	6.56	5.31	4.22	2.18	2.43
12	6.42	6.22	3.68	2.19	2.10
13	6.23	6.14	3.57	2.18	2.17
14	6.27	4.68	2.20	2.18	1.97
15	8.75	6.91	4.20	2.84	2.38
16	7.69	6.10	3.45	3.09	1.00
17	7.98	6.06	3.34	2.35	1.70
18	7.92	6.46	3.85	2.58	1.30
19	7.37	6.02	3.01	2.33	2.12
20	7.32	5.99	3.04	2.55	2.20

Appendix 4.2 (continued)

Table A2.17 The pH change in Nham during 14 days of fermentation affected by starter cultures.

	pH1	pH3	pH7	pH10	pH14
1	6.22	4.46	3.96	3.87	3.84
2	6.00	4.35	4.02	3.95	3.86
3	5.92	3.95	3.82	3.77	3.84
4	6.05	4.59	4.22	4.22	4.18
5	6.18	4.40	4.18	4.01	4.12
6	5.72	3.88	3.84	3.81	3.91
7	5.92	4.31	4.20	4.23	4.36
8	5.82	3.99	3.83	3.88	3.80
9	5.80	4.32	4.45	4.25	4.34
10	5.85	4.16	3.90	3.90	3.92
11	6.02	4.00	3.78	3.80	3.80
12	5.96	4.36	4.12	4.21	4.19
13	6.20	4.21	3.96	3.87	3.90
14	6.31	4.21	4.00	4.06	4.09
15	5.82	4.08	4.02	4.10	4.10
16	6.05	4.28	4.21	4.26	4.31
17	5.98	4.05	3.94	3.94	4.12
18	5.78	4.16	4.20	4.24	4.26
19	6.20	4.20	3.98	3.81	3.84
20	6.11	4.41	3.96	3.90	3.91

Table A 2.18 The gas production (Gas; ml CO_2/ 100 g at NTP) during 6 days of Nham fermentation affected by starter cultures.

	Gas2	Gas3	Gas4	Gas5	Gas6
1	56.42	100.16	116.87	122.56	126.02
2	26.55	32.12	35.59	37.04	38.84
3	51.09	82.36	90.04	90.04	90.19
4	67.99	94.96	132.19	146.21	192.07
5	21.17	28.14	35.66	38.21	39.47
6	70.79	78.65	79.19	79.53	79.60
7	104.17	147.32	161.78	167.27	167.49
8	29.48	37.49	38.75	39.33	39.46
9	50.39	94.43	112.98	141.62	145.77
10	17.57	30.86	45.03	49.93	51.65
11	25.28	30.79	34.05	34.28	35.41
12	62.03	70.89	96.28	101.39	107.97
13	16.24	36.21	38.06	45.56	58.00
14	17.25	35.41	55.68	61.22	68.68
15	36.14	56.07	59.58	63.33	65.06
16	72.45	111.59	138.89	143.95	145.81
17	67.22	81.03	82.87	83.73	83.94
18	64.17	82.13	84.06	85.19	86.13
19	52.89	67.51	69.50	70.35	72.34
20	51.80	65.72	69.31	70.27	71.71

Table A2.19 The compression force (CF; Newtons) during 14 days of Nham fermentation affected by starter cultures.

	CF1	CF3	CF7	CF10	CF14
1	15.85	69.75	70.65	61.56	43.99
2	17.53	71.39	181.37	186.17	175.68
3	10.82	96.69	136.83	80.25	61.14
4	12.19	51.89	68.25	66.53	61.04
5	13.86	57.93	97.81	70.49	81.15
6	12.77	112.57	103.83	78.96	93.99
7	12.84	69.94	89.07	65.43	66.94
8	12.29	102.46	103.28	94.53	81.97
9	13.38	68.30	76.09	63.39	61.48
10	13.38	95.08	115.30	93.17	85.25
11	9.93	99.26	177.92	144.37	122.79
12	13.69	82.13	79.23	76.99	69.62
13	10.04	52.40	68.60	67.95	56.20
14	10.18	76.07	76.64	74.18	69.97
15	11.75	94.40	75.14	81.70	83.06
16	13.04	60.39	69.94	56.56	46.72
17	12.71	83.34	65.03	87.97	69.40
18	13.11	75.41	64.48	72.40	71.86
19	12.55	77.27	73.64	77.32	57.16
20	10.29	89.84	71.67	69.12	74.40

Appendix 4.2 (continued)

Table A 2.20 The compression energy (CE; Joules) during 14 days of Nham fermentation affected by starter cultures.

	CE1	CE3	CE7	CE10	CE14
1	0.083	0.552	0.418	0.388	0.194
2	0.083	0.519	1.295	1.410	1.148
3	0.062	0.675	0.962	0.478	0.295
4	0.062	0.312	0.448	0.388	0.339
5	0.067	0.320	0.749	0.443	0.524
6	0.063	0.694	0.574	0.535	0.656
7	0.056	0.399	0.568	0.557	0.415
8	0.064	0.713	0.617	0.858	0.645
9	0.067	0.369	0.489	0.459	0.380
10	0.062	0.590	0.770	0.601	0.596
11	0.041	0.724	1.076	1.016	0.694
12	0.063	0.492	0.486	0.508	0.437
13	0.049	0.342	0.404	0.434	0.336
14	0.055	0.530	0.432	0.473	0.415
15	0.053	0.481	0.442	0.656	0.412
16	0.072	0.377	0.442	0.478	0.352
17	0.060	0.426	0.459	0.590	0.418
18	0.064	0.366	0.443	0.475	0.415
19	0.064	0.478	0.448	0.489	0.470
20	0.060	0.533	0.470	0.500	0.473

Appendix 4.2 (continued)

Table A2.21 The tristimulus values (x, y, z) of Nham during 14 days of fermentation affected by starter cultures.

	X1	Y1	Z1	X3	Y3	Z3
1	21.65	19.80	17.80	24.95	22.45	21.15
2	22.00	19.85	17.95	28.20	25.60	25.35
3	21.10	19.05	17.10	28.50	25.85	26.25
4	20.65	18.55	16.85	26.25	23.55	23.30
5	23.85	21.45	20.15	23.95	21.85	22.05
6	25.35	22.85	21.65	28.75	26.55	26.85
7	24.70	22.00	20.50	24.80	22.60	22.30
8	21.90	19.30	18.05	25.40	23.00	22.90
9	23.20	20.60	18.70	24.20	21.90	22.00
10	23.05	20.95	18.45	24.45	22.05	21.05
11	23.35	20.90	19.15	29.25	26.55	26.80
12	20.05	17.85	16.10	26.55	23.80	23.55
13	21.05	19.25	17.35	26.55	24.10	23.45
14	19.40	17.50	15.45	24.70	21.95	21.60
15	23.45	20.45	18.85	26.65	24.35	24.15
16	22.15	19.65	18.35	25.75	35.35	23.45
17	22.85	20.85	18.85	26.30	24.10	24.50
18	22.75	20.25	19.05	27.05	24.95	24.85
19	22.00	19.75	18.10	27.60	25.10	24.85
20	21.55	19.35	17.55	26.40	23.70	23.75

Appendix 4.2 (continued)

Table A2.21 (continued)

	X7	Y7	Z7	X10	Y10	Z10
1	27.50	24.90	25.05	27.20	24.40	24.00
2	28.15	25.35	25.00	28.45	25.45	25.20
3	27.80	24.85	24.95	26.30	23.35	22.85
4	25.75	22.90	22.65	28.55	25.70	25.50
5	26.45	24.15	24.30	26.85	23.95	24.25
6	29.25	26.40	26.50	28.45	25.95	26.35
7	25.50	22.70	22.30	25.20	22.20	22.50
8	28.20	25.50	25.30	26.30	23.60	23.50
9	26.35	23.65	23.55	26.75	24.05	23.95
10	28.00	25.45	24.85	27.75	25.05	24.45
11	28.35	25.50	25.25	28.35	25.25	24.85
12	25.30	22.30	22.10	26.70	23.35	23.15
13	26.50	23.85	23.50	27.05	24.10	23.55
14	25.05	22.00	21.65	25.40	22.35	21.65
15	27.85	25.15	25.25	25.75	22.85	22.75
16	27.75	25.15	25.40	27.55	24.85	25.05
17	27.15	24.75	24.55	25.35	22.85	22.65
18	28.25	25.55	25.70	25.65	23.15	23.45
19	27.40	24.60	24.55	26.10	23.15	22.75
20	27.80	25.00	24.95	26.75	23.85	23.65

Table A2.21 (continued)

	X14	Y14	Z14
1	27.30	24.45	24.35
2	28.00	25.15	24.60
3	29.10	26.20	26.05
4	26.00	23.10	22.40
5	25.55	22.65	23.15
6	29.15	26.45	27.05
7	24.70	22.00	22.55
8	25.80	23.10	22.90
9	24.15	21.45	21.85
10	26.75	23.85	23.55
11	28.55	25.45	25.15
12	25.05	22.00	21.55
13	25.05	21.90	21.05
14	24.95	21.95	21.00
15	25.85	22.95	23.45
16	25.25	22.65	22.85
17	25.15	22.20	23.20
18	25.40	22.60	23.20
19	26.70	24.00	23.50
20	25.55	22.85	22.40

Table A2.22 Residual nitrite (RN; ppm) during 14 days of Nham fermentation affected by starter cultures.

	RN1	RN3	RN7	RN10	RN14
1	82.61	43.16	14.27	2.13	1.91
2	78.23	27.99	20.34	3.82	1.46
3	56.54	34.51	32.37	4.04	1.01
4	68.57	43.21	17.31	5.17	1.68
5	67.65	84.75	22.59	5.39	1.00
6	73.27	13.04	12.25	3.93	0.45
7	87.77	38.10	14.95	7.75	0.78
8	48.89	15.73	12.14	4.16	0.89
9	38.55	49.79	11.69	4.49	0.33
10	34.95	21.35	12.70	7.08	0.45
11	54.72	27.09	31.13	3.03	1.91
12	56.87	26.97	18.09	4.27	0.89
13	51.59	30.35	10.90	3.70	0.89
14	46.08	29.79	20.79	3.70	1.57
15	69.90	16.52	14.72	8.09	0.33
16	42.82	104.54	18.88	2.58	0.56
17	52.91	37.32	8.20	4.38	0.33
18	56.30	45.94	6.97	4.16	0.33
19	50.34	42.14	8.20	3.70	0.56
20	58.21	48.48	8.54	3.59	0.33

Tabel A2.23 The log number of Enterobacteriaceae (EN) during 14 days of Nham fermentation affected by starter cultures.

	EN1	EN3	EN7	EN10	EN14
1	7.05	6.85	1.48	0.00	0.00
2	6.98	3.30	0.00	0.00	0.00
3	6.32	2.74	0.00	0.00	0.00
4	6.71	5.19	3.94	0.00	0.00
5	8.29	6.76	4.68	0.00	0.00
6	7.67	5.20	1.00	0.00	0.00
7	8.61	6.68	3.20	3.47	3.74
8	7.88	6.10	1.30	0.00	0.00
9	8.29	6.32	4.07	2.03	2.05
10	7.49	2.89	0.00	0.00	0.00
11	7.19	2.40	0.00	0.00	0.00
12	7.17	2.54	0.00	0.00	0.00
13	6.44	4.16	0.00	0.00	0.00
14	6.73	2.60	0.00	0.00	0.00
15	8.49	6.71	1.60	0.00	0.00
16	8.16	6.15	3.58	1.18	1.48
17	7.72	5.93	1.30	0.70	0.00
18	8.57	6.26	1.30	1.00	0.00
19	6.78	6.36	1.00	0.00	0.00
20	6.85	6.35	1.18	0.00	0.00

Table A2.24 The weight loss (W; %) during 14 days of Nham fermentation affected by starter cultures.

	W3	W7	W10	W14
1	4.43	4.69	6.77	9.06
2	1.30	5.79	6.80	7.32
3	3.74	4.79	5.16	5.67
4	3.72	5.48	8.02	9.07
5	1.48	6.38	9.44	10.47
6	2.70	4.89	5.24	5.94
7	2.49	6.38	10.50	11.40
8	3.11	7.31	8.40	9.33
9	3.11	6.66	8.22	8.83
10	2.66	9.27	10.87	11.50
11	3.61	6.38	6.54	6.84
12	3.84	9.00	9.70	10.65
13	3.90	9.94	11.15	11.49
14	4.71	9.62	10.74	11.17
15	5.83	10.92	12.42	13.18
16	2.07	5.37	8.60	9.20
17	3.87	6.30	8.43	8.81
18	4.85	5.87	8.64	9.05
19	3.79	8.81	9.65	10.14
20	3.93	7.24	9.95	10.24

APPENDIX 4.3

Regression equation and regression statistics from the 2^4 factorial design (Chapter 4) and an example of the method used to test for goodness of fit.

Table A 3.1: Empirical model and regression statistics for pH on the third day of fermentation.

Empirical method:

$$pH_3 = 4.218 - 0.162\,(LP) + 0.058\,(LB)$$

Correlation coefficient:

R-squared = 70.93%

Analysis of variance:

Due to	SS	df	MS = SS/df
Regression	0.473	3	0.158
Residual	0.194	17	0.011
Total	0.667	20	

Test for goodness of fit

Due to	SS	df	MS = SS/df
Residual	0.194	17	0.011
Pure error	0.068	3	0.023
Lack of fit	0.126	14	9×10^{-3}

$$\frac{\text{MS lof}}{\text{MS pure}} = \frac{9.0 \times 10^{-3}}{0.023} = 0.39^{ns}$$

As $F_{(14,\,3)\ at\ 0.05} = 8.71$; cannot accept the hypothesis that there is significant lack of fit of the model at the 5% level.

APPENDIX 4.4

The multiple regression equations for dependent variables affected by starter cultures.

Table A4.1

The multiple regression equations for overall acceptability of Nham during 14 days of fermentation affected by starter cultures.

Overall acceptability* 3 = 0.725 + 0.001 (MV) + 0.026 (PC) + 0.037 (LP) - 0.028 (LB)
+ 0.003 (MVPC) + 0.019 (MVLP) + 0.017 (MVLB)
- 0.001 (PCLP) + 0.014 (PCLB) - 0.017 (LPLB)
+ 0.024 (MVPCLP) - 0.023 (MVPCLB) - 0.014 (MVLPLB)
+ 0.028 (PCLPLB) + 0.001 (MVPCLPLB) - 0.008 (BLOCK)
(R^2 = 97.06%)

Overall acceptability 7 = 0.755 + 0.006 (MV) + 0.006 (PC) + 0.038 (LP) - 0.041 (LB)
- 0.010 (MVPC) - 0.021 (MVLP) + 0.000 (MVLB)
- 0.021 (PCLP) - 0.020 (PCLB) + 0.021 (LPLB)
+ 0.020 (MVPCLP) + 0.001 (MVPCLB) - 0.000 (MVLPLB)
+ 0.015 (PCLPLB) + 0.001 (MVPCLPLB) - 0.002 (BLOCK)
(R^2 = 97.66%)

Overall acceptability 10 = 0.678 + 0.015 (MV) + 0.036 (PC) + 0.026 (LP) - 0.044 (LB)
- 0.011 (MVPC) - 0.001 (MVLP) - 0.006 (MVLB)
- 0.015 (PCLP) - 0.027 (PCLB) + 0.005 (LPLB)
+ 0.005 (MVPCLP) + 0.033 (MVPCLB) + 0.005 (MVLPLB)
+ 0.014 (PCLPLB) - 0.056 (MVPCLPLB) + 0.042 (BLOCK)
(R^2 = 90.32%)

Overall acceptability 14 = 0.668 + 0.023 (MV) + 0.001 (PC) + 0.009 (LP) - 0.028 (LB)
+ 0.008 (MVPC) + 0.007 (MVLP) - 0.028 (MVLB)
- 0.013 (PCLP) - 0.013 (PCLB) + 0.033 (LPLB)
+ 0.007 (MVPCLP) + 0.039 (MVPCLB) - 0.014 (MVLPLB)
+ 0.013 (PCLPLB) - 0.094 (MVPCLPLB) + 0.072 (BLOCK)
(R^2 = 93.44%)

* mean ideal ratio scores

MV = M. varians, PC = P. cerevisiae, LP = L. plantarum, LB = L. brevis

Table A 4.2

The multiple regression equations for each starter culture during 14 days of Nham fermentation affected by starter cultures inoculations.

Log LP_1 = 5.378 - 0.133 (MV) + 0.272 (PC) + 1.149 (LP) - 0.183 (LB)
+ 0.156 (MVPC) + 0.321 (MVLP) + 0.213 (MVLB) - 0.182 (PCLP)
+ 0.071 (PCLB) + 0.006 (LPLB) + 0.034 (MVPCLP) + 0.172 (MVPCLB)
- 0.266 (MVLPLB) - 0.041 (PCLPLB) + 0.141 (MVPCLPLB)
- 0.173 (BLOCK)
(R^2 = 98.28%)

Log LP_3 = 8.447 + 0.083 (MV) - 0.059 (PC) + 0.159 (LP) - 0.002 (LB)
- 0.079 (MVPC) + 0.046 (MVLP) - 0.082 (MVLB) + 0.182 (PCLP)
+ 0.084 (PCLB) - 0.064 (LPLB) + 0.095 (MVPCLP) + 0.199 (MVPCLB)
+ 0.089 (MVLPLB) - 0.142 (PCLPLB) + 0.572 (MVPCLPLB)
- 0.462 (BLOCK)
(R^2 = 90.71%)

Log LP_7 = 8.204 - 0.043 (MV) + 0.031 (PC) - 0.136 (LP) - 0.114 (LB)
- 0.104 (MVPC) - 0.061 (MVLP) - 0.064 (MVLB) + 0.083 (PCLP)
+ 0.077 (PCLB) + 0.010 (LPLB) + 0.142 (MVPCLB) + 0.043 (MVPCLB)
+ 0.060 (MVLPLB) + 0.061 (PCLPLB) + 0.204 (MVPCLPLB)
- 0.007 (BLOCK)
(R^2 = 87.49%)

Log LP_{10} = 8.164 - 0.062 (MV) + 0.059 (PC) - 0.136 (LP) - 0.025 (LB)
+ 0.103 (MVPC) + 0.062 (MVLP) + 0.021 (MVLB) - 0.009 (PCLP)
+ 0.047 (PCLB) + 0.000 (LPLB) + 0.053 (MVPCLP) + 0.076 (MVPCLB)
- 0.026 (MVLPLB) + 0.013 (PCLPLB) + 0.019 (MVPCLPLB)
+ 0.030 (BLOCK)
(R^2 = 99.45%)

Appendix 4.4 Table 4.2 (continued)

$\text{Log } LP_{14}$ = 7.946 - 0.023 (MV) + 0.011 (PC) - 0.181 (LP) - 0.082 (LB)
- 0.093 (MVPC) - 0.077 (MVLP) + 0.032 (MVLB) - 0.061 (PCLP)
+ 0.151 (PCLB) + 0.074 (LPLB) + 0.061 (MVPCLP) + 0.112 (MVPCLB)
- 0.047 (MVLPLB) - 0.036 (PCLPLB) + 0.028 (MVPCLPLB)
+ 0.142 (BLOCK)
(R^2 = 83.39%)

$\text{Log } LB_1$ = 5.371 - 0.194 (MV) + 0.305 (PC) - 0.012 (LP) + 0.991 (LB)
+ 0.110 (MVPC) + 0.260 (MVLP) + 0.249 (MVLB) + 0.096 (PCLP)
- 0.295 (PCLB) + 0.008 (LPLB) + 0.101 (MVPCLP) - 0.025 (MVPCLB)
- 0.290 (MVLPLB) - 0.066 (PCLPLB) - 0.014 (MVPCLPLB)
+ 0.028 (BLOCK)
(R^2 = 99.90%)

$\text{Log } LB_3$ = 8.120 - 0.029 (MV) - 0.087 (PC) - 0.254 (LP) + 0.132 (LB)
+ 0.011 (MVPC) + 0.071 (MVLP) - 0.123 (MVLB) + 0.156 (PCLP)
- 0.006 (PCLB) + 0.117 (LPLB) + 0.003 (MVPCLP) + 0.102 (MVPCLB)
+ 0.057 (MVLPLB) - 0.118 (PCLPLB) + 0.122 (MVPCLPLB)
+ 0.028 (BLOCK)
(R^2 = 61.00%)

$\text{Log } LB_7$ = 8.046 + 0.043 (MV) - 0.053 (PC) - 0.184 (LP) + 0.209 (LB)
+ 0.039 (MVPC) + 0.006 (MVLP) + 0.002 (MVLB) + 0.087 (PCLP)
+ 0.076 (PCLB) - 0.088 (LPLB) - 0.118 (MVPCLP) + 0.018 (MVPCLB)
+ 0.132 (MVLPLB) - 0.002 (PCLPLB) + 0.198 (MVPCLPLB)
+ 0.055 (BLOCK)
(R^2 = 87.24%)

$\text{Log } LB_{10}$ = 7.850 - 0.044 (MV) - 0.075 (PC) - 0.091 (LP) - 0.055 (LB)
+ 0.009 (MVPC) + 0.023 (MVLP) - 0.016 (MVLB) + 0.024 (PCLP)
+ 0.137 (PCLB) - 0.029 (LPLB) + 0.047 (MVPCLP) + 0.016 (MVPCLB)
+ 0.020 (MVLPLB) + 0.036 (PCLPLB) - 0.040 (MVPCLPLB)
+ 0.235 (BLOCK)
(R^2 = 97.53%)

$\text{Log } LB_{14}$ = 7.741 + 0.061 (MV) - 0.049 (PC) - 0.061 (LP) + 0.001 (LB)
- 0.098 (MVPC) - 0.059 (MVLP) + 0.057 (MVLB) - 0.049 (PCLP)
+ 0.129 (PCLB) + 0.103 (LPLB) - 0.011 (MVPCLP) - 0.007 (MVPCLB)
+ 0.047 (MVLPLB) + 0.049 (PCLPLB) + 0.326 (MVPCLPLB)
- 0.010 (BLOCK)
(R^2 = 80.67%)

$\text{Log } PC_1$ = 5.165 - 0.100 (MV) + 1.354 (PC) + 0.080 (LP) - 0.190 (LB)
+ 0.231 (MVPC) + 0.060 (MVLP) + 0.050 (MVLB) - 0.061 (PCLP)
- 0.341 (PCLB) + 0.003 (LPLB) - 0.459 (MVPCLP) - 0.079 (MVPCLB)
+ 0.238 (MVLPLB) + 0.056 (PCLPLB) + 0.364 (MVPCLPLB)
- 0.070 (BLOCK)
(R^2 = 92.99%)

$\text{Log } PC_3$ = 7.792 + 0.009 (MV) + 0.069 (PC) - 0.209 (LP) + 0.154 (LB)
+ 0.098 (MVPC) - 0.208 (MVLP) + 0.058 (MVLB) + 0.182 (PCLP)
+ 0.023 (PCLB) + 0.193 (LPLB) - 0.074 (MVPCLP) - 0.156 (MVPCLB)
+ 0.116 (MVLPLB) - 0.224 (PCLPLB) + 0.570 (MVPCLPLB)
- 0.132 (BLOCK)
(R^2 = 87.06%)

$\text{Log } PC_7$ = 7.205 + 0.011 (MV) - 0.121 (PC) - 0.205 (LP) + 0.111 (LB)
+ 0.076 (MVPC) - 0.065 (MVLP) - 0.036 (MVLB) - 0.030 (PCLP)
- 0.089 (PCLB) + 0.325 (LPLB) - 0.152 (MVPCLP) + 0.091 (MVPCLB)
+ 0.217 (MVLPLB) - 0.287 (PCLPLB) - 0.032 (MVPCLPLB)
+ 0.270 (BLOCK)
(R^2 = 89.21%)

$\text{Log } PC_{10}$ = 7.056 + 0.160 (MV) - 0.063 (PC) - 0.219 (LP) + 0.088 (LB)
+ 0.094 (MVPC) + 0.152 (MVLP) - 0.164 (MVLB) - 0.110 (PCLP)
+ 0.181 (PCLB) + 0.140 (LPLB) - 0.181 (MVPCLP) + 0.137 (MVPCLB)
+ 0.006 (MVLPLB) - 0.004 (PCLPLB) + 0.180 (MVPCLPLB)
- 0.130 (BLOCK)
(R^2 = 68.04%)

Appendix 4.4 Table 4.2 (continued)

$\text{Log } PC_{14} = 6.823 - 0.088\,(MV) + 0.036\,(PC) - 0.091\,(LP) + 0.276\,(LB)$
$+ 0.092\,(MVPC) + 0.127\,(MVLP) + 0.000\,(MVLB) + 0.009\,(PCLP)$
$+ 0.132\,(PCLB) + 0.231\,(LPLB) - 0.117\,(MVPCLP) - 0.085\,(MVPCLB)$
$- 0.002\,(MVLPLB) - 0.119\,(PCLPLB) - 0.023\,(MVPCLPLB)$
$- 0.025\,(BLOCK)$
$(R^2 = 83.58\%)$

$\text{Log } MV_1 = 7.341 + 0.294\,(MV) + 0.032\,(PC) - 0.053\,(LP) - 0.047\,(LB)$
$- 0.066\,(MVPC) + 0.052\,(MVLP) + 0.051\,(MVLB) + 0.122\,(PCLP)$
$+ 0.068\,(PCLB) - 0.127\,(LPLB) + 0.009\,(MVPCLP) - 0.012\,(MVPCLB)$
$+ 0.056\,(MVLPLB) + 0.251\,(PCLPLB) + 1.158\,(MVPCLPLB)$
$- 0.303\,(BLOCK)$
$(R^2 = 97.01\%)$

$\text{Log } MV_3 = 6.081 + 0.423\,(MV) - 0.014\,(PC) - 0.263\,(LP) - 0.083\,(LB)$
$- 0.149\,(MVPC) + 0.139\,(MVLP) + 0.147\,(MVLB) + 0.029\,(PCLP)$
$+ 0.114\,(PCLB) - 0.184\,(LPLB) - 0.123\,(MVPCLP) + 0.059\,(MVPCLB)$
$+ 0.278\,(MVLPLB) + 0.128\,(PCLPLB) + 0.468\,(MVPCLPLB)$
$- 0.128\,(BLOCK)$
$(R^2 = 99.03\%)$

$\text{Log } MV_7 = 3.521 + 0.119\,(MV) + 0.262\,(PC) - 0.241\,(LP) - 0.159\,(LB)$
$+ 0.260\,(MVPC) + 0.396\,(MVLP) + 0.179\,(MVLB) - 0.237\,(PCLP)$
$- 0.142\,(PCLB) - 0.154\,(LPLB) + 0.077\,(MVPCLP) - 0.152\,(MVPCLB)$
$+ 0.171\,(MVLPLB) + 0.297\,(PCLPLB) + 0.235\,(MVPCLPLB)$
$- 0.285\,(BLOCK)$
$(R^2 = 96.91\%)$

$\text{Log } MV_{10} = 2.609 - 0.074\,(MV) - 0.093\,(PC) - 0.085\,(LP) + 0.010\,(LB)$
$- 0.098\,(MVPC) + 0.057\,(MVLP) + 0.065\,(MVLB) + 0.074\,(PCLP)$
$+ 0.109\,(PCLB) - 0.179\,(LPLB) + 0.079\,(MVPCLP) - 0.054\,(MVPCLB)$
$+ 0.066\,(MVLPLB) + 0.025\,(PCLPLB) + 0.300\,(MVPCLPLB)$
$- 0.013\,(BLOCK)$
$(R^2 = 94.43\%)$

$\text{Log } MV_{14} = 2.073 + 0.224\,(MV) + 0.094\,(PC) + 0.061\,(LP) + 0.171\,(LB)$
$- 0.208\,(MVPC) - 0.034\,(MVLP) - 0.052\,(MVLB) + 0.013\,(PCLP)$
$+ 0.076\,(PCLB) - 0.411\,(LPLB) + 0.121\,(MVPCLP) - 0.199\,(MVPCLB)$
$+ 0.182\,(MVLPLB) + 0.037\,(PCLPLB) - 0.158\,(MVPCLPLB)$
$+ 0.330\,(BLOCK)$
$(R^2 = 95.09\%)$

MV = M. varians, PC = P. cerevisiae, LP = L. plantarum, LB = L. brevis

Appendix 4.4 (continued)

Table A 4.3

The multiple regression equations for pH of Nham during 14 days of fermentation affected by starter cultures.

pH_3 = 4.218 - 0.009 (MV) - 0.008 (PC) - 0.162 (LP) + 0.058 (LB) + 0.006 (MVPC)
- 0.008 (MVLP) - 0.031 (MVLB) + 0.018 (PCLP) - 0.029 (PCLB)
+ 0.047 (LPLB) - 0.018 (MVPCLP) + 0.012 (MVPCLB) + 0.028 (MVLPLB)
- 0.001 (PCLPLB) - 0.144 (MVPCLPLB) + 1.00 (BLOCK)
(R^2 = 95.65%)

pH_7 = 4.029 - 0.024 (MV) + 0.018 (PC) - 0.138 (LP) + 0.077 (LB) - 0.001 (MVPC)
+ 0.031 (MVLP) - 0.009 (MVLB) - 0.004 (PCLP) + 0.021 (PCLB)
- 0.001 (LPLB) - 0.013 (MVPCLP) - 0.043 (MVPCLB) + 0.023 (MVLPLB)
+ 0.006 (PCLPLB) + 0.097 (MVPCLPLB) - 0.050 (BLOCK)
(R^2 = 93.84%)

pH_{10} = 4.004 - 0.024 (MV) + 0.021 (PC) - 0.113 (LP) + 0.093 (LB) + 0.022 (MVPC)
+ 0.021 (MVLP) + 0.022 (MVLB) + 0.041 (PCLP) + 0.029 (PCLB)
- 0.009 (LPLB) - 0.028 (MVPCLP) - 0.019 (MVPCLB) - 0.016 (MVLPLB)
+ 0.007 (PCLPLB) + 0.161 (MVPCLPLB) - 0.117 (BLOCK)
(R^2 = 90.78%)

pH_{14} = 4.034 - 0.007 (MV) + 0.003 (PC) - 0.115 (LP) + 0.100 (LB) + 0.023 (MVPC)
+ 0.015 (MVLP) + 0.10 (MVLB) + 0.025 (PCLP) + 0.043 (PCLB)
- 0.017 (LPLB) -0.028 (MVPCLP) - 0.060 (MVPCLB) - 0.020 (MVLPLB)
+ 0.022 (PCLPLB) + 0.230 (MVPCLPLB) - 0.157 (BLOCK)
(R^2 = 98.27%)

MV = M. varians, PC = P. cerevisiae, LP = L. plantarum, LB = L. brevis

Appendix 4.4 (continued)

Table A 4.4

The multiple regression equations for gas formation (G) during 6 days of Nham fermentation affected by starter cultures.

G^*_2 = 48.054 + 3.717 (MV) - 11.777 (PC) - 12.333 (LP) + 1.159 (LB)
- 1.098 (MVPC) + 0.416 (MVLP) + 4.456 (MVLB) + 5.834 (PCLP)
+ 6.757 (PCLB) - 12.339 (LPLB) + 0.638 (MVPCLP) + 0.558 (MVPCLB)
- 4.198 (MVLPLB) + 4.081 (PCLPLB) + 11.632 (MVPCLPLB)
- 6.675 (BLOCK)
(R^2 = 94.32%)

G_3 = 68.192 + 1.813 (MV) - 18.548 (PC) - 18.236 (LP) + 4.053 (LB)
- 3.508 (MVPC) + 0.137 (MVLP) + 5.041 (MVLB) + 10.008 (PCLP)
+ 11.979 (PCLB) - 12.896 (LPLB) + 5.048 (MVPCLP) - 4.066 (MVPCLB)
- 0.488 (MVLPLB) + 2.663 (PCLPLB) + 13.836 (MVPCLPLB)
- 7.482 (BLOCK)
(R^2 = 99.12%)

G_4 = 78.818 - 1.730 (MV) - 20.842 (PC) - 24.366 (LP) + 8.284 (LB)
- 0.449 (MVPC) - 0.597 (MVLP) + 2.957 (MVLB) + 12.810 (PCLP)
+ 14.275 (PCLB) - 13.744 (LPLB) + 2.576 (MVPCLP) - 3.979 (MVPCLB)
- 1.397 (MVLPLB) + 1.800 (PCLPLB) + 11.599 (MVPCLPLB)
- 7.030 (BLOCK)
(R^2 = 99.89%)

G_5 = 83.550 - 3.576 (MV) - 20.539 (PC) - 27.189 (LP) + 11.974 (LB)
- 11.674 (MVPC) + 1.348 (MVLP) + 0.897 (MVLB) + 12.177 (PCLP)
+ 15.363 (PCLB) - 14.867 (LPLB) + 3.167 (MVPCLP) - 5.177 (MVPCLB)
+ 0.766 (MVLPLB) + 0.264 (PCLPLB) + 12.379 (MVPCLPLB)
- 7.075 (BLOCK)
(R^2 = 99.41%)

G_6 = 88.280 - 5.841 (MV) - 23.136 (PC) - 29.712 (LP) + 16.368 (LB)
+ 0.236 (MVPC) + 4.352 (MVLP) - 1.616 (MVLB) + 14.282 (PCLP)
+ 12.919 (PCLB) - 16.527 (LPLB) - 0.664 (MVPCLP) - 3.134 (MVPCLB)
+ 3.787 (MVLPLB) + 1.957 (PCLPLB) + 7.576 (MVPCLPLB)
- 6.505 (BLOCK)

(R^2 = 98.78%)

---

* ml CO_2/ 100 g at NTP

MV = M. varians, PC = P. cerevisiae, LP = L. plantarum, LB = L. brevis

Appendix 4.4 (continued)

Table A 4.5

The multiple regression equations for compression force (CF) during 14 days of Nham fermentation affected by starter cultures.

CF^*_1 = 12.612 - 0.130 (MV) + 0.105 (PC) - 1.326 (LP) -0.540 (LB) - 0.389 (MVPC)
- 0.142 (MVLP) + 0.029 (MVLB) - 0.463 (PCLP) - 0.036 (PCLB)
+ 0.482 (LPLB) + 0.464 (MVPCLP) + 0.960 (MVPCLB) - 0.199 (MVLPLB)
+ 0.021 (PCLPLB) + 0.938 (MVPCLPLB) -0.745 (BLOCK)
(R^2 = 94.71%)

CF_3 = 79.325 + 1.007 (MV) + 2.702 (PC) + 12.326 (LP) - 5.014 (LB)
+ 0.931 (MVPC) - 2.466 (MVLP) - 0.066 (MVLB) - 0.771 (PCLP)
+ 3.747 (PCLB) - 6.614 (LPLB) + 4.311 (MVPCLP) + 6.168 (MVPCLB)
- 4.563 (MVLPLB) + 0.069 (PCLPLB) + 1.753 (MVPCLPLB)
+ 2.090 (BLOCK)
(R^2 = 97.64%)

CF_7 = 93.238 - 4.091 (MV) + 9.063 (PC) + 7.821 (LP) - 18.332 (LB) + 3.181 (MVPC)
+ 3.271 (MVLP) + 1.061 (MVLB) - 8.011 (PCLP) - 13.328 (PCLB)
- 4.941 (LPLB) + 15.924 (MVPCLP) + 0.259 (MVPCLB) - 12.291 (MVLPLB)
+ 4.246 (PCLPLB) - 12.014 (MVPCLPLB) + 3.950 (BLOCK)
(R^2 = 87.14%)

CF_{10} = 83.452 - 4.208 (MV) + 13.838 (PC) + 4.249 (LP) - 11.472 (LB)
- 1.382 (MVPC) + 8.064 (MVLP) + 3.558 (MVLB) - 4.532 (PCLP)
- 13.441 (PCLB) + 1.333 (LPLB) + 11.866 (MVPCLP) + 7.312 (MVPCLB)
- 11.839 (MVLPLB) + 2.824 (PCLPLB) - 6.128 (MVPCLPLB)
- 3.482 (BLOCK)
(R^2 = 97.83%)

CF_{14} = 76.690 - 1.594 (MV) + 14.403 (PC) + 2.984 (LP) - 9.617 (LB) - 2.466 (MVPC)
+ 8.808 (MVLP) + 1.354 (MVLB) - 6.752 (PCLP) - 12.566 (PCLB)
+ 1.441 (LPLB) + 8.729 (MVPCLP) + 8.013 (MVPCLB) - 12.558 (MVLPLB)
+ 7.809 (PCLPLB) - 1.317 (MVPCLPLB) - 2.425 (BLOCK)
(R^2 = 96.83%)

---

* Newtons

MV = M. varians, PC = P. cerevisiae, LP = L. plantarum, LB = L. brevis

Table A 4.6

The multiple regression equations for compression energy (CE) during 14 days of Nham fermentation affected by starter cultures.

CE_1* = 0.062 - 0.003 (MV) - 0.001 (PC) - 0.006 (LP) - 0.004 (LB) - 0.002 (MVPC)
- 0.001 (MVLP) + 0.000 (MVLB) - 0.002 (PCLP) + 0.002 (PCLB)
+ 0.003 (LPLB) + 0.001 (MVPCLP) + 0.004 (MVPCLB) + 0.001 (MVLPLB)
- 0.000 (PCLPLB) + 0.000 (MVPCLPLB) + 0.000 (BLOCK)
(R^2 = 99.05%)

CE_3 = 0.495 - 0.005 (MV) + 0.013 (PC) + 0.088 (LP) - 0.066 (LB) - 0.009 (MVPC)
- 0.028 (MVLP) - 0.006 (MVLB) + 0.005 (PCLP) + 0.016 (PCLB)
- 0.042 (LPLB) + 0.033 (MVPCLP) + 0.039 (MVPCLB) - 0.035 (MVLPLB)
- 0.014 (PCLPLB) - 0.067 (MVPCLPLB) + 0.055 (BLOCK)
(R^2 = 96.15%)

CE_7 = 0.600 - 0.046 (MV) + 0.063 (PC) + 0.024 (LP) - 0.131 (LB) + 0.036 (MVPC)
+ 0.010 (MVLP) + 0.016 (MVLB) - 0.080 (PCLP) - 0.105 (PCLB)
- 0.017 (LPLB) + 0.117 (MVPCLP) - 0.005 (MVPCLB) - 0.070 (MVLPLB)
+ 0.048 (PCLPLB) - 0.058 (MVPCLPLB) + 0.004 (BLOCK)
(R^2 = 91.08%)

CE_{10} = 0.587 - 0.038 (MV) + 0.123 (PC) + 0.026 (LP) - 0.096 (LB) - 0.034 (MVPC)
+ 0.067 (MVLP) + 0.067 (MVLB) - 0.003 (PCLP) - 0.108 (PCLB)
+ 0.005 (LPLB) + 0.091 (MVPCLP) + 0.063 (MVPCLB) - 0.092 (MVLPLB)
+ 0.012 (PCLPLB) - 0.013 (MVPCLPLB) - 0.019 (BLOCK)
(R^2 = 97.10%)

CE_{14} = 0.481 - 0.031 (MV) + 0.092 (PC) + 0.016 (LP) - 0.074 (LB) - 0.034 (MVPC)
+ 0.050 (MVLP) + 0.015 (MVLB) - 0.057 (PCLP) - 0.097 (PCLB)
+ 0.007 (LPLB) + 0.027 (MVPCLP) + 0.063 (MVPCLB) - 0.099 (MVLPLB)
+ 0.036 (PCLPLB) - 0.020 (MVPCLPLB) + 0.028 (BLOCK)
(R^2 = 99.13%)

---

* Joules

MV = M. varians, PC = P. cerevisiae, LP = L. plantarum, LB = L. brevis

Appendix 4.4 (continued)

Table A 4.7

The multiple regression equations for the tristimulus values (x, y, z) during 14 days of Nham fermentation affected by starter cultures.

x_1 = 22.302 + 0.625 (MV) - 0.156 (PC) + 0.025 (LP) - 0.362 (LB) - 0.100 (MVPC)
+ 0.344 (MVLP) - 0.256 (MVLB) - 0.150 (PCLP) - 0.263 (PCLB)
- 0.231 (LPLB) + 0.506 (MVPCLP) - 0.044 (MVPCLB) - 0.200 (MVLPLB)
+ 0.256 (PCLPLB) + 1.663 (MVPCLPLB) - 0.512 (BLOCK)
(R^2 = 99.75%)

y_1 = 20.010 + 0.569 (MV) - 0.263 (PC) + 0.031 (LP) - 0.356 (LB) - 0.144 (MVPC)
+ 0.262 (MVLP) - 0.325 (MVLB) - 0.231 (PCLP) - 0.281 (PCLB)
- 0.138 (LPLB) + 0.450 (MVPCLP) - 0.050 (MVPCLB) - 0.194 (MVLPLB)
+ 0.212 (PCLPLB) + 1.406 (MVPCLPLB) - 0.500 (BLOCK)
(R^2 = 99.18%)

z_1 = 18.300 + 0.666 (MV) - 0.228 (PC) - 0.022 (LP) - 0.497 (LB) - 0.153 (MVPC)
+ 0.328 (MVLP) - 0.247 (MVLB) - 0.153 (PCLP) - 0.278 (PCLB)
- 0.234 (LPLB) + 0.284 (MVPCLP) - 0.066 (MVPCLB) - 0.172 (MVLPLB)
+ 0.284 (PCLPLB) + 1.622 (MVPCLPLB) - 0.563 (BLOCK)
(R^2 = 99.48%)

x_3 = 26.312 + 0.250 (MV) - 0.069 (PC) + 0.600 (LP) - 0.662 (LB) + 0.237 (MVPC)
+ 0.769 (MVLP) + 0.369 (MVLB) - 0.212 (PCLP) + 0.075 (PCLB)
- 0.531 (LPLB) + 0.194 (MVPCLP) + 0.219 (MVPCLB) - 0.375 (MVLPLB)
+ 0.294 (PCLPLB) - 0.850 (MVPCLPLB) + 0.163 (BLOCK)
(R^2 = 94.91%)

y_3 = 23.867 + 0.313 (MV) - 0.094 (PC) + 0.581 (LP) - 0.681 (LB) + 0.200 (MVPC)
+ 0.775 (MVLP) + 0.363 (MVLB) - 0.244 (PCLP) + 0.056 (PCLB)
- 0.506 (LPLB) + 0.200 (MVPCLP) + 0.200 (MVPCLB) - 0.338 (MVLPLB)
+ 0.319 (PCLPLB) - 0.450 (MVPCLPLB) - 0.063 (BLOCK)
(R^2 = 92.94%)

z_3 = 23.757 + 0.337 (MV) - 0.025 (PC) + 0.556 (LP) - 0.900 (LB) + 0.250 (MVPC)
+ 0.844 (MVLP) + 0.350 (MVLB) - 0.244 (PCLP) + 0.175 (PCLB)
- 0.669 (LPLB) + 0.181 (MVPCLP) + 0.088 (MVPCLB) - 0.294 (MVLPLB)
+ 0.406 (PCLPLB) - 0.294 (MVPCLPLB) - 0.187 (BLOCK)
(R^2 = 93.89%)

x_7 = 27.217 - 0.022(MV) - 0.147 (PC) + 0.516 (LP) - 0.822 (LB) + 0.047 (MVPC)
+ 0.384 (MVLP) + 0.022 (MVLB) - 0.116 (PCLP) - 0.003 (PCLB)
+ 0.047 (LPLB) + 0.328 (MVPCLP) + 0.391 (MVPCLB) - 0.059 (MVLPLB)
- 0.134 (PCLPLB) + 0.359 (MVPCLPLB) - 0.050 (BLOCK)
(R^2 = 93.71%)

y_7 = 24.485 + 0.006 (MV) - 0.162 (PC) + 0.475 (LP) - 0.862 (LB) + 0.069 (MVPC)
+ 0.381 (MVLP) - 0.006 (MVLB) - 0.138 (PCLP) - 0.062 (PCLB)
+ 0.138 (LPLB) + 0.331 (MVPCLP) + 0.381 (MVPCLB) + 0.006 (MVLPLB)
- 0.175 (PCLPLB) + 0.581 (MVPCLPLB) - 0.175 (BLOCK)
(R^2 = 94.27%)

z_7 = 24.367 + 0.056 (MV) - 0.175 (PC) + 0.431 (LP) - 0.994 (LB) + 0.119 (MVPC)
+ 0.413 (MVLP) + 0.000 (MVLB) - 0.119 (PCLP) + 0.081 (PCLB)
+ 0.150 (LPLB) + 0.300 (MVPCLP) + 0.363 (MVPCLB) + 0.094 (MVLPLB)
- 0.150 (PCLPLB) + 0.644 (MVPCLPLB) - 0.188 (BLOCK)
(R^2 = 92.80%)

Appendix 4.4 Table 4.7 (continued)

x_{10} = 26.822 - 0.094 (MV) - 0.219 (PC) - 0.119 (LP) - 0.394 (LB) + 0.188 (MVPC)
+ 0.575 (MVLP) - 0.375 (MVLB) - 0.250 (PCLP) - 0.275 (PCLB)
- 0.038 (LPLB) - 0.069 (MVPCLP) + 0.356 (MVPCLB) - 0.194 (MVLPLB)
- 0.169 (PCLPLB) - 0.675 (MVPCLPLB) + 0.462 (BLOCK)
(R^2 = 82.54%)

y_{10} = 23.972 - 0.147 (MV) - 0.297 (PC) - 0.091 (LP) - 0.447 (LB) + 0.141 (MVPC)
+ 0.622 (MVLP) - 0.434 (MVLB) - 0.253 (PCLP) - 0.259 (PCLB)
- 0.028 (LPLB) - 0.078 (MVPCLP) + 0.391 (MVPCLB) - 0.153 (MVLPLB)
- 0.178 (PCLPLB) - 0.341 (MVPCLPLB) + 0.250 (BLOCK)
(R^2 = 87.62%)

z_{10} = 23.802 - 0.047 (MV) - 0.309 (PC) - 0.228 (LP) - 0.534 (LB) + 0.134 (MVPC)
+ 0.678 (MVLP) - 0.403 (MVLB) - 0.247 (PCLP) - 0.253 (PCLB)
- 0.109 (LPLB) - 0.153 (MVPCLP) + 0.391 (MVPCLB) - 0.178 (MVLPLB)
- 0.091 (PCLPLB) + 0.053 (MVPCLPLB) + 0.075 (BLOCK)
(R^2 = 88.46%)

x_{14} = 26.200 + 0.075 (MV) - 0.338 (PC) + 0.575 (LP) - 1.013 (LB) + 0.188 (MVPC)
+ 0.175 (MVLP) - 0.225 (MVLB) - 0.275 (PCLP) + 0.025 (PCLB)
- 0.237 (LPLB) + 0.475 (MVPCLP) + 0.412 (MVPCLB) - 0.225 (MVLPLB)
+ 0.338 (PCLPLB) - 0.850 (MVPCLPLB) + 0.425 (BLOCK)
(R^2 = 95.39%)

y_{14} = 23.347 + 0.025 (MV) - 0.369 (PC) + 0.525 (LP) - 1.056 (LB) + 0.150 (MVPC)
+ 0.181 (MVLP) - 0.212 (MVLB) - 0.250 (PCLP) + 0.056 (PCLB)
- 0.262 (LPLB) + 0.481 (MVPCLP) + 0.425 (MVPCLB) - 0.231 (MVLPLB)
+ 0.350 (PCLPLB) - 0.831 (MVPCLPLB) + 0.513 (BLOCK)
(R^2 = 95.98%)

z_{14} = 23.290 + 0.194 (MV) - 0.387 (PC) + 0.431 (LP) - 1.169 (LB) + 0.175 (MVPC)
+ 0.206 (MVLP) - 0.219 (MVLB) - 0.263 (PCLP) + 0.175 (PCLB)
- 0.344 (LPLB) + 0.600 (MVPCLP) + 0.387 (MVPCLB) - 0.194 (MVLPLB)
+ 0.437 (PCLPLB) + 0.200 (MVPCLPLB) - 0.125 (BLOCK)
(R^2 = 98.19%)

MV = M. varians, PC = P. cerevisiae, LP = L. plantarum, LB = L. brevis

Table A 4.8

The multiple regression equations for residual nitrite (RN) during 14 days of Nham fermentation affected by starter cultures.

RN_1* = 58.844 + 8.109 (MV) - 2.326 (PC) - 5.445 (LP) - 3.153 (LB) - 3.436 (MVPC)
- 0.232 (MVLP) + 1.638 (MVLB) + 2.730 (PCLP) - 1.607 (PCLB)
- 0.708 (LPLB) + 2.969 (MVPCLP) + 4.224 (MVPCLB) + 0.599 (MVLPLB)
+ 8.563 (PCLPLB) - 1.797 (MVPCLPLB) - 0.165 (BLOCK)
(R^2 = 96.64%)

RN_3 = 39.043 - 2.933 (MV) - 3.102 (PC) - 14.385 (LP) - 5.920 (LB) + 6.937 (MVPC)
+ 1.134 (MVLP) - 1.092 (MVLB) + 1.837 (PCLP) + 1.859 (PCLB)
+ 6.875 (LPLB) - 5.617 (MVPCLP) - 11.933 (MVPCLB) + 1.823 (MVLPLB)
- 1.943 (PCLPLB) + 3.207 (MVPCLPLB) + 1.842 (BLOCK)
(R^2 = 98.33%)

RN_7 = 15.855 - 0.457 (MV) + 1.117 (PC) + 0.555 (LP) - 2.677 (LB) + 3.154 (MVPC)
- 0.667 (MVLP) - 0.021 (MVLB) + 0.204 (PCLP) + 0.063 (PCLB)
- 0.920 (LPLB) + 1.201 (MVPCLP) - 2.592 (MVPCLB) - 0.822 (MVLPLB)
+ 1.595 (PCLPLB) - 3.225 (MVPCLPLB) + 0.394 (BLOCK)
(R^2 = 67.12%)

RN_{10} = 4.463 + 0.204 (MV) + 0.035 (PC) + 0.134 (LP) + 0.948 (LB) + 0.373 (MVPC)
- 0.232 (MVLP) + 0.218 (MVLB) - 0.007 (PCLP) - 0.428 (PCLB)
- 0.021 (LPLB) + 0.471 (MVPCLP) + 0.246 (MVPCLB) + 0.063 (MVLPLB)
+ 0.653 (PCLPLB) + 1.160 (MVPCLPLB) - 0.309 (BLOCK)
(R^2 = 97.26%)

RN_{14} = 0.888 + 0.013 (MV) + 0.042 (PC) 0.070 (LP) - 0.140 (LB) - 0.028 (MVPC)
- 0.056 (MVLP) - 0.154 (MVLB) + 0.197 (PCLP) - 0.126 (PCLB)
+ 0.014 (LPLB) + 0.014 (MVPCLP) + 0.000 (MVPCLB) - 0.000 (MVLPLB)
+ 0.028 (PCLPLB) - 0.464 (MVPCLPLB) + 0.056 (BLOCK)
(R^2 = 78.07%)

---

* ppm

MV = M. varians, PC = P. cerevisiae, LP = L. plantarum, LB = L. brevis

Table A 4.9

The multiple regression equations for Enterobacteriaceae (EN) during 14 days of Nham fermentation affected by starter cultures.

EN^*_1 = 7.469 + 0.147 (MV) + 0.161 (PC) - 0.191 (LP) + 0.024 (LB) + 0.011 (MVPC)
+ 0.024 (MVLP) + 0.039 (MVLB) + 0.136 (PCLP) + 0.018 (PCLB)
- 0.013 (LPLB) + 0.086 (MVPCLP) - 0.037 (MVPCLB) - 0.033 (MVLPLB)
+ 0.008 (PCLPLB) + 1.308 (MVPCLPLB) - 0.665 (BLOCK)
(R^2 = 96.56%)

EN_3 = 5.074 + 0.376 (MV) - 0.196 (PC) - 0.687 (LP) - 0.151 (LB) - 0.364 (MVPC)
+ 0.142 (MVLP) + 0.011 (MVLB) + 0.548 (PCLP) + 0.102 (PCLB)
+ 0.141 (LPLB) - 0.051 (MVPCLP) + 0.061 (MVPCLB) + 0.817 (MVLPLB)
+ 0.111 (PCLPLB) + 0.934 (MVPCLPLB) + 0.130 (BLOCK)
(R^2 = 87.72%)

EN_7 = 1.481 - 0.058 (MV) - 0.097 (PC) - 1.066 (LP) + 0.048 (LB) + 0.172 (MVPC)
+ 0.221 (MVLP) - 0.343 (MVLB) + 0.334 (PCLP) - 0.087 (PCLB)
- 0.136 (LPLB) - 0.259 (MVPCLP) - 0.388 (MVPCLB) + 0.581 (MVLPLB)
+ 0.249 (PCLPLB) + 0.981 (MVPCLPLB) + 0.105 (BLOCK)
(R^2 = 99.08%)

EN_{10} = 0.419 + 0.016 (MV) - 0.164 (PC) - 0.417 (LP) + 0.270 (LB) - 0.270 (MVPC)
- 0.016 (MVLP) + 0.164 (MVLB) + 0.164 (PCLP) - 0.016 (PCLB)
- 0.270 (LPLB) + 0.270 (MVPCLP) - 0.417 (MVPCLB) - 0.164 (MVLPLB)
+ 0.016 (PCLPLB) + 0.842 (MVPCLPLB) - 0.425 (BLOCK)
(R^2 = 99.71%)

EN_{14} = 0.363 + 0.013 (MV) - 0.198 (PC) - 0.454 (LP) + 0.269 (LB) - 0.269 (MVPC)
- 0.013 (MVLP) + 0.198 (MVLB) + 0.198 (PCLP) - 0.013 (PCLB)
- 0.269 (LPLB) + 0.269 (MVPCLP) - 0.454 (MVPCLB) - 0.198 (MVLPLB)
+ 0.013 (PCLPLB) + 0.454 (MVPCLPLB) + 0.000 (BLOCK)
(R^2 = 96.28%)

---

* log number

MV = M. varians, PC = P. cerevisiae, LP = L. plantarum, LB = L. brevis

Appendix 4.4 (continued)

Table A 4.10
The multiple regression equation for weight loss (WL) during 14 days of Nham fermentation affected by starter cultures.

WL_3 (%) = 3.457 + 0.241 (MV) + 0.080 (PC) + 0.489 (LP) + 0.489 (LB)
+ 0.075 (MVPC) - 0.014 (MVLP) - 0.009 (MVLB) + 0.452 (PCLP)
+ 0.510 (PCLB) + 0.004 (LPLB) + 0.102 (MVPCLP) + 0.155 (MVPCLB)
+ 0.371 (MVLPLB) - 0.047 (PCLPLB) - 0.112 (MVPCLPLB)
- 0.250 (BLOCK)
(R^2 = 89.06%)

WL_7 (%) = 7.054 + 0.268(MV) + 0.703 (PC) + 0.836 (LP) + 1.354 (LB)
+ 0.144 (MVPC) - 0.126 (MVLP) + 0.383 (MVLB) - 0.036 (PCLP)
- 0.062 (PCLB) + 0.693 (LPLB) - 0.194 (MVPCLP) + 0.114 (MVPCLB)
- 0.033 (MVLPLB) - 0.273 (PCLPLB) - 0.877 (MVPCLPLB)
+ 0.970 (BLOCK)
(R^2 = 98.04%)

WL_{10} (%) = 8.762 + 0.309 (MV) + 0.372 (PC) + 0.155 (LP) + 1.541 (LB)
+ 0.183 (MVPC) - 0.287 (MVLP) + 0.431 (MVLB) + 0.339 (PCLP)
- 0.305 (PCLB) + 0.938 (LPLB) - 0.251 (MVPCLP) - 0.132 (MVPCLB)
+ 0.038 (MVLPLB) - 0.121 (PCLPLB) - 0.081 (MVPCLPLB)
+ 0.632 (BLOCK)
(R^2 = 98.79%)

WL_{14} (%) = 9.468 + 0.434 (MV) + 0.279 (PC) - 0.055 (LP) + 1.466 (LB)
+ 0.128 (MVPC) - 0.461 (MVLP) + 0.335 (MVLB) + 0.461 (PCLP)
- 0.232 (PCLB) + 0.979 (LPLB) - 0.220 (MVPCLP) + 0.061 (MVPCLB)
+ 0.193 (MVLPLB) - 0.167 (PCLPLB) - 0.094 (MVPCLPLB)
+ 0.630 (BLOCK)
(R^2 = 99.89%)

MV = M. varians, PC = P. cerevisiae, LP = L. plantarum, LB = L. brevis

APPENDIX 4.5

Analysis of variance of overall acceptability during Nham fermentation

Analysis of variance

SOV	df	3 days		7 days		10 days		14 days	
		SS	MS	SS	MS	SS	MS	SS	MS
Rep-block	15	0.225	0.015**	0.150	0.010*	0.331	0.022**	0.459	0.031**
Treatment	14	0.769	0.055**	0.731	0.052**	0.854	0.061**	0.712	0.051**
Error	98	0.626	6.39×10^{-3}	0.529	5.4×10^{-3}	0.689	7.03×10^{-3}	0.668	6.83×10^{-3}
Total	127								

Combined analysis

AOV	Error SS	df	Treat. SS	df	Rep-block SS	df
3 days	0.626	98	0.769	14	0.225	15
7 days	0.529	98	0.731	14	0.150	15
10 days	0.689	98	0.854	14	0.331	15
14 days	0.668	98	0.712	14	0.459	15
	2.512	392	3.066	56	1.165	60
	= Pooled error		= Treatment/time		= Rep-block/time	

$$\text{Time SS} = \frac{\left(92.21^2 + 96.78^2 + 88.52^2 + 86.91^2\right)}{128} - \frac{(364.42)^2}{512} = 0.45$$

$$\text{Rep-block SS} = \frac{\left(23.05^2 + 24.07^2 + \ldots + 22.89^2\right)}{32} - \text{CF} = 0.646$$

Rep-block x Time SS = (Rep-block/time SS) - (Rep-block SS) = 0.519

$$\text{Treatment SS} = \left[\frac{\left(19.83^2 + 23.74^2 + \ldots + 22.16^2\right)}{32} - \text{CF}\right] - \left[\left(\frac{179.46^2 + 184.96^2}{64 \times 4}\right) - \text{CF}\right]$$

$$= 261.26 - 259.44 = 1.82$$

Treatment x Time SS = Treatment/time SS - Treatment SS = 1.246

SOV	df	SS	MS	F-TEST
Time	3	0.450	0.150	23.40**
Rep-block	15	0.646	0.043	6.71**
Rep-block x Time	45	0.519	0.012	1.87**
Treatment	14	1.820	0.130	20.28**
Treatment x Time	42	1.246	0.030	4.68**
Pooled error	392	2.512	6.41×10^{-3}	
Total	511			

Significance level at
95% (392, 3) = 2.621 *
99% (392, 3) = 3.832 **
95% (392, 14) = 1.721 *
99% (392, 14) = 2.122 **
95% (392, 15) = 1.696 *
99% (392, 15) = 2.082 **
95% (392, 42) = 1.413 *
99% (392, 42) = 1.474 **
95% (392, 45) = 1.401 *
99% (392, 45) = 1.511 **

Appendix 4.5 (continued)

Orthogonal comparison test

Comparison	1 b 26.26	2 abc 25.38	3 ac 24.78	4 ab 24.34	5 c 24.09	6 bc 23.41	7 a 22.97	8 cd 22.91	9 bcd 22.75	10 acd 22.16	11 abd 21.11	12 ad 20.61	13 d 20.24	14 bd 19.84	15 (1) 19.83	$Ecij^2$	Li	SS	F-test
1 vs 2-15	+14	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	210	53.22	0.421	65.70**
2 vs 3-15	0	+13	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	182	40.90	0.287	44.79**
3 vs 4-15	0	0	+12	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	156	33.10	0.219	34.18**
4 vs 5-15	0	0	0	+11	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	132	27.82	0.183	28.56**
5 vs 6-15	0	0	0	0	+10	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	110	25.07	0.178	27.78**
6 vs 7-15	0	0	0	0	0	+9	-1	-1	-1	-1	-1	-1	-1	-1	-1	90	18.27	0.116	18.10**
7 vs 8-15	0	0	0	0	0	0	+8	-1	-1	-1	-1	-1	-1	-1	-1	72	14.31	0.089	13.89**
8 vs 9-15	0	0	0	0	0	0	0	+7	-1	-1	-1	-1	-1	-1	-1	56	13.83	0.107	16.70**
9 vs 10-15	0	0	0	0	0	0	0	0	+6	-1	-1	-1	-1	-1	-1	42	12.71	0.120	18.73**
10 vs 11-15	0	0	0	0	0	0	0	0	0	+5	-1	-1	-1	-1	-1	30	9.17	0.088	13.73**
11 vs 12-15	0	0	0	0	0	0	0	0	0	0	+4	-1	-1	-1	-1	20	3.92	0.024	3.74ns
12 vs 13-15	0	0	0	0	0	0	0	0	0	0	0	+3	-1	-1	-1	12	1.92	$9.6x10^{-3}$	1.49ns
13 vs 14-15	0	0	0	0	0	0	0	0	0	0	0	0	+2	-1	-1	6	0.81	$3.4x10^{-3}$	0.53ns
14 vs 15	0	0	0	0	0	0	0	0	0	0	0	0	0	+1	-1	2	0.01	$1.5x10^{-6}$	$2.3x10^{-4}$ns

Significant level at 95% (392, 1) = 3.861*

99% (392, 1) = 6.702**

APPENDIX 5.1

Results for 2^3 factorial design used for multiple regression.

Table A1.1 Matrix for 2^3 factorial design and example

Run	TREATMENTS	Independent variables							Dependent variables					
		CR	G	CRG	LP	CRLP	GLP	CRGLP	X1	X2	X3	X4	X5	X6
1	(1)	-	-	+	-	+	+	-	x_1	x_1	x_1	x_1	x_1	x_1
2	a	+	-	-	-	-	+	+	x_2	x_2	x_2	x_2	x_2	x_2
3	b	-	+	-	-	+	-	+	x_3	x_3	x_3	x_3	x_3	x_3
4	ab	+	+	+	-	-	-	-	x_4	x_4	x_4	x_4	x_4	x_4
5	c	-	-	+	+	-	-	+	x_5	x_5	x_5	x_5	x_5	x_5
6	ac	+	-	-	+	+	-	-	x_6	x_6	x_6	x_6	x_6	x_6
7	bc	-	+	-	+	-	+	-	x_7	x_7	x_7	x_7	x_7	x_7
8	abc	+	+	+	+	+	+	+	x_8	x_8	x_8	x_8	x_8	x_8
9	cp1	0	0	0	0	0	0	0	x_9	x_9	x_9	x_9	x_9	x_9
10	cp2	0	0	0	0	0	0	0	x_{10}	x_{10}	x_{10}	x_{10}	x_{10}	x_{10}

CR=Cooked rice (a); G= Glucose (b); LP= L.plantarum (c) cp= centrepoint
(1)= Control

Appendix 5.1 (continued)

Table A1.2 The pH during 7 days of Nham fermentation affected by carbon sources and L. plantarum.

	pH.0h	pH.6h	pH.12h	pH.18h	pH.24h	pH.2d
1	6.89	6.81	6.70	5.29	5.12	4.48
2	6.92	6.75	6.66	5.18	5.00	4.36
3	6.85	6.71	6.66	5.20	5.05	4.44
4	6.92	6.71	6.60	5.64	5.17	4.46
5	6.92	6.79	6.58	5.10	5.08	4.40
6	6.82	6.70	6.59	5.24	5.07	4.54
7	6.95	6.66	6.56	5.13	4.97	4.40
8	6.91	6.64	6.50	5.60	5.15	4.46
9	6.88	6.68	6.56	5.50	5.11	4.47
10	6.81	6.71	6.59	5.58	5.10	4.49

	pH.3d	pH.4d	pH.5d	pH.6d	pH.7d
1	4.27	4.18	4.14	4.02	4.02
2	4.21	4.12	4.11	4.00	4.00
3	4.26	4.20	4.14	4.02	4.01
4	4.30	4.24	4.12	4.04	4.04
5	4.25	4.15	4.10	4.00	3.95
6	4.24	4.20	4.18	4.04	4.04
7	4.24	4.14	4.14	4.00	3.98
8	4.29	4.22	4.11	4.09	4.08
9	4.29	4.20	4.12	4.04	4.03
10	4.28	4.15	4.10	4.03	4.04

Appendix 5.1 (continued)

Table A 1.3 The reducing sugars (RS; %) change during 7 days of Nham fermentation affected by carbon sources and L. plantarum.

	RS.0h	RS.6h	RS.12h	RS.18h	RS.24h	RS.2d
1	0.48	1.39	1.51	1.14	1.11	1.11
2	0.50	1.78	1.61	1.30	1.17	1.13
3	0.97	1.85	2.09	1.67	1.41	1.30
4	0.98	1.81	1.89	1.71	1.62	1.54
5	0.52	1.29	1.23	1.10	1.02	1.02
6	0.50	1.36	1.33	1.14	1.12	1.14
7	0.98	1.52	2.00	1.52	1.29	1.19
8	1.01	1.62	1.76	1.70	1.47	1.38
9	0.73	1.59	1.73	1.55	1.20	1.07
10	0.73	1.61	1.70	1.49	1.24	1.00

	RS.3d	RS.4d	RS.5d	RS.6d	RS.7d
1	1.13	0.89	0.85	0.81	0.77
2	1.17	1.12	1.05	1.05	1.07
3	1.22	1.26	1.26	1.26	1.06
4	1.35	1.28	1.29	1.35	1.41
5	0.94	0.99	0.88	0.89	0.73
6	1.05	1.00	0.92	1.00	1.00
7	1.09	1.11	1.13	1.02	0.99
8	1.21	1.50	1.41	1.31	1.26
9	1.16	1.20	1.16	1.14	1.05
10	1.18	1.24	1.10	1.10	1.10

Table A1.4 The cooked rice (CR; %) change during 7 days of Nham fermentation affected by carbon sources and L. plantarum.

	CR.0h	CR.6h	CR.12h	CR.18h	CR.24h	CR.2d
1	7.92	5.94	4.03	3.88	2.68	2.21
2	9.89	6.58	4.70	3.97	3.22	2.58
3	7.70	5.82	4.91	4.88	4.29	2.73
4	9.89	6.58	4.98	4.65	4.63	2.96
5	7.52	5.51	3.67	3.26	2.98	2.60
6	9.56	6.22	4.31	3.35	3.16	3.03
7	7.73	5.74	4.56	3.55	3.40	3.01
8	9.52	7.11	4.76	4.28	3.83	3.52
9	8.78	6.56	4.68	3.99	3.64	2.93
10	8.68	6.50	4.60	4.05	3.46	3.03

	CR.3d	CR.4d	CR.5d	CR.6d	CR.7d
1	1.93	1.77	1.77	1.72	1.71
2	2.30	1.92	1.87	1.87	1.84
3	2.57	2.31	2.10	1.71	1.68
4	2.61	2.40	2.30	2.31	1.97
5	2.31	2.27	1.93	1.86	1.81
6	2.96	2.87	2.39	2.25	2.16
7	2.13	1.90	1.68	1.66	1.30
8	3.47	2.87	2.33	2.11	1.97
9	2.52	3.49	1.98	1.93	1.85
10	2.58	2.40	2.02	1.95	1.80

Table A1.5 The weight loss (WL; %) change during 7 days of Nham fermentation affected by carbon sources and L. plantarum.

	WL.1d	WL.2d	WL.3d	WL.4d	WL.5d	WL.6d	WL.7d
1	0.11	0.78	2.94	4.58	5.42	6.91	6.78
2	0.09	0.56	2.08	2.56	3.25	4.54	5.60
3	0.09	0.90	3.32	4.62	5.32	6.52	6.92
4	0.09	1.20	3.56	5.11	6.35	7.16	7.76
5	0.07	0.54	1.89	2.65	3.76	4.32	5.14
6	0.09	1.08	2.94	4.28	5.52	6.12	6.92
7	0.11	1.04	3.17	3.91	5.98	6.35	7.08
8	0.08	0.80	3.20	5.23	6.23	6.99	7.78
9	0.07	0.60	2.54	4.41	5.61	6.36	7.42
10	0.08	0.75	3.41	4.70	5.74	6.30	7.40

Table A1.6 The compression force (CF; Newtons) change during 7 days of Nham fermentation affected by carbon sources and L. plantarum.

	CF.1d	CF.2d	CF.3d	CF.4d	CF.5d	CF.6d	CF.7d
1	51.74	87.98	100.63	115.40	128.80	127.58	115.72
2	65.68	105.98	134.80	137.88	140.53	134.67	125.55
3	70.67	98.10	123.30	124.47	132.34	124.41	124.85
4	56.61	101.21	104.32	114.88	120.40	103.96	106.05
5	78.75	139.81	151.48	152.81	138.08	130.73	123.84
6	76.74	94.02	118.68	118.31	98.50	98.92	97.25
7	81.89	136.55	140.44	144.45	135.02	120.83	120.40
8	59.46	112.84	129.54	128.59	107.90	96.00	97.73
9	54.34	93.74	112.15	126.91	121.04	120.84	111.69
10	51.01	98.94	116.43	125.62	118.10	119.25	113.40

Appendix 5.1 (continued)

Table A1.7 The tristimulus values (x, y, z) during 7 days of Nham fermentation affected by carbon sources and L. plantarum.

	X1	Y1	Z1	X2	Y2	Z2
1	20.45	17.95	16.80	24.35	21.60	20.95
2	22.65	20.05	18.85	25.45	22.85	22.20
3	22.00	19.80	18.70	25.00	22.40	21.50
4	23.45	21.05	19.45	24.75	22.15	21.20
5	21.30	18.95	17.50	24.60	21.85	21.35
6	22.85	20.60	19.50	24.15	21.60	20.65
7	23.05	20.85	19.10	24.95	22.05	21.50
8	22.05	20.25	18.80	25.35	22.65	22.00
9	22.65	20.65	19.30	25.20	22.60	21.80
10	22.65	20.40	19.10	25.05	22.45	21.85
	X3	Y3	Z3	X4	Y4	Z4
1	25.10	22.35	21.85	25.60	22.75	22.45
2	25.95	23.35	22.75	25.75	23.05	22.75
3	25.75	22.95	22.60	25.60	22.95	22.65
4	25.50	22.65	22.20	25.85	23.05	22.90
5	25.75	23.15	22.95	25.65	22.80	22.40
6	26.75	24.15	23.70	26.75	23.80	23.50
7	25.85	23.00	22.50	25.65	22.85	22.30
8	25.80	23.20	22.50	26.15	23.30	22.85
9	26.55	24.10	23.55	26.65	23.80	23.45
10	26.75	24.05	23.60	26.80	24.05	23.55
	X5	Y5	Z5	X6	Y6	Z6
1	25.85	23.15	22.65	25.30	22.60	22.40
2	26.65	23.90	23.70	25.95	23.35	22.95
3	25.55	22.80	22.15	25.30	22.50	22.05
4	25.10	22.25	21.80	24.80	22.10	21.50
5	25.65	22.70	22.10	26.10	23.35	23.15
6	26.20	23.40	22.80	25.90	22.95	22.75
7	26.20	23.15	22.95	25.70	23.15	22.55
8	25.90	22.90	22.80	25.20	22.45	22.15
9	26.60	23.85	23.40	25.85	23.35	22.60
10	26.65	23.70	23.40	25.70	22.95	22.55

	X7	Y7	Z7
1	25.35	22.65	22.30
2	25.05	22.50	22.10
3	24.85	22.25	21.50
4	24.65	21.80	21.25
5	25.20	22.50	21.95
6	25.50	22.70	22.20
7	25.75	23.05	22.65
8	22.20	22.50	22.15
9	25.95	23.40	23.80
10	25.85	23.20	23.00

Appendix 5.1 (continued)

Table A1.8 (a) The sensory evaluation on 3 days of Nham fermentation affected by carbon sources and L. plantarum.

	Colour *	Visual Texture	Air Pockets	Firmness	Juiciness	Smoothness
1	0.95	0.96	1.02	0.95	0.98	0.95
2	1.00	0.93	1.02	1.02	0.91	0.93
3	0.97	0.91	1.01	0.97	0.99	0.95
4	0.96	0.92	1.05	0.92	0.96	0.92
5	0.97	0.90	1.02	1.03	0.95	0.93
6	1.01	0.95	1.04	0.88	1.08	0.93
7	0.98	0.94	1.06	1.01	1.01	0.94
8	0.98	0.93	1.01	0.91	0.97	0.93
9	1.01	0.95	1.03	0.96	0.99	0.92
10	1.01	0.98	1.01	0.96	1.00	0.94

	Sourness	Saltiness	Spiciness	Pork Flavour	Overall Acceptability
1	0.89	1.03	0.93	0.94	0.85
2	0.99	1.00	0.93	0.97	0.93
3	0.91	1.05	0.94	0.96	0.90
4	0.86	1.02	0.96	0.98	0.90
5	0.92	1.00	0.96	0.97	0.93
6	0.93	1.02	0.93	0.97	0.85
7	0.92	1.01	0.95	1.00	0.92
8	0.88	0.98	0.96	0.97	0.91
9	0.88	0.99	0.96	0.96	0.89
10	0.88	1.02	0.96	0.98	0.90

* = mean ideal ratio score

Appendix 5.1 (continued)

Table A1.8(b) The sensory evaluation on 7 days of Nham fermentation affected by carbon sources and L. plantarum.

	Colour *	Visual Texture	Air Pockets	Firmness	Juiciness	Smoothness
1	0.99	0.96	1.05	0.98	0.99	0.97
2	1.00	0.92	1.06	1.06	0.97	0.94
3	0.98	0.98	1.02	1.04	0.99	0.98
4	0.97	0.94	1.07	0.98	1.01	0.95
5	0.99	0.93	1.07	1.04	1.00	0.94
6	0.99	0.94	1.05	0.92	1.02	0.97
7	1.00	0.95	1.04	1.00	1.03	0.97
8	0.98	0.98	1.04	0.94	1.05	0.96
9	1.00	0.95	1.06	0.96	1.00	0.92
10	0.99	0.95	1.06	0.94	1.01	0.95

	Sourness	Saltiness	Spiciness	Pork Flavour	Overall Acceptability
1	0.98	1.02	1.01	0.98	0.91
2	1.00	0.98	0.95	0.98	0.94
3	0.99	0.99	0.97	0.96	0.92
4	0.96	1.04	0.96	0.96	0.91
5	1.04	1.02	0.99	0.96	0.94
6	0.96	1.02	0.98	0.98	0.91
7	1.02	1.00	0.98	0.99	0.92
8	0.94	1.01	1.00	0.97	0.91
9	0.98	1.01	0.98	0.99	0.89
10	0.98	1.00	0.98	0.98	0.89

* = mean ideal ratio score

APPENDIX 5.2

The multiple regression equations for dependent variables affected by carbon sources and L. plantarum

Table A2.1 The stepwise multiple regression for pH during 0-48 hours of Nham fermentation affected by carbon sources and L. plantarum.

0-48 hours;

Transformation selected: independent variable = $\sqrt{\text{Time} + 1}$

dependent variable = 1/pH

constant = 0.0186

Variables	Coeffeicients	Beta	F-ratio	Probability
CR	-0.0008	-0.0226	0.2903	0.5924
G	-0.0004	-0.0108	0.0662	0.7980
LP	0.0004	0.0133	0.1005	0.7525
CRG	-0.0011	-0.0332	0.6252	0.4328
CRLP	-0.0006	-0.0172	0.1683	0.6834
GLP	0.0002	0.0059	0.0196	0.8891
CRGLP	0.0004	0.0134	0.1020	0.7507
$\sqrt{\text{Time} + 1}$	0.1207	0.9526	513.8493	0.000

Final equation is $1/pH = 0.0186 + 0.1207 \sqrt{\text{Time} + 1}$ (R^2 = 90.75%)

CR = Cooked rice; G = Glucose; LP = L. plantarum

Table A 2.2 The multiple regression equations for reducing sugars (RS) during 2 days of Nham fermentation affected by carbon sources and L. plantarum.

RS* (6 hrs) = 1.582 + 0.065 (CR) + 0.122 (G) - 0.050 (CRG) - 0.130 (LP) - 0.023 (CRLP) + 0.000 (GLP) + 0.058 (CRGLP) (R^2 = 99.70%)

RS (12 hrs) = 1.685 - 0.030 (CR) + 0.258 (G) - 0.080 (CRG) - 0.098 (LP) - 0.005 (CRLP) + 0.043 (GLP) - 0.005 (CRGLP) (R^2 = 99.60%)

RS (18 hrs) = 1.432 + 0.053 (CR) + 0.240 (G) + 0.003 (CRG) - 0.045 (LP) + 0.003 (CRLP) + 0.005 (GLP) + 0.032 (CRGLP) (R^2 = 96.00%)

RS (24 hrs) = 1.265 + 0.069 (CR) + 0.172 (G) + 0.029 (CRG) - 0.051 (LP) + 0.001 (CRLP) - 0.016 (GLP) - 0.009 (CRGLP) (R^2 = 98.10%)

RS (2 days) = 1.188 + 0.071 (CR) + 0.126 (G) + 0.036 (CRG) - 0.044 (LP) + 0.006 (CRLP) - 0.024 (GLP) - 0.019 (CRGLP) (R^2 = 76.78%)

* (%)

CR = Cooked rice, G = Glucose, LP = L. plantarum.

Table A 2.3 The multiple regression equations for cooked rice (CR) during 2 days of Nham fermentation affected by carbon sources and L. plantarum.

CR* (6 hrs) = 6.256 + 0.435 (CR) + 0.125 (G) + 0.097 (CRG) - 0.043 (LP) + 0.085 (CRLP) + 0.155 (GLP) + 0.068 (CRGLP) (R^2 = 91.41%)

CR (12 hrs) = 4.520 + 0.197 (CR) + 0.312 (G) - 0.130 (CRG) - 0.165 (LP) + 0.012 (CRLP) + 0.023 (GLP) + 0.020 (CRGLP) (R^2 = 97.38%)

CR (18 hrs) = 3.986 + 0.085 (CR) + 0.363 (G) + 0.040 (CRG) - 0.368 (LP) + 0.120 (CRLP) - 0.058 (GLP) + 0.120 (CRGLP) (R^2 = 99.81%)

CR (24 hrs) = 3.529 + 0.186 (CR) + 0.514 (G) + 0.006 (CRG) - 0.181 (LP) - 0.034 (CRLP) - 0.241 (GLP) + 0.056 (CRGLP) (R^2 = 99.45%)

CR (2 days) = 2.860 + 0.192 (CR) + 0.225 (G) - 0.008 (CRG) + 0.210 (LP) + 0.043 (CRLP) - 0.000 (GLP) + 0.027 (CRGLP) (R^2 = 96.33%)

---

* (%)
CR = Cooked rice, G = Glucose, LP = L. plantarum.

Table A 2.4 The multiple regression equations for weight loss (WL) during 2 days of Nham fermentation affected by carbon sources and L. plantarum.

WL* (1 day) = 0.088 - 0.004 (CR) + 0.001 (G) - 0.004 (CRG) - 0.004 (LP) + 0.001 (CRLP) + 0.006 (GLP) - 0.009 (CRGLP) (R^2 = 73.15%)

WL (2 days) = 0.825 + 0.048 (CR) + 0.122 (G) - 0.033 (CRG) + 0.002 (LP) + 0.028 (CRLP) - 0.068 (GLP) - 0.162 (CRGLP) (R^2 = 85.57%)

---

* (%)
CR = Cooked rice, G = Glucose, LP = L. plantarum

Table A 2.5 The multiple regression equations for compression force (CF) during 2 days of Nham fermentation affected by carbon sources and L. plantarum.

CF* (1 day) = 64.689 - 3.070 (CR) - 0.535 (G) - 6.052 (CRG) + 6.518 (LP) - 3.040 (CRLP) - 3.000 (GLP) + 0.947 (CRGLP) (R^2 = 70.21%)

CF (2 days) = 107.117 - 5.799 (CR) + 2.364 (G) + 0.649 (CRG) + 11.494 (LP) - 11.076 (CRLP) + 1.026 (GLP) + 4.371 (CRGLP) (R^2 = 89.23%)

---

* (Newtons)
CR = Cooked rice; G = Glucose; LP = L. plantarum

Appendix 5.2 (continued)

Table A 2.6 The multiple regression equations for tristimulus values (x, y, z) during 2 days of Nham fermentation affected by carbon sources and L. plantarum.

X_1* = 22.310 + 0.525 (CR) + 0.412(G) - 0.413 (CRG) + 0.087 (LP)
- 0.387 (CRLP) - 0.175 (GLP) - 0.225 (CRGLP)
(R^2 = 95.95%)

Y_1 = 20.055 + 0.550 (CR) + 0.550 (G) - 0.387 (CRG) + 0.225 (LP)
- 0.287 (CRLP) - 0.163 (GLP) - 0.175 (CRGLP)
(R^2 = 92.84%)

Z_1 = 18.710 + 0.563 (CR) + 0.425 (G) - 0.450 (CRG) + 0.138 (LP)
- 0.138 (CRLP) - 0.200 (GLP) - 0.125 (CRGLP)
(R^2 = 91.09%)

X_2 = 24.885 + 0.100 (CR) + 0.188 (G) - 0.063 (CRG) - 0.063 (LP)
- 0.112 (CRLP) + 0.200 (GLP) + 0.275 (CRGLP)
(R^2 = 90.33%)

Y_2 = 22.220 + 0.169 (CR) + 0.169 (G) - 0.081 (CRG) - 0.106 (LP)
- 0.081 (CRLP) + 0.144 (GLP) + 0.294 (CRGLP)
(R^2 = 86.08%)

Z_2 = 21.500 + 0.094 (CR) + 0.131 (G) - 0.044 (CRG) - 0.044 (LP)
- 0.144 (CRLP) + 0.244 (GLP) + 0.344 (CRGLP)
(R^2 = 87.31%)

CR = Cooked rice; G = Glucose; LP = L. plantarum

* x_1:x-value at 1 day.

Appendix 5.2 (continued)

Table A 2.7 The multiple regression equations for overall acceptability on 3 and 7 days of Nham fermentation affected by carbon sources and L. plantarum.

Overall acceptability* 3 = 0.898 - 0.001 (CR) + 0.009 (G) - 0.001 (CRG)
+ 0.004 (LP) - 0.021 (CRLP) + 0.004 (GLP) + 0.019 (CRGLP)
(R^2 = 99.01%)

Overall acceptability 7 = 0.913 - 0.004 (CR) - 0.006 (G) - 0.004 (CRG)
- 0.001 (LP) - 0.009 (CRLP) - 0.001 (GLP) + 0.006 (CRGLP)
(R^2 = 52.94%)

---

* overall acceptability$_3$ = overall acceptability of Nham at 3 day (mean ideal ratio scores)

CR = Cooked rice; G = Glucose; LP = L. plantarum

APPENDIX 6.1

Results of 2^2 factorial design for multiple regression.

Table A 1.1 The maxtrix of 2^2 factorial design for multiple regression analysis (e.g. pH)

Run(s)	TREATMENTS	INDEPENDENT VARIABLES			DEPENDENT VARIABLES			
		A	B	AB	pH (18 hrs)	pH (24 hrs)	pH (48 hrs)	pH (72hrs)
1	(1)	-	-	+	6.56	6.49	5.18	4.72
2	a	+	-	-	5.96	5.19	4.60	4.44
3	b	-	+	-	6.49	6.47	5.09	4.70
4	ab	+	+	+	5.90	5.02	4.52	4.35
5	cp_1	0	0	0	6.22	6.12	4.74	4.52
6	cp_1	0	0	0	6.32	6.22	4.85	4.59

A = Temperature, B = Relative humidity

Appendix 6.1 (continued)

Table A 1.2 The pH value during 72 hours of Nham fermentation at different temperatures and relative humidities.

	pH 0hrs	pH 6hrs	pH12 hrs	pH 18hrs
1	6.75	6.65	6.59	6.56
2	6.68	6.64	6.60	5.96
3	6.68	6.60	6.59	6.49
4	6.68	6.62	6.52	5.90
5	6.66	6.62	6.62	6.22
6	6.69	6.62	6.62	6.32
	pH 24hrs	pH 48hrs	pH 72hrs	
1	6.49	5.18	4.72	
2	5.19	4.60	4.44	
3	6.47	5.09	4.70	
4	5.02	4.52	4.35	
5	6.12	4.74	4.52	
6	6.22	4.85	4.59	

Appendix 6.1 (continued)

Table A1.3 The weight loss (WL; %) during 72 hours of Nham fermentation at different temperatures and relative humidities.

	WL 24hrs	WL 48hrs	WL 72hrs
1	0.07	0.14	0.43
2	0.06	0.29	1.01
3	0.04	0.06	0.09
4	0.03	0.05	0.24
5	0.05	0.07	0.31
6	0.04	0.09	0.28

Appendix 6.1 (continued)

Table A1.4 The compression force (CF; Newtons) during 72 hours of Nham fermentation at different temperatures and relative humidities.

	CF 0hrs	CF 24hrs	CF 48hrs	CF 72hrs
1	10.35	12.70	23.36	69.77
2	10.35	61.89	124.69	151.03
3	10.35	11.48	31.15	75.61
4	10.35	33.00	134.63	138.73
5	10.35	15.98	81.05	103.79
6	10.35	20.18	90.22	106.56

Appendix 6.1 (continued)

Table A1.5 The tristimulus values (x, y, z) during 72 hours of Nham fermentation at different temperatures and relative humidities.

	X 0hrs	Y 0hrs	Z 0hrs	X 24hrs	Y 24hrs	Z 24hrs
1	17.75	15.25	13.35	18.65	15.95	13.95
2	17.75	15.25	13.35	19.85	17.50	16.35
3	17.75	15.25	13.35	18.65	16.35	14.95
4	17.75	15.25	13.35	19.60	17.55	16.05
5	17.75	15.25	13.35	18.35	15.85	14.25
6	17.75	15.25	13.35	18.65	16.55	14.70

Table A1.5 (continued)

	X 48hrs	Y 48hrs	Z 48hrs	X 72hrs	Y 72hrs	Z 72hrs
1	19.50	17.05	15.50	21.75	19.60	18.70
2	23.20	21.00	20.25	25.10	22.15	21.85
3	19.60	17.30	16.05	20.65	18.80	18.00
4	23.70	21.80	21.40	25.65	22.70	22.70
5	21.35	19.40	18.55	23.55	21.45	21.05
6	21.25	20.40	19.55	24.20	22.30	21.75

Appendix 6.1 (continued)

Table A1.6 The Nham characteristics at 72 hours of fermentation at different temperatures and relative humidities.

	Colour	Visual texture	Air pockets	Firmness	Juiciness	Smoothness
1	0.86*	0.90	1.06	0.82	1.08	0.94
2	1.04	0.96	1.02	1.02	0.99	0.99
3	0.88	0.89	1.06	0.87	1.04	0.92
4	1.01	0.93	1.00	1.05	0.96	0.94
5	0.98	0.91	1.03	0.98	0.97	0.95
6	0.96	0.92	1.04	0.97	1.00	0.93

	Sourness	Saltiness	Spiciness	Pork flavour	Overall acceptability
1	0.77	1.01	0.94	1.04	0.69
2	0.95	1.02	0.99	0.99	0.96
3	0.82	0.99	0.94	1.01	0.73
4	0.96	1.00	0.99	0.98	0.96
5	0.91	1.02	0.98	0.98	0.85
6	0.92	1.03	0.99	0.99	0.86

* (mean ideal ratio scores)

APPENDIX 6.2

The multiple regression equations for dependent variables affected by temperature and relative humidity

Table A2.1 The multiple regression equations for pH with all independent variables during 72 hours of Nham fermentation at different temperatures and relative humidities.

pH (0hrs) = 6.690 - 0.018 (T) - 0.018 (RH) + 0.018 (TRH) (R^2 = 76.56%)
pH (6 hrs) = 6.625 + 0.003 (T) - 0.018 (RH) + 0.007 (TRH) (R^2 = 95.16%)
pH (12 hrs) = 6.590 - 0.015 (T) - 0.020 (RH) - 0.020 (TRH) (R^2 = 60.29%)
pH (18 hrs) = 6.242 - 0.298 (T) - 0.033 (RH) + 0.002 (TRH) (R^2 = 97.97%)
pH (24 hrs) = 5.918 - 0.688 (T) - 0.048 (RH) - 0.037 (TRH) (R^2 = 90.72%)
pH (48 hrs) = 4.830 - 0.287 (T) - 0.042 (RH) + 0.002 (TRH) (R^2 = 97.20%)
pH (72 hrs) = 4.553 - 0.157 (T) - 0.027 (RH) - 0.017 (TRH) (R^2 = 97.68%)

T = Temperature, RH = Relative humidity

Table A 2.2 The multiple regression equations for weight loss (WL) with all independent variables during 72 hours of Nham fermentation at different temperatures and relative humidities.

WL* (24 hrs) = 0.048 - 0.005 (T) - 0.015 (RH) + 0.000 (TRH) (R^2 = 92.31%)
WL (48 hrs) = 0.117 + 0.035 (T) - 0.080 (RH) - 0.040 (TRH) (R^2 = 89.71%)
WL (72 hrs) = 0.393 + 0.183 (T) - 0.277 (RH) - 0.107 (TRH) (R^2 = 94.30%)

* (%); T = Temperature, RH = Relative humidity

Table A 2.3 The multiple regression equations for compression force (CF) with all independent variabls during 72 hours of Nham fermentation at different temperatures and relative humdities.

CF* (24 hrs) = 25.872 + 17.677 (T) - 7.527 (RH) - 6.917 (TRH) (R^2 = 89.73%)
CF (48 hrs) = 80.850 + 51.202 (T) + 4.432 (RH) + 0.538 (TRH) (R^2 = 98.96%)
CF (72 hrs) = 107.582 + 36.095 (T) - 1.615 (RH) - 4.535 (TRH) (R^2 = 99.60%)

* (Newtons); T = Temperature, RH = Relative humidity

Table A 2.4 The multiple regression equations for tristimulus values (x, y, z) with all independent variables during 72 hours of Nham fermentation at different temperatures and relative humidities.

24 hrs; x = 18.958 + 0.537 (T) - 0.063 (RH) - 0.063 (TRH) (R^2 = 63.74%)
y = 16.625 + 0.688 (T) + 0.113 (RH) - 0.088 (TRH) (R^2 = 71.48%)
z = 15.042 + 0.875 (T) + 0.175 (RH) - 0.325 (TRH) (R^2 = 77.21%)

48 hrs; x = 21.433 + 1.950 (T) + 0.150 (RH) + 0.100 (TRH) (R^2 = 99.62%)
y = 19.492 + 2.113 (T) + 0.262 (RH) + 0.137 (TRH) (R^2 = 94.79%)
z = 18.550 + 2.525 (T) + 0.425 (RH) + 0.150 (TRH) (R^2 = 95.47%)

72hrs; x = 23.483 + 2.088 (T) - 0.137 (RH) + 0.412 (TRH) (R^2 = 96.44%)
y = 21.167 + 1.612 (T) - 0.063 (RH) + 0.337 (TRH) (R^2 = 85.35%)
z = 20.675 + 1.962 (T) + 0.037 (RH) + 0.387 (TRH) (R^2 = 89.78%)

T = Temperature, RH = Relative humidity

Table A 2.5 The multiple regression equations for bacterial counts with all independent variables at 72 hours of Nham fermentation at different temperatures and relative humidities.

Mesophilic aerobic counts *	= 8.348 - 0.220 (T) + 0.015 (RH) + 0.015 (TRH)	(R^2 = 48.43%)
L. plantarum	= 7.942 + 0.177 (T) + 0.028 (RH) - 0.022 (TRH)	(R^2 = 97.47%)
P. cerevisiae	= 7.222 - 0.087 (T) - 0.007 (RH) + 0.003 (TRH)	(R^2 = 42.25%)
M. varians	= 5.868 - 1.463 (T) + 0.072 (RH) + 0.018 (TRH)	(R^2 = 99.72%)
S. aureus	= 3.772 - 0.450 (T) + 0.030 (RH) + 0.020 (TRH)	(R^2 = 90.45%)
Enterobacteriaceae	= 3.385 - 0.997 (T) - 0.092 (RH) - 0.168 (TRH)	(R^2 = 98.90%)

* (Log number); T = Temperature, RH = Relative humidity

Table A 2.6 The multiple regression equations for Nham characteristics with all independent variables at 72 hours of Nham fermentation at different temperatures and relative humdities.

Colour * = 0.955 + 0.078 (T) - 0.003 (RH) - 0.012 (TRH)	(R^2 = 96.58%)
Visual texture = 0.918 + 0.025 (T) - 0.010 (RH) - 0.005 (TRH)	(R^2 = 97.30%)
Air pockets = 1.035 - 0.025 (T) - 0.005 (RH) - 0.005 (TRH)	(R^2 = 98.18%)
Firmness = 0.952 + 0.095 (T) - 0.020 (RH) - 0.005 (TRH)	(R^2 = 95.74%)
Juiciness = 1.007 - 0.043 (T) - 0.017 (RH) + 0.003 (TRH)	(R^2 = 82.02%)
Smoothness = 0.945 + 0.017 (T) - 0.017 (RH) - 0.008 (TRH)	(R^2 = 90.68%)
Sourness = 0.888 + 0.080 (T) + 0.015 (RH) - 0.010 (TRH)	(R^2 = 92.49%)
Saltiness = 1.012 + 0.005 (T) - 0.010 (RH) + 0.000 (TRH)	(R^2 = 46.15%)
Spiciness = 0.972 + 0.025 (T) + 0.000 (RH) + 0.000 (TRH)	(R^2 = 81.08%)
Pork flavour = 0.998 - 0.020 (T) - 0.010 (RH) + 0.005 (TRH)	(R^2 = 78.26%)
Overall acceptability = 0.842 + 0.125 (T) + 0.010 (RH) - 0.010 (TRH)	(R^2 = 99.09%)

* (mean ideal ratio score)
T = Temperature, RH = Relative humidity

APPENDIX 6.3

Calculations of the rate of firmness and colour development in Nham incubated at different temperatures.

Table A3.1 The rate of firmness development in Nham incubated at different temperatures.

Temperatures		1/T	k	R^2
°C	°K	($°K^{-1}x10^{-3}$)	($hr^{-1}x10^{-3}$)	(%)
20	293	3.41	2.80	91.31
25	298	3.36	3.50	91.76
30	303	3.30	3.70	87.39

Appendix 6.2 (continued)

Table A3.2 The rate of colour development in Nham incubated at different temperatures.

Tristimulus values	Temperatures °C	°K	1/T ($°K^{-1}x10^{-3}$)	k ($hr^{-1}x10^{-3}$)	R^2 (%)
x-value	20	293	3.41	2.41	94.43
	25	298	3.36	4.29	95.71
	30	303	3.30	5.19	97.79
y-value	20	293	3.41	3.13	95.05
	25	298	3.36	5.36	94.25
	30	303	3.30	5.65	94.94
z-value	20	293	3.41	4.34	94.23
	25	298	3.36	7.04	94.88
	30	303	3.30	7.44	95.18

APPENDIX 7.1

THE STORAGE TEST QUESTIONNAIRE

PRODUCT DEVELOPMENT SUB DEPARTMENT
MASSEY UNIVERSITY
NAME:_____ DATE:_____

GOOD MORNING / GOOD AFTERNOON

See your again. Thank you very much for your cooperation at the last time. You have been presented with____ samples of Nham coded_____

Please mark in all samples according to your ideals which are fixed on the scales and also write the coded number of each sample.

A: APPEARANCE:

1.COLOUR

DARK RED |———|———|———|———|———| PALE PINK

B: TEXTURE:

2.FIRMNESS

NOT FIRM |———|———|———|———|———| VERY FIRM

C: FLAVOUR AND TASTE:

3.SOURNESS

NOT SOUR |———|———|———|———|———| VERY SOUR

4.OFF-FLAVOUR

NOT PRESENT |———|———|———|———|———| VERY STRONG

PLEASE DESCRIBE:_____

_____

D: ACCEPTABILITY:

|———|———|———|———|———|

VERY UNACCEPTABLE | UNACCEPTABLE | NEITHER ACCEPTABLE NOR UNACCEPTABLE | ACCEPTABLE | VERY ACCEPTABLE

COMMENT:_____

_____

_____

DEFINITION OF WORDS TO DESCRIBE NHAM CHARACTERISTICS

A: APPEARANCE:

1.COLOUR *:the intensity of red colour both internal and external of Nham.*

B: TEXTURE:

2.FIRMNESS *:the overall force required to compress the Nham between the molar teeth to a give deformation or to penetration.*

C: FLAVOUR AND TASTE:

3.SOURNESS *:the overall strength of fermented sour taste detected in the Nham.*

4.OFF FLAVOUR *:the overall strength of non-meat flavour(s) in the Nham for example rancidity,staleness, putrid or the ammonia flavour or others.*

D: OVERALL ACCEPTABILITY:

5.ACCEPTABILITY *:take 5-6 bites of Nham,chew in molar teeth until ready for swallowing. Swallow Nham and evaluate overall texture,odour,flavour,and acceptability of product Nham.*

APPENDIX 7.2

The results of dependent variables during the storage of Nham at different temperatures (1-30°C).

Table A2.1 The pH change in Nham during storage time at different temperatures (1-30°C).

Storage temperature (°C)	Time (weeks)					
	0	1	2	5	8	10
1	4.32	4.32	4.32	4.31	4.31	4.31
5	4.32	4.30	4.29	4.29	4.28	4.28
10	4.32	4.29	4.27	4.26	4.25	4.26
20	4.32	4.19	3.99			
25	4.32	4.14	3.97			
30	4.32	3.98	3.90			

Appendix 7.2 (continued)

Table A2.2 The cumulative weight loss (%) change in Nham during storage time at different temperatures (1-30°C).

Storage temperature (°C)	Time (weeks)					
	0	1	2	5	8	10
1	0.29	0.40	0.46	0.50	0.51	0.54
5	0.26	0.42	0.44	0.55	0.63	0.78
10	0.28	0.45	0.48	0.74	0.84	1.06
20	0.24	0.75	0.88			
25	0.26	2.47	3.74			
30	0.24	3.45	4.49			

Appendix 7.2 (continued)

Table A2.3 The compression force (Newtons) change in Nham during storage time at different temperatures (1-30°C).

Storage temperature (°C)	Time (weeks)					
	0	1	2	5	8	10
1	134.84	160.78	166.50	140.37	139.86	132.68
5	134.84	158.80	164.44	125.00	124.08	120.62
10	134.84	145.05	143.44	120.90	93.24	85.04
20	134.84	110.54	91.31			
25	134.84	112.30	86.80			
30	134.84	115.90	84.43			

Appendix 7.2 (continued)

Table A2.4 The tristimulus values (x, y, z) changes in Nham during storage time at different temperatures (1-30°C).

Storage temperature (°C)		Time (weeks) 0	1	2	5	8	10
1	x	25.55	24.50	24.45	24.35	24.15	23.70
	y	22.85	21.65	21.45	21.35	21.35	20.35
	z	22.60	21.35	21.04	20.85	20.85	20.05
5	x	25.55	24.00	23.60	23.55	23.20	22.70
	y	22.85	21.50	20.75	20.80	20.85	20.00
	z	22.60	21.00	20.65	20.25	20.40	19.75
10	x	25.55	24.20	23.70	23.15	23.15	22.50
	y	22.85	21.90	20.75	20.40	20.55	19.30
	z	22.60	21.75	20.60	19.75	20.55	19.05
20	x	25.55	23.95	23.40			
	y	22.85	20.90	20.35			
	z	22.60	20.60	20.15			
25	x	25.55	23.60	23.00			
	y	22.85	20.60	20.10			
	z	22.60	20.05	20.05			
30	x	25.55	23.70	22.75			
	y	22.85	20.75	19.75			
	z	22.60	20.75	19.50			

Appendix 7.2 (continued)

Table A2.5 The mesophilic aerobic plate counts (log number) change in Nham during storage time at different temperatures (1-30°C).

Storage temperature (°C)	Time (weeks) 0	1	2	5	8	10
1	8.38	8.42	8.45	8.19	8.00	7.82
5	8.38	8.35	8.37	8.27	8.05	7.95
10	8.38	8.32	8.21	7.97	7.94	7.98
20	8.38	8.48	8.33			
25	8.38	8.34	8.39			
30	8.38	8.36	8.37			

Appendix 7.2 (continued)

Table A2.6 The L. plantarum (log number) change in Nham during storage time at different temperatures (1-30°C).

Storage temperature (°C)	Time (weeks) 0	1	2	5	8	10
1	8.31	8.40	8.25	8.09	7.92	7.68
5	8.31	8.37	8.35	8.17	7.97	7.86
10	8.31	8.30	8.15	7.84	7.61	7.49
20	8.31	8.35	8.32			
25	8.31	8.33	8.36			
30	8.31	8.35	8.37			

Appendix 7.2 (continued)

Table A2.7 The P. cerevisiae (log number) change in Nham during storage time at different temperatures (1-30°C).

Storage temperature (°C)	Time (weeks)					
	0	1	2	5	8	10
1	7.14	7.10	7.01	6.93	6.84	6.77
5	7.14	7.00	6.80	6.78	6.19	6.12
10	7.14	6.80	6.69	6.51	6.32	6.11
20	7.14	6.60	6.10			
25	7.14	6.61	6.13			
30	7.14	6.81	6.19			

Appendix 7.2 (continued)

Table A2.8 The M. varians (log number) change in Nham during storage time at different temperatures (1-30°C).

Storage temperature (°C)	Time (weeks)					
	0	1	2	5	8	10
1	4.75	4.60	4.60	2.27	1.97	1.74
5	4.75	4.65	4.50	2.24	1.15	<1
10	4.75	3.95	3.74	1.77	1.39	<1
20	4.75	3.18	3.02			
25	4.75	2.87	2.17			
30	4.75	2.04	2.06			

Appendix 7.2 (continued)

Table A2.9 The Enterobacteriaceae (log number) change in Nham during storage time at different temperatures (1-30°C).

Storage temperature (°C)	Time (weeks)					
	0	1	2	5	8	10
1	2.30	2.30	2.32	1.81	1.66	<1
5	2.30	2.28	2.22	2.23	<1	<1
10	2.30	2.20	2.16	<1	<1	<1
20	2.30	2.12	<1			
25	2.30	2.38	<1			
30	2.30	<1	<1			

Appendix 7.2 (continued)

Table A2.10 The S. aureus (log number) change in Nham during storage time at different temperatures (1-30°C).

Storage temperature (°C)	Time (weeks)					
	0	1	2	5	8	10
1	3.23	3.19	3.15	3.07	2.46	2.23
5	3.23	3.10	3.00	2.54	1.99	1.23
10	3.23	3.05	2.96	1.54	<1	<1
20	3.23	2.06	1.00			
25	3.23	1.47	<1			
30	3.23	1.15	<1			

Appendix 7.2 (continued)

Table A2.11 The moulds and yeasts (log number) change in Nham during storage time at different temperatures (1-30°C).

Storage temperature (°C)	Time (weeks)					
	0	1	2	5	8	10
1	-	-	-	-	-	-
5	-	-	-	-	-	-
10	-	-	-	-	-	-
20	-	-	-	-	-	-
25	-	-	-			
30	-	-	-			

Appendix 7.2 (continued)

Table A2.12 The firmness (mean ideal ratio score) change in Nham during storage time at different temperatures (1-30°C).

Storage temperature (°C)	Time (weeks)					
	0	1	2	5	8	10
1	1.03	1.04	1.05	1.04	1.03	1.01
5	1.03	1.03	1.02	1.00	0.98	0.95
10	1.03	1.02	1.01	0.97	0.97	0.93
20	1.03	1.04	1.01			
25	1.03	0.99	0.96			
30	1.03	1.02	0.94			

Appendix 7.2 (continued)

Table A2.13 The colour (mean ideal ratio score) change in Nham during storage time at different temperatures (1-30°C).

Storage temperature (°C)	Time (weeks)					
	0	1	2	5	8	10
1	1.01	1.01	0.99	0.96	0.98	0.96
5	1.01	0.99	0.97	0.96	0.92	0.90
10	1.01	0.97	0.95	0.94	0.94	0.89
20	1.01	1.01	1.00			
25	1.01	1.00	0.96			
30	1.01	0.94	0.94			

Appendix 7.2 (continued)

Table A2.14 The sourness (mean ideal ratio score) change in Nham during storage time at different temperatures (1-30°C).

Storage temperature (°C)	Time (weeks)					
	0	1	2	5	8	10
1	0.97	0.96	0.96	0.98	0.99	1.00
5	0.97	0.99	0.99	1.00	0.99	1.02
10	0.97	0.99	1.00	1.02	1.03	1.05
20	0.97	0.95	1.08			
25	0.97	0.99	1.10			
30	0.97	1.10	1.15			

Appendix 7.2 (continued)

Table A2.15 The off flavour (actual score) change in Nham during storage time at different temperatures (1-30°C).

Storage temperature (°C)	Time (weeks)					
	0	1	2	5	8	10
1	1.00	1.00	1.00	1.04	1.08	1.45
5	1.00	1.00	1.00	1.06	1.39	1.55
10	1.00	1.00	1.00	1.07	1.45	1.62
20	1.00	1.13	1.54			
25	1.00	1.14	1.62			
30	1.00	1.72	1.81			

Score 1 = not present; 5 = very strong

Appendix 7.2 (continued)

Table A2.16 The overall acceptability (actural score) change in Nham during storage time at different temperatures (1-30°C).

Storage temperature (°C)	Time (weeks)					
	0	1	2	5	8	10
1	4.75	4.70	4.60	4.50	4.15	3.40
5	4.75	4.75	4.60	4.40	3.75	2.90
10	4.75	4.75	4.70	4.25	4.10	2.60
20	4.75	3.70	2.70			
25	4.75	3.60	2.50			
30	4.75	3.00	2.30			

Score 1 = very unacceptable; 5 = very acceptable

APPENDIX 7.3

The rate of pH, weight loss, firmness, colour, off-flavour and overall acceptability changes during storage of Nham at different temperatures (1-30°C).

Table A3.1 The rate of pH reduction during storage of Nham at different temperatures (1-30°C).

Temperatures °C	°K	$^1/_T \times 10^{-3}$ (°K^{-1})	ln Co	k x 10^{-3} (day^{-1})	R^2
30	303	3.30	1.453	-7.31	89.19
25	298	3.36	1.463	-6.04	99.99
20	293	3.41	1.466	-5.68	98.25
10	283	3.53	1.458	-0.17	64.86
5	278	3.60	1.460	-0.10	71.58
1	274	3.65	1.458	-0.04	81.97

Appendix 7.3 (continued)

Table A 3.2 The rate of weight loss during storage of Nham at different temperatures (1-30°C).

Temperatures °C	°K	$^1/_T \times 10^{-3}$ (°K^{-1})	ln Co	k x 10^{-2} (day^{-1})	R^2
30	303	3.30	-1.027	20.90	81.69
25	298	3.36	-1.041	19.00	86.35
20	293	3.41	-1.264	9.30	84.07
10	283	3.53	-1.037	1.60	90.89
5	278	3.60	-1.105	1.30	86.09
1	274	3.65	-1.022	0.67	67.29

Appendix 7.3 (continued)

Table A3.3 The rate of compression force changes during storage of Nham at different temperatures (1-30°C).

Temperatures °C	°K	$^1/_T \times 10^{-3}$ (°K^{-1})	ln Co	k x 10^{-2} (day^{-1})	R^2
30	303	3.30	4.932	-3.30	96.00
25	298	3.36	4.917	-3.10	99.05
20	293	3.41	4.903	-2.80	99.99
10	283	3.53	5.110	-0.97	98.95
5	278	3.60	5.109	-0.51	74.17
1	274	3.65	5.132	-0.37	82.17

Appendix 7.3 (continued)

Table A3.4 The rate of tristimulus values changes during storage of Nham at different temperatures (1-30°C).

x-value

Temperatures °C	°K	$^1/_T \times 10^{-3}$ (°K^{-1})	ln Co	k x 10^{-3} (day^{-1})	R^2
30	303	3.30	3.235	-8.29	97.18
25	298	3.36	3.232	-7.51	92.02
20	293	3.41	3.234	-6.28	93.10
10	283	3.53	3.207	-1.39	77.91
5	278	3.60	3.204	-1.20	70.66
1	274	3.65	3.219	-0.74	71.10

y-value

Temperatures °C	°K	$^1/_T \times 10^{-3}$ (°K^{-1})	ln Co	k x 10^{-3} (day^{-1})	R^2
30	303	3.30	3.121	-10.00	96.65
25	298	3.36	3.116	-9.16	88.75
20	293	3.41	3.119	-8.28	91.15
10	283	3.53	3.097	-1.85	78.65
5	278	3.60	3.088	-1.27	63.48
1	274	3.65	3.101	-1.10	69.47

z-value

Temperatures °C	°K	$^1/_T \times 10^{-3}$ (°K^{-1})	ln Co	k x 10^{-3} (day^{-1})	R^2
30	303	3.30	3.114	-11.00	99.18
25	298	3.36	3.098	-8.55	75.00
20	293	3.41	3.106	-8.20	88.80
10	283	3.53	3.086	-1.87	71.82
5	278	3.60	3.073	-1.33	65.38
1	274	3.65	3.085	-1.18	71.44

Appendix 7.3 (continued)

Table A3.5 The rate of off-flavour changes during storage of Nham at different temperatures (1-30°C).

Temperatures °C	°K	$^1/_T \times 10^{-3}$ (°K^{-1})	ln Co	k x 10^{-2} (day^{-1})	R^2
30	303	3.30	-0.032	4.20	81.40
25	298	3.36	-0.037	3.40	93.50
20	293	3.41	-0.031	3.10	94.10
10	283	3.53	-0.067	0.73	90.19
5	278	3.60	-0.061	0.66	89.91
1	274	3.65	-0.050	0.48	82.60

Table A3.6 The rate of overall acceptability changes during storage of Nham at different temperatures (1-30°C).

Temperatures °C	°K	$^1/_T \times 10^{-3}$ ($°K^{-1}$)	ln Co	$k \times 10^{-2}$ (day^{-1})	R^2
30	303	3.30	1.526	-5.20	97.67
25	298	3.36	1.573	-4.60	99.39
20	293	3.41	1.569	-4.00	99.56
10	283	3.53	1.626	-0.70	72.82
5	278	3.60	1.612	-0.64	86.11
1	274	3.65	1.587	-0.41	81.72

APPENDIX 7.4

Calculation of the shelf life of Nham at different temperatures (1-30°C).

Table A4.1 Calculation of shelf-life of Nham at different temperatures based on selected product attribute quality.

Temperatures °C	k (day^{-1})	ln C/Co = kt	t (days)
Based on off-flavour			
1	0.51×10^{-2}	$0.445 = 0.51 \times 10^{-2}(t)$	87
5	0.61×10^{-2}	$0.445 = 0.61 \times 10^{-2}(t)$	73
10	0.75×10^{-2}	$0.445 = 0.75 \times 10^{-2}(t)$	59
20	3.03×10^{-2}	$0.445 = 3.03 \times 10^{-2}(t)$	15
25	3.53×10^{-2}	$0.445 = 3.53 \times 10^{-2}(t)$	13
30	4.14×10^{-2}	$0.445 = 4.14 \times 10^{-2}(t)$	11
Based on acceptability			
1	-0.45×10^{-2}	$-0.462 = -0.45 \times 10^{-2}$ (t)	103
5	-0.56×10^{-2}	$-0.462 = -0.56 \times 10^{-2}$ (t)	82
10	-0.73×10^{-2}	$-0.462 = -0.73 \times 10^{-2}$ (t)	63
20	-4.02×10^{-2}	$-0.462 = -4.02 \times 10^{-2}$ (t)	11
25	-4.56×10^{-2}	$-0.462 = -4.56 \times 10^{-2}$ (t)	10
30	-5.22×10^{-2}	$-0.462 = -5.22 \times 10^{-2}$ (t)	9

APPENDIX 8.1

Product testing questionnaire (translated into Thai)

แบบทดสอบผลิตภัณฑ์

ภาควิชาวิทยาศาสตร์และเทคโนโลยีการอาหาร

คณะเกษตรศาสตร์ มหาวิทยาลัยเชียงใหม่

แหนมที่ท่านได้ชิมนี้เป็นแหนมตัวอย่างสำหรับการวิจัย เพื่อ.- ที่จะมีการผลิตออกจำหน่ายในร้านค้าต่างๆ ผมใคร่ขอรบกวนตามข้อคิดเห็นบางประการ โปรดตอบคำถามทุกข้อต่อไปนี้ โดยไม่ต้องเกรงใจเจ้าของแหนมครับ

โปรดทำเครื่องหมาย ✓ ในช่องว่างรูปสี่เหลี่ยม ☐ เพียงหนึ่งช่องที่ท่านคิดว่าเป็นลักษณะของแหนมตัวอย่างที่ท่านกำลังชิมอยู่

ลักษณะของแหนมที่เราต้องการทราบ มีดังนี้

1. สีของแหนม คือความเข้มของสีแดงทั้งภายนอกและภายในของแหนม แล้วบอกความเข้มของสีแดงของตัวอย่างแหนมในแบบทดสอบ
2. ความเหนียวแน่นของแหนม คือเมื่อท่านใช้ในขบเคี้ยวแหนม ท่านมีความรู้สึกว่าต้องออกแรงกัดมากน้อยเพียงไร โปรดคัดสินความรู้สึกนั้นแล้วกรอกลำดับลงในแบบทดสอบ
3. ความเข้มข้นของแหนม ท่านมีความรู้สึกว่าแหนมที่ชิมมีความเปรี้ยวมากน้อยเพียงไรขณะชิมแหนม คัดสินความรู้สึกนั้นแล้วกรอกลำดับ
4. ความเค็มของแหนม ท่านมีความรู้สึกว่าแหนมที่ชิมมีความเค็มมากน้อยเพียงไรขณะชิมแหนม คัดสินความรู้สึกนั้นแล้วกรอกลำดับ
5. ความแรงของกลิ่นเครื่องเทศ ท่านมีความรู้สึกว่าแหนมที่ชิมมีความแรงของกลิ่นกระเทียม พริกไทยมากน้อยเพียงไร คัดสินความรู้สึกนั้นแล้วกรอกลำดับ
6. ความชอบ เมื่อท่านได้ชิมแหนมดังกล่าวจากลักษณะรวม ๆ ทั้งหมด ท่านชอบแหนมนี้มากน้อยเพียงไร คัดสินจากความรู้สึกทั้งหมดแล้วกรอกลำดับ

Appendix 8.1 (continued)

1. ลักษณะของแหนมที่เราต้องการให้ท่านทดลองทางด้านประสาทสัมผัส

1.1 สีของแหนม

- ☐ สีชมพูอ่อน
- ☐ สีชมพู
- ☐ สีชมพูแดง
- ☐ สีแดง
- ☐ สีแดง คล้ำ หรือแดงออกม่วงคล้ำ

โปรดระบุสีที่ท่านชอบมากที่สุดสำหรับผลิตภัณฑ์นี้ .....

1.2 ความเหนียวนุ่มของแหนม

- ☐ เหนียวนุ่มมาก
- ☐ เหนียวนุ่มพอสมควร
- ☐ เหนียวพอดี
- ☐ อ่อนเล็กน้อย
- ☐ อ่อนมาก

1.3 ความเปรี้ยวของแหนม

- ☐ เปรี้ยวมาก
- ☐ เปรี้ยวพอสมควร
- ☐ เปรี้ยวพอดี
- ☐ เปรี้ยวเล็กน้อย
- ☐ ไม่เปรี้ยวเลย

1.4 ความเค็มของแหนม

- ☐ เค็มมาก
- ☐ เค็มพอสมควร
- ☐ เค็มพอดี
- ☐ เค็มเล็กน้อย
- ☐ จืด

1.5 ความแรงของเหล้าเครื่องเทศของแหนม

- ☐ มีกลิ่นเครื่องเทศแรงมาก
- ☐ มีกลิ่นเครื่องเทศแรงพอสมควร
- ☐ มีกลิ่นเครื่องเทศกำลังพอดี
- ☐ มีกลิ่นเครื่องเทศเล็กน้อย
- ☐ ไม่มีกลิ่นของเครื่องเทศเลย

1.6 ความชอบ

- ☐ ชอบมากที่สุด
- ☐ ชอบพอสมควร
- ☐ เฉย ๆ
- ☐ ไม่ค่อยชอบ
- ☐ ไม่ชอบเลย

2. โปรดกรอกข้อมูลเกี่ยวกับผู้ทำแหนมเล็กน้อย

2.1 เพศ ☐ ชาย ☐ หญิง

2.2 อายุ ☐ 15-20 ปี ☐ 21-30 ปี ☐ 31-40 ปี ☐ 41-50 ปี ☐ มากกว่า 50 ปี

2.3 อาชีพ

- ☐ นักเรียนในโรงเรียน
- ☐ นักศึกษาในวิทยาลัยครู หรือมหาวิทยาลัย
- ☐ รับราชการ
- ☐ ทำงานในบริษัทเอกชน
- ☐ ทำงานส่วนตัว
- ☐ อื่น ๆ โปรดระบุ .....

ขอขอบคุณอย่างมากในการที่ท่านสละเวลาอันมีค่าของท่าน เพื่อกรอกแบบทดสอบนี้ ความช่วยเหลือของท่านในครั้งนี้จะเป็นประโยชน์อย่างมากต่อการพัฒนาผลิตภัณฑ์ ขอขอบคุณท่านอีกครั้งหนึ่ง

APPENDIX 8.2

Questionnaire for housewives (translated into Thai)

แบบสอบถามเฉพาะแม่บ้าน

ภาควิชาวิทยาศาสตร์และเทคโนโลยีการอาหาร

คณะเกษตรศาสตร์ มหาวิทยาลัยเชียงใหม่

สวัสดีครับ ท่านแม่บ้าน

โปรดพิจารณาตัวอย่างแหนมที่ท่านได้รับ แล้วกรุณากรอกแบบสอบถามฉบับนี้ด้วย

1. ปกติท่านชอบทานแหนมหรือไม่

☐ ชอบ ☐ ไม่ชอบ

2. ท่านรับประทานแหนมมานานกี่ปี .....

3. ท่านรับประทานแหนมบ่อยเพียงไร

☐ เดือนละครั้ง

☐ เดือนละ 2 ครั้ง

☐ เดือนละ 4 ครั้ง

☐ อื่น ๆ โปรดระบุ.....

4. เหตุใดท่านจึงซื้อแหนม (ตอบได้มากกว่า 1 ข้อ)

☐ ท่านชอบรับประทาน

☐ คนในครอบครัวชอบรับประทาน

☐ เพราะสะดวก หาซื้อมีที่จะทานได้ทันที

☐ ราคาพอสมควร

☐ อื่น ๆ โปรดระบุ.....

5. ปกติท่านซื้อแหนมที่ไหน ตอบได้มากกว่า 1 ข้อ

☐ ตามห้างสรรพสินค้า

☐ ตามตลาด

☐ ตามร้านค้าย่อยทั่วไป

☐ อื่น ๆ โปรดระบุ.....

6. ท่านชอบแหนมขนาดที่มีน้ำหนักเท่าไร

☐ หนึ่งกิโลกรัม

☐ ครึ่งกิโลกรัม

☐ สองขีดครึ่ง

☐ หนึ่งขีด

☐ อื่น ๆ โปรดระบุ.....

7. ปกติท่านชอบการบรรจุแหนมแบบไหน

☐ บรรจุในห่อพลาสติกรูปทางกระบอกเดี่ยว ๆ

☐ บรรจุในถุงพลาสติกผูกห่อด้วยใบตอง

☐ บรรจุในลักษณะอื่น ๆ โปรดระบุ.....

8. สำหรับการบรรจุแบบไส้กรอกเป็นแท่งเล็ก ๆ แล้วใส่กล่องกระดาษ ด้านบนกล่องเป็นพลาสติกของแท้น ด้วยแหนม ท่านคิดว่าจะชอบหรือไม่

☐ ชอบมากกว่าการบรรจุที่เลือกไว้ในข้อ 7.

☐ เฉย ๆ

☐ ชอบน้อยกว่า

☐ ไม่ชอบเลย

โปรดระบุเหตุผลโดยย่อ.....

Apperdix 8.2 (continued)

9. สำหรับการบรรจุแบบใส่กรอกเป็นแท่งเล็ก ๆ แล้วใส่ถุงพลาสติกแบบสูญญากาศ ท่านจะชอบหรือไม่

- ☐ ชอบมากเมื่อเทียบกับการบรรจุในข้อ 7
- ☐ เฉย ๆ
- ☐ ชอบน้อยกว่าข้อ 7.
- ☐ ไม่ชอบเลย
- ☐ ชอบมากกว่าข้อ 8.
- ☒ ชอบน้อยกว่าข้อ 8.

โปรดอธิบายเหตุผลในข้อ.....

10. สำหรับสลากบนห่อแหนม ท่านต้องการรายละเอียดอะไรบ้างบนแจลาก ตอบได้มากกว่า 1 ข้อ

- ☐ น้ำหนักสุทธิ
- ☐ ส่วนประกอบและปริมาณที่ใช้ในการผลิต
- ☐ คุณค่าทางอาหาร
- ☐ วันที่หมดอายุ
- ☐ วิธีเก็บแหนมไว้บริโภค
- ☐ ชื่อและที่อยู่ของผู้ผลิตจำหน่าย
- ☐ อื่น ๆ โปรดระบุ .....

11. ปกติเมื่อท่านไม่รับประทานแหนมทันทีที่ซื้อมาหรือรับประทานไม่หมด ท่านเก็บไว้ที่ใด

- ☐ ในตู้กับข้าว (กรุณาตอบข้อ 12)
- ☐ ในตู้เย็น (กรุณาตอบข้อ 13)

12. ถ้าท่านเก็บในตู้กับข้าวนั้น ท่านคิดว่าจะเก็บไว้ได้นานเท่าไรที่จะยังรับประทานได้อยู่

- ☐ น้อยกว่า 3 วัน
- ☐ 3 - 7 วัน
- ☐ มากกว่า 7 วัน

13. ถ้าท่านเก็บในตู้เย็น ท่านคิดว่าจะเก็บไว้ได้นานเท่าไรจึงยังรับประทานได้อยู่

- ☐ 1 สัปดาห์
- ☐ 2 สัปดาห์
- ☐ 1 เดือน
- ☐ 2 เดือน
- ☐ อื่น ๆ โปรดระบุ .....

14. ท่านเห็นว่าแหนมตัวอย่างนี้มีลักษณะต่างจากแหนมทั่วไปในท้องตลาดหรือไม่ ในด้านลักษณะปรากฏที่เห็นด้วยตา

- ☐ แตกต่าง คือ .....
- ☐ ไม่แตกต่างเลย

15. จากแหนมตัวอย่างที่ท่านได้ชิมนี้ ท่านมีแนวโน้มจะซื้อในราคาเท่าใด เมื่อเทียบกับขนาดของแหนมที่มีน้ำหนักเท่ากันนี้ที่ท่านเคยซื้อ

- ☐ สูงกว่า
- ☐ เท่ากัน
- ☐ ต่ำกว่า

Appendix 8.2 (continued)

16. จากลักษณะของแหนมตัวอย่างนี้ ทั้งลักษณะที่ปรากฏแก่สายตา รส กลิ่น ความเหนียว ฯลฯ ท่านคิดว่า ท่านจะซื้อแหนมนี้หรือไม่

- ☐ ซื้อแน่นอน
- ☐ บางทีอาจจะซื้อ
- ☐ บางทีอาจไม่ซื้อ
- ☐ ไม่ซื้อแน่นอน

17. ถ้าท่านมีคำแนะนำที่เกี่ยวข้องกับผลิตภัณฑ์แหนมตัวอย่างนี้ โปรดชี้แจงด้วย จะเป็นพระคุณยิ่ง

_____

_____

_____

_____

_____

ขอขอบคุณท่านอย่างมากที่สละเวลาและให้ความร่วมมือในการกรอกแบบสอบถามนี้ ข้อมูลที่ได้รับจากท่านนับว่ามีประโยชน์อย่างมากต่อการพัฒนาผลิตภัณฑ์อาหาร

ภาควิชาวิทยาศาสตร์และเทคโนโลยีการอาหาร

คณะเกษตรศาสตร์ มหาวิทยาลัยเชียงใหม่

โทร. 221699 ต่อ 4060

APPENDIX 8.3

APPENDIX 8.3(a) Product testing questionnaire (translated into English)

Consumer Test Form

Food Science and Technology Department,
Faculty of Agriculture, Chiang Mai University.

Nham tested is a sample Nham for this study (or expect to produce for sell in the future). I would like you to taste and ask you some opinion, please answer these questions.

For question 1. Please mark (√) in only one box (☐) provided to express a degree of each characteristic of the product.

There are only six characteristics you will evaluate. They are

1. Colour of Nham is to see the intensity of red colour both internal and external of Nham and then evaluate and complete the questionnaire.

2. Firmness of Nham is that when you eat the Nham, you feel to use the overall force required to compress the Nham to give deformation and then evaluate and complete the questionnaire.

3. Sourness of Nham is referred to the overall degree of fermented sour taste detected in the Nham.

4. Saltiness of Nham is referred to the overall degree of salty taste detected in the Nham.

5. Spiciness of Nham is referred to the overall degree of garlic and white pepper flavour detected in the Nham.

6. Overall acceptability is referred to how much you like the Nham by overall consideration of all characteristics of the Nham.

Appendix 8.3(a) (continued)

Please be careful to evaluate all characteristics of the Nham.

1. Sensory characteristics

1.1 Colour of Nham

- ☐ pale pink
- ☐ pink
- ☐ red pink
- ☐ red
- ☐ dark red

which colour of Nham do you accept it very much, please specify

_____.

1.2 Firmness of Nham

- ☐ very firm
- ☐ slightly firm
- ☐ just right
- ☐ slightly soft
- ☐ very soft

1.3 Sourness of Nham

- ☐ very sour
- ☐ slightly sour
- ☐ just right
- ☐ slightly lacking in sourness
- ☐ very lacking in sourness

1.4 Saltiness of Nham

- ☐ very salty
- ☐ slightly salty
- ☐ just right
- ☐ slightly lacking in saltiness
- ☐ very lacking in saltiness

1.5 Spiciness of Nham

- ☐ very spicy
- ☐ slightly spicy
- ☐ just right
- ☐ slightly lacking in spiciness
- ☐ very lacking in spiciness

1.6 Overall acceptability of Nham

- ☐ like it very much
- ☐ like it slightly
- ☐ neither like nor dislike it
- ☐ dislike it slightly
- ☐ dislike it very much.

2. Personal data

2.1 Sex:

☐ Male ☐ Female

2.2 Age group:

☐ 15-20 ☐ 21-30
☐ 31-40 ☐ 41-50
☐ more than 50

2.3 Career:

- ☐ school pupil
- ☐ college or university student
- ☐ government official
- ☐ working at company
- ☐ private work
- ☐ others, please specify _____

Thank you very much for you time and cooperation.

Food Science and Technology Department,
Faculty of Agriculture, Chiang Mai University
Tel. 221 699 Ext 4060.

APPENDIX 8.3(b) Questionnaire for housewives (translated into English)

A Questionnaire for a Housewife

Department of Food Science and Technology,
Faculty of Agriculture, Chiang Mai University.

Good morning/Good afternoon.

Please consider the Nham sample which you received and complete the questionnaire.

1. Do you normally prefer to eat Nham?
   - ☐ Yes
   - ☐ No

2. How long have you eaten the Nham?
   - ☐ 0 year
   - ☐ 1-10 years
   - ☐ 11-20 years
   - ☐ 21-30 years
   - ☐ more than 30 years.

3. How often have you eaten the Nham?
   - ☐ Once a month
   - ☐ Once a fortnight
   - ☐ Once a week
   - ☐ Others, please specify _____

4. What is the major influence on your buying decision?
   (mark more than one)
   - ☐ you like to eat it
   - ☐ family preference
   - ☐ convenience, ready to eat
   - ☐ reasonably price
   - ☐ others, please specify _____

5. Where do you buy the Nham from usually?
   (You may mark more than one)
   - ☐ Supermarkets
   - ☐ Local markets
   - ☐ Service Stations or retail shops
   - ☐ Others, please specify _____

6. Which weight of Nham would you prefer to buy?
   - ☐ one kilogram
   - ☐ a half kilogram
   - ☐ 250 grams
   - ☐ 100 grams
   - ☐ others, please specify _____

7. Which packaging of Nham would you like?
   - ☐ packed in the individual cylindrical plastic bags
   - ☐ packed in plastic bags and wrapped with banana leaves
   - ☐ others, please specify _____

8. If the Nham was packed as small sausages and put in a paper-box so that you can see the Nham at the top of the package, do you like it?
   - ☐ like it more than the one in question 7
   - ☐ neither like nor dislike
   - ☐ like it less than the one in question 7
   - ☐ not like it at all.

   please give a reason for the choice selected
   _____

9. If the Nham was stuffed in cylindrical plastic bag and packed together in a vacuum bag, do you like it?
   - ☐ like it very much when compared with the one in question 7
   - ☐ neither like nor dislike
   - ☐ like it less than the one in question 7
   - ☐ not like it at all
   - ☐ like it very much when compared with the one in question 8
   - ☐ like it less than the one in question 8.

   please give a reason for the choice selected
   _____

Appendix 8.3(b) (continued)

10. When you buy this product, what information do you need to known that should be shown on the label? You may mark more than one.

- ☐ net weight
- ☐ ingredients
- ☐ nutritional values
- ☐ expiry date
- ☐ method to keep it for consumption
- ☐ name and address of factory
- ☐ others, please specify _____

11. If you do not eat Nham that you buy it suddenly or you cannot finish to eat it, where do you keep it?

- ☐ kitchen cabinet (go to question 12)
- ☐ refrigerator (go to question 13)

12. Regarding question 11, if you keep the Nham in the kitchen cabinet, how long do you think the product is still suitable to eat?

- ☐ less than 3 days
- ☐ 3-7 days
- ☐ more than 7 days

13. Regarding question 11, if you keep the Nham in the refrigerator, how long you do think the product is still suitable to eat?

- ☐ a week
- ☐ two weeks
- ☐ a month
- ☐ two months
- ☐ others, please specify _____

14. Do you think the Nham sample is different from the Nham which is in the market in terms of general appearance?

- ☐ difference that is _____
- ☐ no difference

15. According to the Nham sample tasted, please consider this product, would you buy the product at the price

- ☐ greater than
- ☐ equal to
- ☐ less than

the price of the local Nham which is the same weight that you have bought it in the market.

16. Having considered all characteristics, please mark only one √ in a box provided below to express you willingness to purchase this product.

- ☐ definitely would buy
- ☐ probably would buy
- ☐ probably would not buy
- ☐ definitely would not buy

17. If you have any comments, related to this product, I would appreciate hearing them, please explain.

_____

_____

_____

_____

_____

Thank you very much for your time and cooperation to complete this questionnaire. Your answers are very useful for this product development.

Food Science and Technology Department,
Faculty of Agriculture, Chiang Mai University
Tel. 221 669 Ext 4060.

APPENDIX 8.4

The overall acceptability of developed Nham.

Tests of PARTIAL associations.

Effect Name	DF	Partial Chisq	Prob
SOV*SEX*AGE*CAR	40	.229	1.0000
SOV*SEX*AGE*OA	24	.948	1.0000
SOV*SEX*CAR*OA	30	8.549	1.0000
SOV*AGE*CAR*OA	120	4.214	1.0000
SEX*AGE*CAR*OA	60	19.591	1.0000
SOV*SEX*AGE	8	29.043	.0003
SOV*SEX*CAR	10	23.598	.0087
SOV*AGE*CAR	40	34.370	.7211
SEX*AGE*CAR	20	8.829	.9848
SOV*SEX*OA	6	.413	.9987
SOV*AGE*OA	24	17.650	.8197
SEX*AGE*OA	12	8.390	.7540
SOV*CAR*OA	30	20.635	.8990
SEX*CAR*OA	15	2.471	.9999
AGE*CAR*OA	60	16.004	1.0000
SOV*SEX	2	3.426	.1803
SOV*AGE	8	28.611	.0004
SEX*AGE	4	.616	.9613
SOV*CAR	10	207.770	.0000
SEX*CAR	5	15.688	.0078
AGE*CAR	20	241.375	.0000
SOV*OA	6	5.688	.4591
SEX*OA	3	1.402	.7050
AGE*OA	12	9.437	.6652
CAR*OA	15	24.651	.0548
SOV	2	60.810	.0000
SEX	1	3.560	.0592
AGE	4	84.188	.0000
CAR	5	375.454	.0000
OA	3	413.855	.0000

SOV = Source of consumers
SEX = Sex of consumers
AGE = Age group
CAR = Career
OA = Overall acceptability of developed Nham

Appendix 8.4 (continued)

The CAR * OA was significant at P = 0.0548

Therefore, the crosstable between the career and overall acceptability was tabulated and analysed.

Career * overall acceptability

	Career	5	4	3	2	Total Row
(1)	Pupil	19 (55.9%)	11 (32.4%)	3 (8.8%)	1 (2.9%)	34 (7.6%)
(2)	Student	14 (63.6%)	8 (36.4%)	-	-	22 (4.9%)
(3)	Government officer	40 (33.9%)	70 (59.3%)	7 (5.9%)	1 (0.8%)	118 (26.2%)
(4)	Working at company	29 (63.0%)	13 (28.3%)	4 (8.7%)	-	46 (10.2%)
(5)	Private work	107 (49.1%)	97 (44.5%)	12 (5.5%)	2 (0.9%)	218 (48.4%)
(6)	Housewife	4 (33.3%)	7 (58.3%)	-	1 (8.3%)	12 (2.7%)
Total column		213 (47.3%)	206 (45.8%)	26 (5.8%)	5 (1.1%)	450 (100.0%)

5 = like it very much
4 = like it slightly
3 = neither like nor dislike it
2 = dislike it slightly

Chi-square = 24.6510 df = 15 significance = 0.0548

Comparisons	Chi-square	df	significance
group (1) & (2)	2.78784	3	0.4255
group (2) & (3)	7.49722	3	0.0576
group (3) & (4)	13.75847	3	0.0033
group (4) & (5)	4.90010	3	0.1793
group (5) & (6)	6.52240	3	0.0888
group (1) + (2) & (3)	10.77162	3	0.0130
group (1) + (2) & (4)	1.56053	3	0.6684
group (1) + (2) + (4) & (3)	17.83265	3	0.0005
group (1) + (2) + (4) & (5)	4.98888	3	0.1726
group (1) + (2) + (4) + (5) & (3)	13.32787	3	0.0040
group (3) & (6)	4.68220	3	0.1966
group (3) + (6) & (1) + (2) + (4) + (5)	14.60247	3	0.0020

APPENDIX 8.5

APPENDIX 8.5(a) Eating pattern for Nham

Q1: Do you normally prefer to eat Nham?

Source	Yes	No	Total Row
Village 1	29 (100%)		29 (25.9%)
Village 2	26 (100%)		26 (23.2%)
City	57 (100%)		57 (50.9%)
Column Total	112 (100%)		112 (100%)

** Statistics cannot be computed when the number of non-empty or column is one **

Q2: How long have you eaten the Nham?

Source	1-10 yrs	11-20 yrs	21-30 yrs	>30 yrs	Total Row
Village 1	19 (65.5%)	6 (20.7%)	2 (6.9%)	2 (6.9%)	29 (25.9%)
Village 2	15 (57.7%)	6 (23.1%)	3 (11.5%)	2 (7.7%)	26 (23.2%)
City	9 (15.8%)	15 (26.3%)	13 (22.8%)	20 (35.1%)	57 (50.9%)
Column total	43 (38.4%)	27 (24.1%)	18 (16.1%)	24 (21.4%)	112 (100%)

Chi-square = 29.50289 df = 6 significance = 0.0000

Comparisons	Chi-square	df	Significance
Village 1 & 2	0.50847	3	0.9170
Village 1 & City	23.60884	3	0.0000
Village 2 & City	17.14824	3	0.0007
Village 1 + 2 & City	29.06399	3	0.0000

Source	1-10 yrs	11-20 yrs	21-30 yrs	>30 yrs	Total Row
Village 1 + 2	34 (61.8%)	12 (21.8%)	5 (9.1%)	4 (7.3%)	54 (49.1%)
City	9 (15.8%)	15 (26.3%)	13 (22.8%)	20 (35.1%)	57 (50.9%)

Q3: How often have you eaten the Nham?

Source	Once a month	Once a fortnight	Once a week	Un-certain	Total Row
Village 1		5 (17.2%)	15 (51.8%)	9 (31.0%)	29 (25.9%)
Village 2	8 (30.8%)	8 (30.8%)	6 (23.1%)	4 (15.3%)	26 (23.2%)
City	7 (12.3%)	25 (43.9%)	9 (15.8%)	16 (28.0%)	57 (50.9%)
Column Total	15 (13.4%)	38 (33.9%)	30 (26.8%)	29 (25.9%)	112 (100%)

Chi-square = 24.86100 df = 6 significance = 0.0004

Comparisons	Chi-square	df	Significance
Village 1 & 2	14.35159	3	0.0025
Village 1 & City	16.41735	3	0.0009
Village 2 & City	5.86394	3	0.1184
Village 2 + City & Village 1	17.93823	3	0.0050

Source	Once a month	Once a fortnight	Once a week	Un-certain	Total Row
Village 2 + City	15 (18.1%)	33 (39.8%)	15 (18.1%)	20 (24.0%)	83 (74.1%)
Village 1		5 (17.2%)	15 (51.7%)	9 (31.1%)	29 (25.9%)

APPENDIX 8.5 (b) Criteria for Nham buying choice

Q4: What is the major influence on your buying decision?

Source	1	2	3	4	5	Total Row
Village 1	12 (20.0%)	24 (40.0%)	20 (33.3%)	4 (6.7%)		60 (27.6%)
Village 2	4 (9.8%)	13 (31.7%)	18 (43.9%)	6 (14.6%)		41 (18.9%)
City	33 (28.4%)	36 (31.0%)	22 (19.0%)	15 (12.9%)	10 (8.7%)	116 (53.5%)
Total column	49 (22.6%)	73 (33.6%)	60 (27.6%)	25 (11.5%)	10 (4.7%)	217 (100%)

1 = you like to eat it
2 = family preference
3 = convenience
4 = reasonable price
5 = others (as a souvenir)

Chi-square = 24.19404 df = 8 significance = 0.0021

Comparisons	Chi-square	df	significance
Village 1 & 2	4.35541	4	0.2256
Village 1 & City	12.06715	4	0.0169
Village 2 & City	15.48954	4	0.0038
Village 1+2 & City	20.23816	4	0.0004

Therefore;

Source	1	2	3	4	5	Total Row
Village 1+2	16 (15.8%)	37 (36.6%)	38 (37.6%)	10 (10.0%)		101 (46.5%)
City	33 (28.4%)	36 (31.0%)	22 (19.0%)	15 (13.0%)	10 (8.6%)	116 (53.5%)

Q5: Where do you buy the Nham from usually?
(you may mark more than one).

Source	1	2	3	4	Total Row
Village 1		23 (51.1%)	22 (48.9%)		45 (27.4%)
Village 2		23 (71.9%)	9 (28.1%)		32 (19.5%)
City	12 (13.8%)	49 (56.3%)	18 (20.7%)	8 (9.2%)	87 (53.0%)
Total Column	12 (7.3%)	95 (57.9%)	49 (29.9%)	8 (4.9%)	164 (100%)

1 = Supermarkets
2 = Local markets
3 = Service stations or retail shops
4 = others (from factory)

Chi-square = 27.11004 df = 6 Significance = 0.0001

Comparisons	Chi-square	df	significance
Village 1 & 2	3.35236	3	0.0671
Village 1 & City	18.27546	3	0.0004
Village 2 & City	8.86172	3	0.0312
Village 1+2 & City	23.01955	3	0.0000

Therefore,

Source	1	2	3	4	Total Row
Village 1+2		46 (59.7%)	31 (40.3%)		77 (47.0%)
City	12 (13.8%)	49 (56.3%)	18 (20.7%)	8 (9.2%)	87 (53.0%)

APPENDIX 8.5 (c) Packaging preference for Nham

Q6: Which weight of Nham would you prefer to buy?

Source	1 kg	0.5 kg	250 g	100 g	Others (50 g)	Total Row
Village 1		4 (13.8%)	10 (34.5%)	15 (51.7%)		29 (25.9%)
Village 2	1 (3.8%)	6 (23.1%)	10 (38.5%)	9 (34.6%)		26 (23.2%)
City	1 (1.7%)	14 (24.6%)	26 (45.6%)	14 (24.6%)	2 (3.5%)	57 (50.9%)
Total Column	2 (1.8%)	24 (21.4%)	46 (41.1%)	38 (33.9%)	2 (1.8%)	112 (100%)

Chi-square = 8.96969 df = 8 Significance = 0.3449

Q 7: Which packaging of Nham would you like?

Source	1	2	Total Row
Village 1	25 (86.2%)	4 (13.8%)	29 (25.9%)
Village 2	15 (57.7%)	11 (42.3%)	25 (23.2%)
City	42 (73.7%)	15 (26.3%)	57 (50.9%)
Total Column	82 (73.2%)	30 (26.8%)	112 (100.0%)

1 = cylindrical plastic bags
2 = plastic bags and wrapped with banana leaves

Chi-square = 5.69694 df = 2 Significance = 0.0579

Q 8: If the Nham was packed as small sausages and put in a paper-box so that you can see the Nham at the top of package, do you like it?

Source	1	2	3	4	Total Row
Village 1	19 (65.5%)	1 (3.4%)	4 (13.8%)	5 (17.3%)	29 (25.9%)
Village 2	16 (61.5%)	5 (19.2%)	3 (11.5%)	2 (7.8%)	25 (23.2%)
City	24 (42.1%)	17 (29.9%)	8 (14.0%)	8 (14.0%)	57 (50.9%)
Total Column	59 (52.7%)	23 (20.5%)	15 (13.4%)	15 (13.4%)	112 (100.0%)

1 = like it more than the one selected in Q7.
2 = neither like nor dislike
3 = like it less than one selected in Q7.
4 = not like it at all.

Chi-square = 10.10115 df = 6 Significance = 0.1205

Q9: If the Nham was stuffed in cylindrical plastic bag and packed together in a vacuum bag, do you like it?

Source	1	2	3	4	5	6	Total Row
Village 1	10 (34.5%)	2 (6.9%)	4 (13.8%)	3 (10.3%)	8 (27.6)	2 (6.9%)	29 (25.9%)
Village 2	14 (53.8%)	4 (15.4%)	4 (15.4%)	2 (7.8%)	1 (3.8%)	1 (3.8%)	26 (23.2%)
City	10 (17.5%)	18 (31.6%)	12 (21.1%)	6 (10.5%)	9 (15.8%)	2 (3.5%)	57 (50.9%)
Total Column	34 (30.4%)	24 (21.4%)	20 (17.9%)	11 (9.8%)	18 (16.1%)	5 (4.4%)	112 (100.0%)

1 = like it very much compared with one selected in Q7.
2 = neither like nor dislike
3 = like it less than the one selected in Q7.
4 = not like it at all
5 = like it very much compared with the one selected in Q8.
6 = like it less than the one selected in Q8.

Chi-square = 20.18900 df = 10 significance 0.0275

Appendix 8.5(c) (continued)

Comparisons	Chi-square	df	significance
Village 1 & 2	7.16881	5	0.2084
Village 1 & City	9.77917	5	0.0817
Village 2 & City	12.47036	5	0.0289
Village 1+2 & City	12.82399	5	0.0251

Therefore,

Source	1	2	3	4	5	6	Total Row
Village 1+2	24 (43.6%)	6 (10.9%)	8 (14.5%)	5 (9.1%)	9 (16.4%)	3 (5.5%)	55 (49.1%)
City	10 (17.5%)	18 (31.6%)	12 (21.1%)	6 (10.5%)	9 (15.8%)	2 (3.5%)	57 (50.9%)

Q10: When you buy the Nham, what information that you need to know should be shown on the label?

Source	1	2	3	4	5	6	7	Total Row
Village 1	13 (14.2%)	16 (17.6%)	7 (7.7%)	24 (26.4%)	7 (7.7%)	24 (26.4%)		91 (23.7%)
Village 2	6 (11.5%)	4 (7.7%)	7 (13.5%)	15 (28.8%)	4 (7.7%)	16 (30.8%)		52 (13.5%)
City	42 (17.4%)	38 (15.8%)	17 (7.1%)	53 (22.0%)	32 (13.3%)	46 (19.1%)	13 (5.3%)	241 (62.8%)
Total Column	61 (15.9%)	58 (15.1%)	31 (8.1%)	92 (24.0%)	43 (11.2%)	86 (22.4%)	13 (3.3%)	384 (100.0%)

1 = net weight
2 = ingredients
3 = nutritional values
4 = expiry date
5 = method to keep it
6 = address, name of factory
7 = others, price and date to eat

Chi-square = 20.44645 df = 12 significance = 0.0591

APPENDIX 8.5 (d) Predicted keeping quality of Nham

Q11: If you do not eat Nham that you buy it suddenly or you want to keep for eating later, where do you keep it?

Source	Kitchen Cabinet	Refrigerator	Total Row
Village 1	7 (24.1%)	22 (75.9%)	29 (25.9%)
Village 2	13 (50.0%)	13 (50.0%)	26 (23.2%)
City	1 (1.8%)	56 (98.2%)	57 (50.9%)
Total Column	21 (18.8%)	91 (81.2%)	112 (100.0%)

Chi-square = 28.02674 df = 2 Significance = 0.0000

Comparisons	Chi-square	df	significance
Village 1 & 2	3.96244	1	0.0465
Village 1 & City	11.41417	1	0.0007
Village 2 & City	29.63909	1	0.0000

Q12: Regarding Q11, how long do you think the Nham is still suitable to eat?

Source	<3 days	3-7 days	> 7 days	Total Row
Village 1	3 (42.9%)	4 (57.1%)		7 (33.3%)
Village 2	12 (92.3%)	1 (7.7%)		13 (61.9%)
City	1 (100%)			
Total Column	16 (76.2%)	5 (23.8%)		21 (100.0%)

Chi-square = 6.46154 df = 2 Significance = 0.0395

Comparisons

Village 1 & 2	FISHER's EXACT TEST		TWO TAIL = 0.03070
Village 1 & City	FISHER's EXACT TEST		TWO TAIL = 1.000
Village 2 & City	FISHER's EXACT TEST		TWO TAIL = 1.000
Village 2+City & Village 1	CHI-SQUARE = 3.97021	df = 2	Significance = 0.0463

Source	< 3 days	3-7 days	> 7 days	Total Row
Village 2 + City	13 (92.9%)	1 (7.1%)		14 (66.7%)
Village 1	3 (42.9%)	4 (57.1%)		7 (33.3%)

Q13: Regarding Q11, how long do you think the Nham is still suitable to eat?

Source	a week	2 weeks	a month	2 months	other	Total Row
Village 1	15 (68.2%)	7 (31.8%)				22 (24.2%)
Village 2	8 (61.5%)	3 (23.1%)	2 (15.4%)			13 (14.3%)
City	36 (64.3%)	16 (28.6%)	4 (7.1%)			56 (61.4%)
Total Column	59 (64.8%)	26 (28.6%)	6 (6.6%)			91 (100.0%)

Chi-square = 3.28093 df = 4 Significance = 0.5120

APPENDIX 8.5(e) Buying prediction for experimental Nham

Q14: Do you think the Nham sample is difference from the Nham which is in the market in terms of general appearance?

Source	1	2	Total Row
Village 1	16 (55.2%)	13 (44.8%)	29 (25.9%)
Village 2	10 (38.5%)	16 (61.5%)	26 (23.2%)
City	32 (56.1%)	25 (43.9%)	57 (50.9%)
Total Column	58 (51.8%)	54 (48.2%)	112 (100.0%)

1 = Difference
2 = No difference

Chi-square 2.41483 df = 2 Significance = 0.2290

Q15: According to the Nham sample tasted, please answer this product, would you buy the product at the price (greater, equal to, or less than) the price of the commercial Nham which is the same weight?

Source	greater than	equal to	less than	Total Row
Village 1	9 (31.0%)	17 (58.6%)	3 (10.4%)	29 (25.9%)
Village 2	2 (7.7%)	20 (76.9%)	4 (15.4%)	26 (23.2%)
City	4 (7.0%)	45 (79.0%)	8 (14.0%)	57 (50.9%)
Total Column	15 (13.4%)	82 (73.2%)	15 (13.4%)	112 (100.0%)

Chi-square = 10.54386 df = 4 Significance = 0.0322

Comparisons	Chi-square	df	significance
Village 1 & 2	4.69097	2	0.0958
Village 1 & City	8.64062	2	0.0133
Village 2 & City	0.04308	2	0.9787
Village 2+City & Village 1	10.50351	2	0.0052

Therefore,

Source	greater than	equal to	less than	Total Row
Village 2 + City	6 (7.2%)	65 (78.3%)	12 (14.5%)	83 (74.1%)
Village 1	9 (31.0%)	17 (58.6%)	3 (10.4%)	29 (25.9%)

Q16: Having considered all characteristics of Nham sample, please mark only one √ in a box provided below to express your willingness to purchase this product.

Source	1	2	3	4	Total Row
Village 1	18 (62.1%)	11 (37.9%)			29 (25.9%)
Village 2	18 (69.2%)	8 (30.8%)			26 (23.2%)
City	35 (61.4%)	20 (35.0%)	1 (1.8%)	1 (1.8%)	57 (50.9%)
Total Column	71 (63.4%)	39 (34.8%)	1 (0.9%)	1 (0.9%)	112 (100.0%)

1 = definitely would buy
2 = probably would buy
3 = probably would not buy
4 = definitely would not buy

Chi-square = 2.31750 df = 6 Significance 0.8883