CONTROLLED FERMENTATION OF *NHAM* INOCULATED WITH *LACTOBACILLUS PLANTARUM* BCC 9546 BY LIMITATION OF CARBON SOURCES

SUJITRA PILUK

A THESIS SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE IN FOOD TECHNOLOGY

GRADUATE SCHOOL

MAEJO UNIVERSITY

2009

APPROVAL SHEET

GRADUATE SCHOOL, MAEJO UNIVERSITY

THE DEGREE OF MASTER OF SCIENCE IN FOOD TECHNOLOGY

Title

CONTROLLED FERMENTATION OF *NHAM* INOCULATED WITH *LACTOBACILLUS PLANTARUM* BCC 9546 BY LIMITATION OF CARBON SOURCES

By

SUJITRA PILUK

APPROVED BY

Advisory Committee Chairperson .....
(Assistant Professor Dr. Kornpaka Arkanit)
28 / 10 / 2009

Advisory Committee Member .....
(Dr. Wonnop Visessanguan)
15 / 10 / 2009

Advisory Committee Member .....
(Dr. Pongsuda Pongtanya)
15 / 10 / 2009

Chairperson, Committee on .....
Master of Science in Food Technology
(Dr. Thanes Keokamnerd)
19 / 10 / 2009

CERTIFIED BY

GRADUATE SCHOOL .....
(Associate Professor Dr. Thep Phongparnich)
2 / Nov. / 2009

Title Controlled Fermentation of Nham Inoculated with *Lactobacillus plantarum* BCC 9546 by Limitation of Carbon Sources

Author Miss Sujitra Piluk

Degree of Master of Science in Food Technology

Advisory Committee Chairperson Assistant Professor Dr. Kornpaka Arkanit

ABSTRACT

Post-acidification is a major problem that affects on physico-chemical properties, shelf-life and acceptability of Nham, a Thai fermented pork sausage. The aim of this study was to develop a strategy to control the post-acidification changes in Nham inoculated with *Lactobacillus plantarum* BCC 9546 by limitation of carbohydrate sources. Glucose and fructose either in free or bound forms were the major sugars found in Nham in which cooked rice and garlic contributed to glucose and fructose, respectively. The varying amounts of cooked rice and garlic added to Nham not only affected the initial amounts of total glucose (T_G) and total fructose (T_F) but also influenced the utilization of these sugars during fermentation. Limitation of total glucose and fructose (T_{G+F}) resulted in higher percentage utilization of T_{G+F}. However, the amount of utilized T_G and T_F varied largely depending on the initial amounts of T_G and T_F. Garlic was found to play a major role as carbohydrate source for Nham fermentation as higher proportion of garlic resulted in an increase in rate of pH drop, lactic acid production and less production of biogenic amine in Nham. Meanwhile, decrease in garlic proportion led to higher production of tyramine. Incorporating varying levels of sucrose had no effect on biogenic amine formation. The omission of cooked rice resulted in lower amounts of T_{G+F} and T_G thus causing in lower production of lactic acid but higher biogenic amine production. This showed that cooked rice was also another essential carbohydrate substrate for Nham fermentation. Based on principal component analysis (PCA), Nham successfully fermented was discriminated from those with fail fermentation (Ultimate pH, $pH_U > 4.6$) by higher amount of garlic added, T_F, free fructose, lactic acid, and lower pH_U. Nham produced by using standard formulation (SF) was discriminated from

those processed with carbohydrate limitation strategies by higher T_{G+F}, T_G, cooked rice, bound glucose, and lower pH_U and changes in post-acidification pH (pH_{PA}). To minimize pH changes and excessive lactic acid production during post-acidification in Nham inoculated with *L. plantarum* BCC 9546 and fermented at 30 ºC, garlic and cooked rice should be added in order to control T_G, T_F and T_{G+F} at 3.17±0.14, 7.97±0.40 and 12.70±2.40 g kg^{-1}, respectively. In comparison with Nham produced by standard formulation, Nham fermented with limited carbohydrate sources exhibited better physico-chemical properties, longer shelf-life, and lower dietary biogenic amine content suggesting that limitation of carbohydrate sources could control post-acidification changes in Nham.

ชื่อเรื่อง การควบคุมการหมักของแหนมที่หมักโดยใช้ต้นเชื้อบริสุทธิ์ *Lactobacillus plantarum* BCC 9546 โดยการจำกัดแหล่งคาร์บอน

ชื่อผู้เขียน นางสาวสุจิตรา พิลึก

ชื่อปริญญา วิทยาศาสตรมหาบัณฑิต สาขาวิชาเทคโนโลยีทางอาหาร

ประธานกรรมการที่ปรึกษา ผู้ช่วยศาสตราจารย์ ดร.กรผกา อรรคนิตย์

บทคัดย่อ

กระบวนการสร้างกรดหลังการหมักเป็นปัญหาสำคัญที่ส่งผลกระทบต่อลักษณะของแหนมทั้งคุณสมบัติทางเคมี-กายภาพ ตลอดจนอายุการเก็บรักษา และการยอมรับของผู้บริโภค การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาวิธีควบคุมการเปลี่ยนแปลงคุณภาพหลังการหมักในแหนมเติมต้นเชื้อ *Lactobacillus plantarum* BCC 9546 โดยการจำกัดแหล่งคาร์โบไฮเดรต ข้าวและกระเทียมในแหนมเป็นแหล่งสำคัญของกลูโคสและฟรุกโตสทั้งที่อยู่ในรูปอิสระและไม่อิสระ การแปรปริมาณข้าวและกระเทียมที่เติมลงในแหนมไม่เพียงแต่ส่งผลต่อปริมาณเริ่มต้นของกลูโคสทั้งหมด (T_G) และฟรุกโตสทั้งหมด (T_F) เท่านั้นยังส่งผลต่อการใช้น้ำตาลเหล่านี้ในระหว่างการหมักด้วย การจำกัดปริมาณกลูโคสและฟรุกโตสทั้งหมด (T_{G+F}) ทำให้ร้อยละของการใช้ T_{G+F} สูงขึ้น อย่างไรก็ตามปริมาณ T_G และ T_F ที่ใช้มีการผันแปรอย่างมากขึ้นอยู่กับปริมาณ T_G และ T_F เริ่มต้น กระเทียมเป็นแหล่งคาร์โบไฮเดรตที่มีบทบาทสำคัญในกระบวนการหมักแหนม การเพิ่มปริมาณกระเทียมมีผลให้อัตราการลดลงของ pH และการสร้างกรดแลคติกเพิ่มขึ้น ตลอดจนการสร้างสารประกอบเอมีนในแหนมลดลง การลดปริมาณกระเทียมส่งผลให้มีการสร้าง tyramine เพิ่มขึ้น ส่วนการแปรปริมาณน้ำตาลซูโครสไม่มีผลต่อการสร้างสารประกอบเอมีน การไม่เติมข้าวมีผลทำให้ T_{G+F} และ T_G ลดลง ซึ่งส่งผลให้มีการสร้างกรดแลคติกลดลงและการสร้างสารประกอบเอมีนเพิ่มขึ้น แสดงให้เห็นว่าข้าวเป็นแหล่งคาร์โบไฮเดรตอีกแหล่งหนึ่งที่จำเป็นต่อการหมักแหนม จากการวิเคราะห์ข้อมูลโดยใช้ Principal component analysis (PCA) พบว่าสามารถแยกแหนมที่เกิดการหมักแบบสมบูรณ์จากแหนมที่หมักไม่สมบูรณ์ (pH ที่จุดสิ้นสุดการหมัก, $pH_U > 4.6$) โดยพิจารณาจากปริมาณกระเทียม ปริมาณ T_F ปริมาณฟรุกโตสอิสระ ปริมาณของกรดแลคติกที่สูงกว่าและ pH_U ที่ต่ำกว่า สำหรับแหนมที่ผลิตโดยใช้สูตรมาตรฐาน (SF) จะมีความแตกต่างจากแหนมที่ผลิตโดยการจำกัดคาร์โบไฮเดรต คือมีปริมาณ T_{G+F} ปริมาณ T_G ปริมาณข้าว และปริมาณกลูโคสในรูปไม่อิสระที่สูง

กว่าและ pH_U และ การเปลี่ยนแปลง pH หลังการหมัก (pH_{PA}) ที่ต่ำกว่า ดังนั้นเพื่อที่จะลดการเปลี่ยนแปลง pH ให้น้อยที่สุดและลดการผลิตกรดแลคติกที่มากเกินไปภายหลังการหมักแหนมที่เติมต้นเชื้อ *L. plantarum* BCC 9546 ที่อุณหภูมิ 30 °C ต้องเติมกระเทียมและข้าวโดยควบคุมให้มี T_G, T_F และ T_{G+F} เริ่มต้นเท่ากับ 3.17±0.14, 7.97±0.40 และ 12.70±2.40 กรัม กิโลกรัม$^{-1}$ ตามลำดับ จากการเปรียบเทียบกับแหนมที่ผลิตด้วยสูตรมาตรฐานแสดงให้เห็นว่าแหนมที่ผลิตด้วยการจำกัดแหล่งคาร์โบไฮเดรตมีคุณสมบัติทางเคมี-กายภาพที่ดีกว่า มีอายุการเก็บรักษาที่ยาวนานกว่า และมีสารประกอบเอมีนต่ำกว่า ผลการศึกษาที่ได้แสดงให้เห็นว่าการจำกัดแหล่งคาร์โบไฮเดรตสามารถควบคุมกระบวนการเปลี่ยนแปลงของแหนมภายหลังการหมักได้

ACKNOWLEDGEMENT

I would like to express my deepest sincere gratitude and appreciation to my advisor, Assistant Professor Dr. Kornpaka Arkanit, for her guidance, invaluable advice, continuous supervision, constant encouragement and kind attention throughout this study. I also express my deepest gratitude to my co-advisors, Dr. Wonnop Visessanguan and Dr. Pongsuda Pongtanya for their advise providing me helpful guidance, data interpretation and suggestion during my study.

I would like also to express my appreciation to the Thailand Graduate Institute of Science and Technology (TGIST) scholarship from 2007-2008. This work was supported by BIOTEC under Grant No. F30039B21

I appreciate Food Biotechnology Laboratory, National Center for Genetic Engineering and Biotechnology (BIOTEC) for their kind helpful, financial support and providing all the facilities used in my study.

My sincere thankfulness is also to all staff of Food Biotechnology Laboratory at BIOTEC for their technical support, help and suggestion during my study.

I would like to thank all staff of Graduate school, Maejo University for their kind suggestion until my thesis successfully completed.

Finally, I would like to express my deepest gratitude and appreciation to my family for their infinite love, support and encouragement during my study.

Sujitra Piluk

October, 2009

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

g	Acceleration gravity
α	Alpha
~	Approximately
β	Beta
cm	Centimeter
CFU	Colony forming unit
°C	Degree Celcius
et al.	Et Alii (Latin), and others
etc.	Et cetera
F	Fahrenheit
e. g.	For example
F_G	Free glucose
F_F	Free fructose
F_S	Free sucrose
F_M	Free maltose
g	Gram
h	Hour
i. e.	id est (Latin), that is
i. d.	Internal diameter
kg	Kilogram
kgy	Kilogray
l	Liter
mg	Milligram
min	Minute
ml	Milliliter
mm	Millimeter
µg	Microgram
µl	Microliter

N	Normality
ppm	Part per million
/	Per
%	Percent
s	Second
S.D.	Standard deviation
T_G	Total glucose
T_F	Total fructose
T_{G+F}	Total glucose and fructose
v/v	Volume per volume
w/v	Weight per volume
w/w	Weight per weight

CHAPTER 1

INTRODUCTION

Nham is one of the most famous traditional fermented pork products which is widely consumed in Thailand. Nham fermentation is known to be initiated by lactic acid bacteria (LAB) naturally present in Nham ingredients and/or selected starter LAB added. All ingredients are mixed, wrap in a microaerobic condition, and allowed to ferment at ambient temperature approximately 48-72 h. During the fermentation, acid production is suggested to be a desirable process that contributes to the formation of acid aroma, sour taste, cohesive texture and the safety for consumption. However, the continuous and excessive decrease in pH of Nham during fermentation and storage or post-acidification generally leads to unacceptable characteristics of Nham which eventually results in quality losses which can be noticeable by dripping, discoloration, texture softening and off-flavor. Lactic acid bacteria (LAB) are the microorganisms that play important roles on Nham fermentation and became dominant microorganisms throughout the end of fermentation. With the excess amount of carbohydrate substrates used in Nham processing, they can continuously grow, produce lactic acid and be involved in post-acidification. Therefore, this study aimed to develop a strategy to minimize quality losses in Nham through the control of post-acidification changes in Nham by limitation of carbohydrate substrates. In this study, major Nham ingredients i.e. minced pork, pork rind, cooked rice, garlic and chilli were collected and subjected to total and free sugar analysis. Several batches of Nham were prepared according to standard Nham formulation with inoculation of *L. plantarum* BCC 9546 at the level of $1x10^4$ CFU/g. Total glucose and fructose (T_{G+F}) required for the completion of Nham fermentation were determined. Total lactic acid bacteria ($Total_{LAB}$), pH and organic acid were analyzed. Based on the amount of T_{G+F} required to reach pH 4.6, the pH of Nham with various controlled fermentation strategies were evaluated during prolonged incubation period. Finally, changes in physico-chemical properties and consumer acceptability of Nham produced by controlled fermentation strategies and standard formulation during storage were compared. The information gained from this study would be beneficial to the Nham producers in the way that they can apply this strategy to control and minimize loss in quality caused by post-acidification.

CHAPTER 2

LITERATURE REVIEWS

1. Nham

Fermentation is one of the oldest food preservation practices of mankind. Nham is one of the most popular traditional fermented pork sausages of Thailand. It is prepared from ground pork, cooked pork rind, garlic, cooked rice, salt, chili, sugar and sodium nitrite. The mixture is thoroughly mixed and tightly packed in banana leaves or plastic sheet. The product is allowed to ferment at ambient temperature over 3-4 days to attain a final pH of 4.5 (Valyasevi and Rolle, 2002). The processing of Nham shares a basic technology as shown in Figure 1.

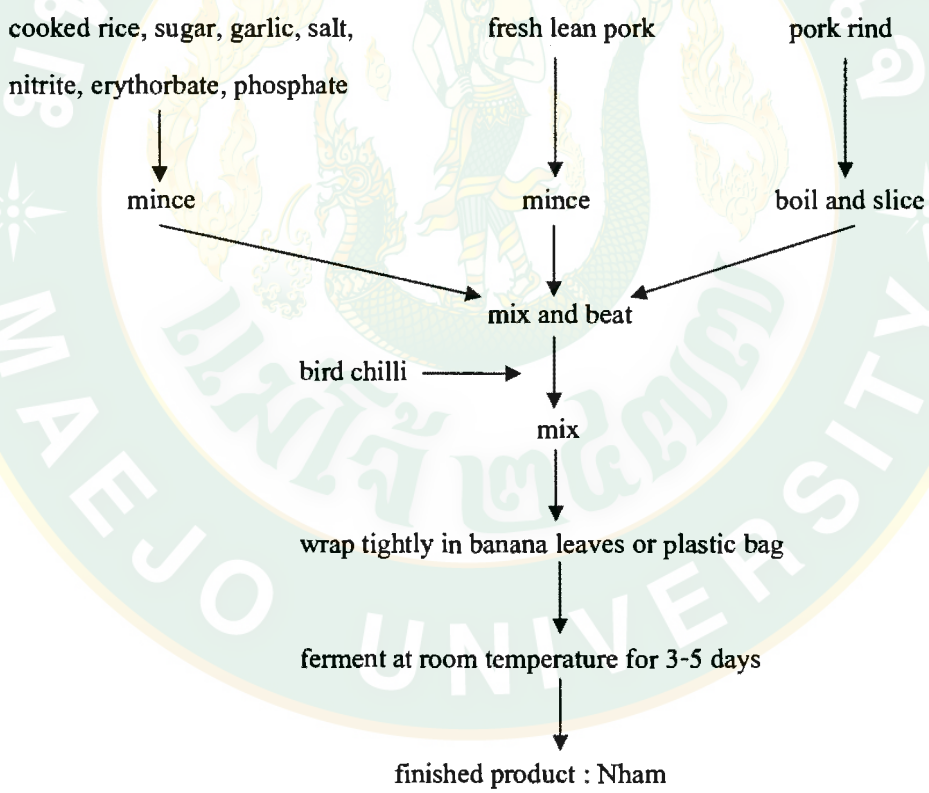

Figure 1 The processing of Nham (Modified from Phithakpol et al., 1995).

1.1 The ingredients of Nham

1.1.1 Pork and cooked pork rind

Among typical ingredients used in Nham, minced pork and cooked pork rind are two major ingredients which comprise over 90% of raw mix. As they are an important source of protein (about 60% of Nham dry weight), they are mainly responsible for the unique characteristics of Nham particularly, texture and color. Changes in Nham characteristics may be associated with physico-chemical and biochemical changes in meat and cooked pork rind. (Visessanguan et al., 2005). The quality of minced pork used is also important to the quality of products. To minimize excess water affecting drip loss in Nham, DFD (dark, firm and dry) and normal meat are generally used (Warner et al., 1997). Myofibrillar proteins play the most critical role during meat processing as they are responsible for cohesive structure and the firm texture of meat products. Nham is mainly induced by the slow lowering of pH affecting the conformation of the muscle proteins and thus their functional properties. It is likely that acid-induced gelation of these proteins was mainly responsible for the formation of Nham texture (Visessanguan et al., 2004). Proteolysis is one of the most important biochemical changes occurring during ripening of fermented sausages (Hughes et al., 2002; Kaban, 2009; Roseiro et al., 2008; Spazini et al., 2009). It influences both texture and flavor development due to the formation of several low molecular weight compounds, including peptides, amino acids, aldehydes, organic acids and amines, which are important flavor compounds, or precursors of flavor compounds. These phenomena are determined by both endogenous muscle enzymes and microbial enzymes, the contribution of which depends on the type of process used (Fernández et al., 2000). The importance of muscle proteinases in the initial breakdown of sarcoplasmic and myofibrillar proteins degradation has been reported (Molly et al., 1997).

All the changes occurring on meat proteins during processing are very important because they will affect not only the texture but also the interactions between the generated flavor compounds and the proteins and peptides and, finally, these interactions will be responsible for different sensory perceptions (Toldrá and Flores, 1998). Therefore, the interaction between pork meat proteins and volatiles compounds has been studied. Actomyosin was able to bind hexanal and octanal that are volatile compounds present in dry-cured ham (Pérez-Juan et al., 2007). The sarcoplasmic homogenates bound higher quantities of the volatile compounds (3-methylbutanal,

2- methylbutanal, 2-pentanone, hexanal, methional and octanal than myofibrillar homogenates (Pérez-Juan et al., 2008). Collagen is the main component of the skin. Collagen and other connective tissue proteins play a negligible in gel formation in sausage batters. During Nham fermentation, most proteolytic enzymes have little activity against native collagen, although they readily degrade denatured collagen (Visessanguan et al., 2004). However, Berge et al. (2001) reported a direct effect of lactic acid on collagen is a swelling of perimysium. The changes found in collagen stability were probably the result of an increased collagenolytic activity of the cathepsins due to their earlier release from the lysosomes and the favorable conditions due to acidification.

1.1.2 Cooked rice

The cooked rice and glutinous rice can be used as the carbon sources for microorganisms in Nham. Wiriyacharee et al. (1993) found that 3% cooked rice and 1% cooked glutinous rice in Nham formulation, when fermented at 30 °C, for 48 h caused rapid pH reduction (pH 4.3) and lactic acid increase to 1.09 % (w/w). In addition, Wiriyacharee et al. (1994) reported that cooked rice is the suitable source of carbohydrate for Nham production which using starter cultures (*L. plantarum, P. cerevisiae* and *Micrococcus varians*). Rice is most often used and the ability of LAB to ferment rice starch is believed to be required for the reduction of pH in product prepared without indigenous starter cultures (Lee, 1997). The lightly salt fermented fish products always contain a carbohydrate source and in Som-fak, rice starch is assumed to be the substrate for fermentation by LAB (Østergaard et al., 1998). However, increasing percentage of rice in Som-fak from 5 to 20% caused only a slight extra decrease in pH (Saisithi et al., 1986). Likewise, rice has been assumed to be the major carbohydrate source in Burong isda, an indigenous fermented fish product of Philipphines (Olympia et al., 1995).

1.1.3 Garlic

Garlic is usually added in Nham but the actual amount of this ingredient may be varied in various Nham formulations. Garlic affected growth of starter cultures as well as their lactic acid production. Three commercial meat starter cultures, *L. curvatus*, *L. sakei* and *P. acidilactici*, were used as starter cultures in Nham. It was found that garlic enhanced both the

growth of lactic acid bacteria and their lactic acid production. Nham mixture containing 5% garlic had higher lactic acid than that without garlic (Swetwivathana et al., 1999). The result of an increasing in lactic acid could be due to microbial catabolism of carbohydrates from garlic. Baumgartner et al. (2000) reported characterization of a high molecular weight fructan isolated from garlic. It was found that the garlic fructan belongs to the neoketose family. It has a (2→1)-linked β-D-fructose backbone with (2→6)-linked β-D-fructose side chains. A structural model was postulated for a degree of polymerization of about 58, as shown in Figure 2. The major volatile compounds formed in Nham were di-2-propenyl disulfide, methyl thiirane and methyl-2-propenyl disulfide, respectively. Formation of these compounds might be due to changes in sulfur-containing compound in garlic by activity of microorganisms (Valyasevi et al., 2003). In addition, garlic possesses an antimicrobial activity that could be used as a natural preservative, to control the microbial growths against food-borne pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Listeria monocytogenes*. Though, it was found that *E. coli* was the most sensitive and *L. monocytogenes* was the least sensitive (Kumar and Berwal, 1998). Recently, chemical characterization of their sulfur compounds has allowed stating that they are main active antimicrobial agent (Corzo-Martínez et al., 2007). To fully eliminate *Salmonella* in the product a combination of 8% garlic and use of *L. plantarum* 509 as a starter culture was necessary (Bernbom et al., 2009) Besides having antimicrobial activity, several components of garlic and garlic extracts such as alliin, diallyl sulfide, allyl sulfide and propyl sulfide have antioxidant activity which is responsible for the antioxidant effect in dose-dependent manner (Yang et al., 1993). Sallam et al. (2004) suggested that fresh garlic and garlic powder, through their combined antioxidant and antimicrobial effects, are potentially useful in preserving meat products.

1.1.4 Sugar

Sucrose is usually used as carbon source for growth and acid production of lactic acid bacteria. Nham with 0.5% glucose, after fermentation at 30 °C for 48 h, are acceptable as determined by sensory analysis. The pH and percentage of lactic acid were found to be 4.23 and 1.04%, respectively (Wiriyacharee et al., 1994). Sugar (glucose and occasionally lactose or saccharose) are usually included for the industrial manufacture of fermented meat products also

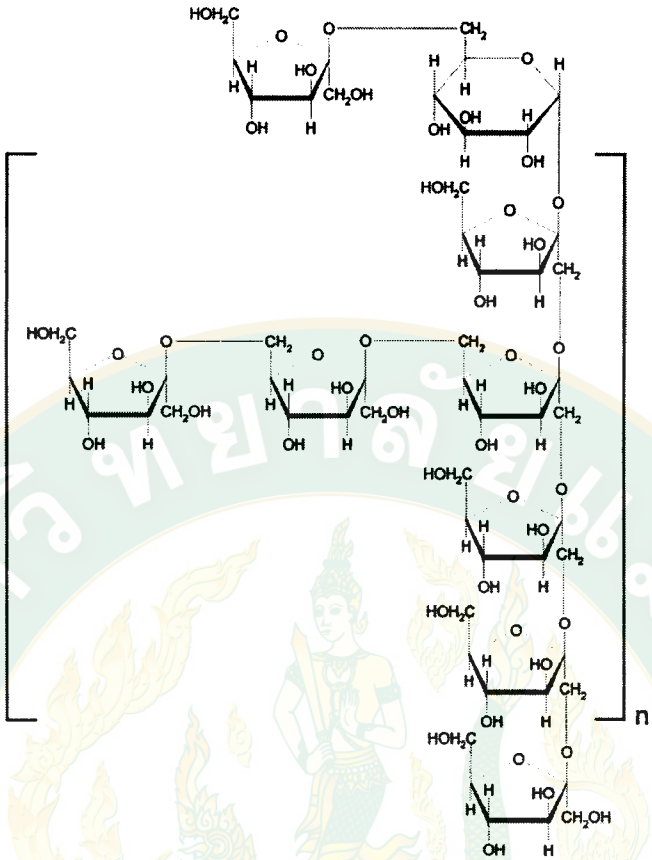

Figure 2 Suggested structure of the garlic fructan ; n=9 for a dp (degree of polymerization) of 58 (Baumgartner et al., 2000).

traditionally in Spain, Chorizo is manufactured with little or no added sugar. During fermentation and ripening, lactic acid bacteria convert glucose (their primary energy source) to lactic acid, which is the main component responsible for the pH decrease (Bover-Cid et al., 2001). González-Fernández et al. (2006) reported about the effect of added glucose on textural properties, hardness and chewiness of Chorizos-Spanish dry-cured sausage at the end of ripening revealed lower values in the 0.1% glucose batch than in samples of 0.5% and 1% glucose batches.

1.1.5 Sodium chloride

NaCl is usually added to the mixture at a concentration of 2-3% depending on Nham formulation. Salt gives the taste of product and helps the binding between meat pieces due to the function of two important proteins. Actin and myosin are proteins in meat which can

dissolve in the present of slight amount of salt. Increased solubilization of proteins found in whole muscles treated with NaCl. NaCl addition results in protein conformational changes, probably by altering hydrophobic and electrostatic interactions that stabilize the protein structure. These changes collaborate in the binding and posterior retention of water inside the tissue by any of the mechanisms proposed for the action of NaCl. This event would be the reason of the increase water holding capacity of meat (Pighin et al., 2008). Cook loss in chicken batter showed a decrease when salt level was increased. The high salt level helped to extract more proteins which improve the water binding (Somboonpanyakul et al., 2007).

Salt, which is multifunctional ingredients in dry-cured ham elaboration, affect both quality and safety. The NaCl reduction increase proteolytic activity during the traditional process of dry-cured ham. Consequently, proteolysis increases the incidence of ham with excessive softness (Costa-Corredor et al., 2009). The concentration of salt also has influence on proteolysis during ripening and storage of dry fermented sausage. Painho de Portalegre dry fermented sausage prepared with 6% of salt addition show lower water activity (a_w) values at the end of processing and in the early storage stages than 3% of salt. This could be determined by the influence of NaCl on the muscle structure, traduced as higher water binding capacity. The extension of myosin hydrolysis in Painho de Portalegre with 6% of salt addition exhibit fewer changes than 3% of salt addition. This evidence demonstrates the inhibition of cathepsin by salt (Roseiro et al., 2008).

An impressive difference between the dry fermented pork sausage with two concentrations of salt (3% and 6%) was observed in the study of Roseiro et al. (2006). Samples with the 3% salt presented higher biogenic amines levels than the 6% salt. The effect of NaCl content on the biogenic amines formation in Painho de Portalegre could be related with differences observed in the microbial development pattern, with relevance to those microrganisms that affect the production of biogenic amines. In addition, salt also promotes the growth of lactic acid bacteria while delaying the growth of some microorganisms that cause the product to be rotten (Prescott and Dunn, 1959). Generally, the amount of salt in fermented sausage is in the range of 2.0-3.5% depending on the type of product. However, the study of Paludan-Müller et al. (2002a) indicates an optimal growth of LAB is dependent on the salt concentration. Salt concentration should not be higher than 6 to 7% (w/w) for the fermentation of Plaa-som to occur

within 4 or 7 days because LAB exhibit slower growth when prepare Plaa-som with high salt concentration (9 and 11%).

1.1.6 Nitrite and nitrate compounds

Nitrite and nitrate compound are important and widely used as food additives. They are related to the development of pink or red color in meat products. Naturally, meat has a purple-red pigment which is called myoglobin. In the first step of color development in meat product, nitrate is reduced to nitrite by temperature, bacteria or acid condition. Then nitrite is reduced to nitric oxide in acidic condition. The occurring nitric oxide reacts with myoglobin to form nitrosomyoglobin which has a red color (Price and Schweigert, 1973). The mechanism of color development is shown in Figure 3. Muscles cured with 100 mg nitrite/kg meat formed products with higher a values (redness) than those cured with the lower (25 mg kg^{-1} meat) nitrite level (Dineen et al., 2000). Staphylococci/Micrococci were dominant at early phase of Nham fermentation (0 to 12 h) (Visessanguan et al., 2006). Wiriyacharee et al. (1990) proposed that *Micrococcus varians* produces nitrate reductase which is important in converting nitrate to nitroso-haemoglobin, thus, imparting a pink color to the product.

The effect of nitrite in cured products has been studied for many years and can be summarized as: formation of the characteristic red color; growth inhibition of spoilage and pathogenic bacteria such as *Clostridium botulinum*; contribution of development of typical cured meat flavor and delaying the oxidative rancidity (Flores and Toldrá, 1993). González and Díez, (2002) reported sodium nitrite and starter culture have significant inhibitory effect on *Enterobacteriaceae* count but did not on *Micrococcaceae*. Microbiological stability of Chorizo after processing depends on the combination of several hurdle (action of nitrite, water activity, and acidification).

Curing salts had a pronounced effect on the level of volatile compounds. In particular, curing with nitrate instead of nitrite resulted in a striking difference. Generally, nitrate increased the level of volatile compounds compared to nitrite (Olesen et al., 2004). It is known that nitrites added to meat products become rapidly depleted as a result of such factors as heat formation of nitrite by inhibiting the growth of nitrate reducing bacteria, such as *Enterobacteria*, during Paocai fermentation.

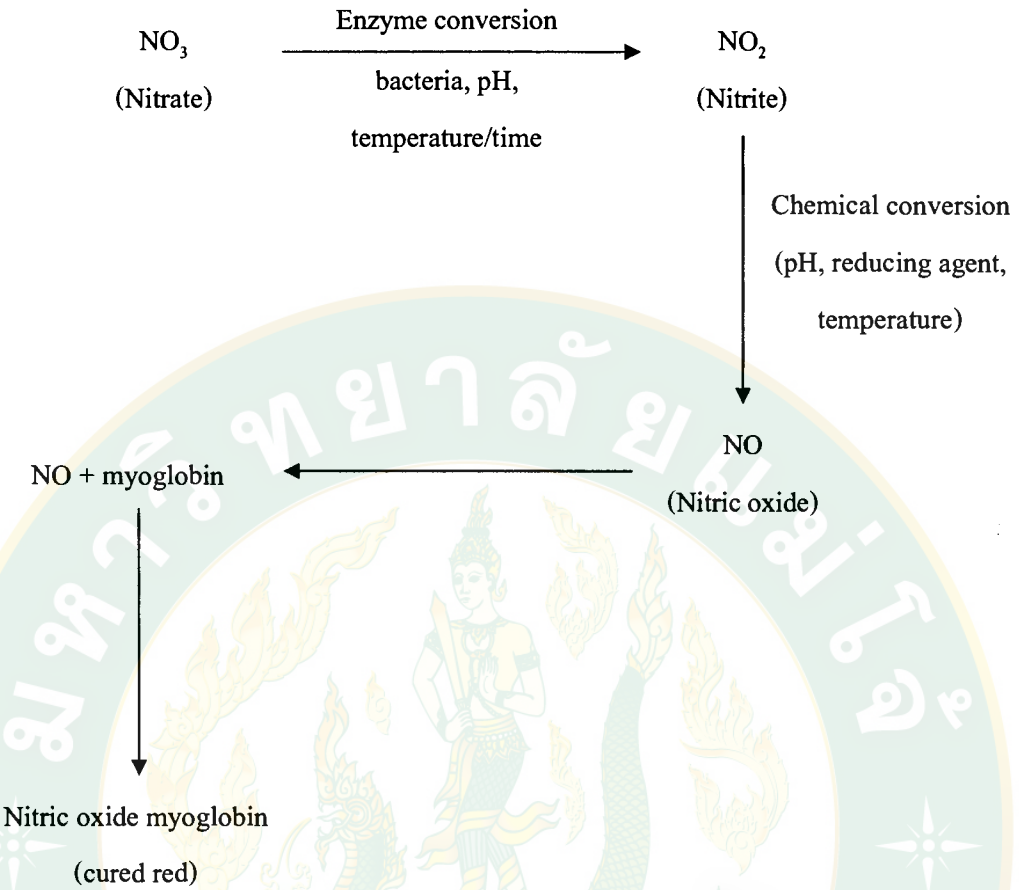

Figure 3 Simplified mechanism of curing agent for color development in sausage (Modified from Kunawasen, 2000).

treatment, product composition, storage temperature, etc. Residual nitrite levels in foodstuffs are extremely important, partly because of their potential to react with amines and amides to form carcinogens, and partly because of their contribution as a source of nitrite in human nutrition (Lee et al., 1976). Honikel (2008) reported that nitrite can cause negative effect on human health such as formation of carcinogenic nitrosamines by reaction between nitrite and biogenic amines. Therefore, many countries have the regulations to limit the residual amount of nitrate and nitrite including Thailand. The maximum allowable level of nitrite and nitrate residues in Nham is 125 and 500 ppm, respectively (Thailand Industrial Standard, 2003). However, the concentration of nitrite could be reduced by using LAB starter cultures. Yan et al. (2008) reported about the LAB starters, namely *L. pentosus* and *Leuconostoc mesenteroides*, are effective in reducing the

formation of nitrite by inhibiting the growth of nitrate reducing bacteria, such as *Enterobacteria*, during Paocai fermentation.

1.1.7 Erythorbate and ascorbic acid compounds

Sodium erythorbate, sodium iso-ascorbate, sodium ascorbate, ascorbic acid and iso-ascorbic acid are used in meat product for improvement of color. They are catalyst in development of pink-red color in meat and maintenance of the occurring color during storage time. There is the report which showed the effect of using the combination of ascorbic acid and nitrite compound in pork product. The color, flavor, and taste of pork product were improved when used both of ascorbic acid and nitrite compound (Watts and Lehmann, 1952). Ascorbic acid possesses antioxidant properties, although it can act as an antioxidant or as a prooxidant depending on the concentration, the presence of metal ions and the tocopherol content (Schaefer et al., 1995).

The efficiency of ascorbic acid to retard oxidation of meat pigment has been reported. The use of ascorbic acid (500 ppm) in beef patties was effective in inhibiting metmyoglobin formation, resulting in higher a* value than sample without ascorbic acid (Sánchez-Escalante et al., 2001). Addition of ascorbic acid prevented color changes in irradiated beef. The ground beef added with ascorbic acid had lower oxidation-reduction potential (ORP) than control, and the low ORP of meat helped maintaining the heme pigments in reduced form (Ahn and Nam, 2004). Ascorbic acid at 1%, 3% and 10% can inhibit a rapid discoloration in beef, 3% and 10% were more affective at maximizing color stability (Mancini et al., 2007). Erythorbate-treated beef patties had greater cooked internal a* value than untreated patties at 60 and 66 °C for all storage conditions (Suman et al., 2005). The optimum level of sodium ascorbate was found to be 500 ppm for preblending with ground buffalo meat to improve its quality, especially meat colour and odour, by minimizing pigment and lipid oxidation processes during refrigerated storage. Its shelf-life is extended from 4 to 8 days under refrigerated storage (Sahoo and Anjaneyulu, 1997). Powdered sodium erythorbate and glucono-delta-lactone/sodium erythorbate reduce microbial counts and improve color during the storage of meats (Barringer et al., 2005). In addition, the presence of ascorbate produces an increase in the headspace concentration of the volatile compounds in dry cured ham, particularly 3-methyl butanal and 2-pentanone (Flores et al., 2007).

1.1.8 Phosphate compounds

Phosphate refer to compounds in which the P atoms are surrounded tetrahedrally by four oxygen atoms. The linear chain illustrated in Figure 4. Phosphoric acid and its salts could be transformed into various forms classification of phosphates into three types: orthophosphates, pyrophosphates and metaphosphates. Orthophosphates are compounds containing discrete PO_4^{3-} ions. The pyrophosphates and metaphosphates are now known as condensed phosphates, which are formed by repeated condensation (polymerization) of tetrahedral [PO_4] units. This results in chains of tetrahedra, each sharing the O atom at one or two corners of the [PO_4] tetrahedron. Diphosphate (pyrophosphate), $P_2O_7^{4-}$ is the simplest condensed phosphate anion, formed by condensation of two orthophosphate anions. The term metaphosphate refers to cyclic anion which have the exact composition $(PO_3)_n^{n-}$. In older literature, long chain polyphosphates are frequently referred to as metaphosphates because they have approximately the same composition (Rashchi and Finch, 2000).

Polyphosphate and pyrophosphate compounds are widely used in meat products. Phosphates function by the sequestration of metal ions and the dissociation of the actomyosin complex bringing about an increase in water-holding capacity (WHC). Another important reason for using phosphates is their ability to increase meat pH and to slow discoloration by stabilizing vitamin C. Several polyphosphates are used in the meat industry and they differ from one another in their properties. Those with a low degree of polymerization (mono and diphosphates) increase water-holding capacity in meat but their use is limited by their low and slow solubility, especially in cold brine. In contrast, triphosphate is soluble and dissolves rapidly but its effect on water-holding capacity in meat is slow because of the need for conversion (enzymic and acid hydrolysis) to pyrophosphate. For this reason the use of a mixture of polyphosphates with different chain lengths is more effective (Dušek et al., 2003). These compounds improve the quality of product in chemical and physical properties such as texture, color, stability and water absorption of product. Addition of phosphate not only raises the pH of meat mixture, causing a shift away from the pI of myofibrillar proteins, but also increases the total negative charges on the myofibrillar proteins (Pearson and Gillett, 1996). Both actions accounted for increased WHC. There are many studies reported about the use of phosphate to improve water holding capacity of meat products. Trisodiumphosphate could improve water holding capacity of low-fat Chinese

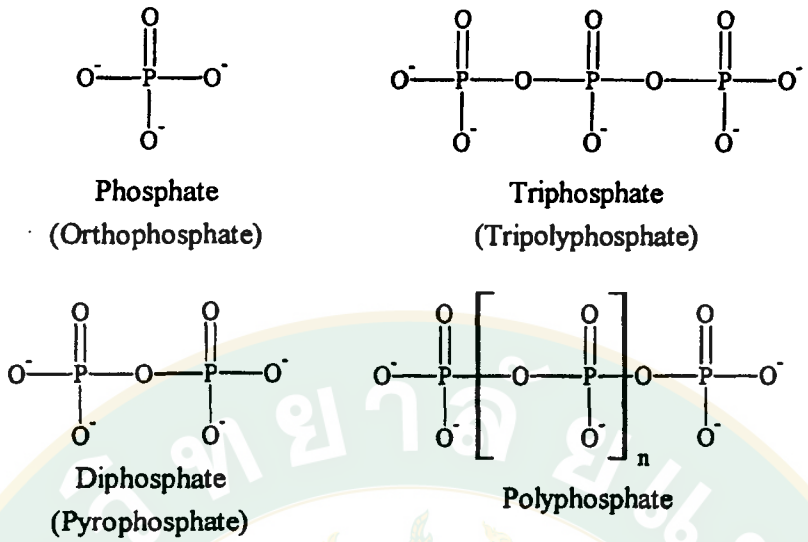

Figure 4 Linear polyphosphate ions (Rashchi and Finch, 2000).

style sausage (Lin and Lin, 2002). Polyphosphate improved water holding, are generally produced more tender and more juicy than control stake. Steaks containing 5% polyphosphate and cooked to 80 °C were more tender and as juicy as steaks without polyphosphate cooked to the lower centre temperature (Sheard et al., 1999).

Phosphate compounds also have buffering capacity in meat product. They prevent an increase in pH during the early stage of meat fermentation, and extend time before the drop in pH (Bacus, 1984). Pretreatment with pyrophosphate resulted in the retarded protein denaturation as evidenced by the reduced changes in sulfhydryl content and surface hydrophobicity during the extended storage of seabass slices. Increase in water uptake ability accompanied by the decreased exudates loss was observed in samples pretreated with phosphates, especially pyrophosphate (Masniyom et al., 2005). The effectiveness of phosphates as antimicrobial agents in meat products depends on the type of phosphate, the amount used, specific food product and conditions under which they are used (Sofos, 1986). Wager and Busta (1985) reported that pyrophosphate was more inhibitory toward microorganisms than tripolyphosphate or longer phosphates in a sausage. Trisodiumphosphate was not only maintains the redness, it could reduce the number of *E. coli*, *Salmonella* spp., coliforms and aerobic bacteria in ground beef (Pohlman et al., 2002). The amount of added phosphates in Nham is limited to 3000 ppm (expressed as P_2O_5) (Thailand Industrial Standard, 1994) because they may chelate

important metals ions (calcium and magnesium) (Dušek et al., 2003).

1.1.9 Other ingredients

Pepper and bird chilli are usually added in Nham but the actual amount of these ingredients may be varied in various Nham formulations. Wiriyacharee et al. (1994) found that 0.05% of pepper and 1% of minced bird chili had significant effects on the production of lactic acid. Moreover, the spices could increase the efficient use of carbohydrates for lactic acid bacteria (Ingolf and Skjelkvale, 1982). In addition, monosodium glutamate is frequently used to enhance umami taste.

2. Lactic acid bacteria (LAB)

Lactic acid bacteria have been used to ferment or culture food for at least 4000 years. The taxonomy of lactic acid bacteria has been based on the Gram reaction and the production of lactic acid from various fermentable carbohydrates. Lactobacilli vary in morphology from long, slender rods to short coccobacilli, which frequently form chains. Typical LAB are gram-positive, nonsporing, catalase-negative, devoid of cytochromes, anaerobic but aerotolerant cocci or rods that are acid tolerant and produce lactic acid as the major end product during sugar fermentation. Besides, they can grow at temperatures from 5 to 45 °C. The growth is optimum at pH 5.5-6.5. LAB consist of bacterial genera within the phylum Firmicutes comprised of about 20 genera. The genera *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Pediococcus*, *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus* and *Weisella* are the main members of the LAB. *Lactobacillus* is largest of these genera, comprising around 80 recognized species (Axelsson, 2004).

Lactic acid bacteria are widely classified into 2 groups by using final products from carbohydrate metabolism (Reddy et al., 2008).

a) Homofermentative lactic acid bacteria (lactococci, pediococci, streptococci and homofermentative lactobacilli) refer to the group that use Embden-Meyerhof-Parnas (EMP) pathway for glucose fermentation. Homolactic fermentation of glucose results in 2 moles of lactic acid and a net gain of 2 ATP per mole of glucose consumed (Figure 5).

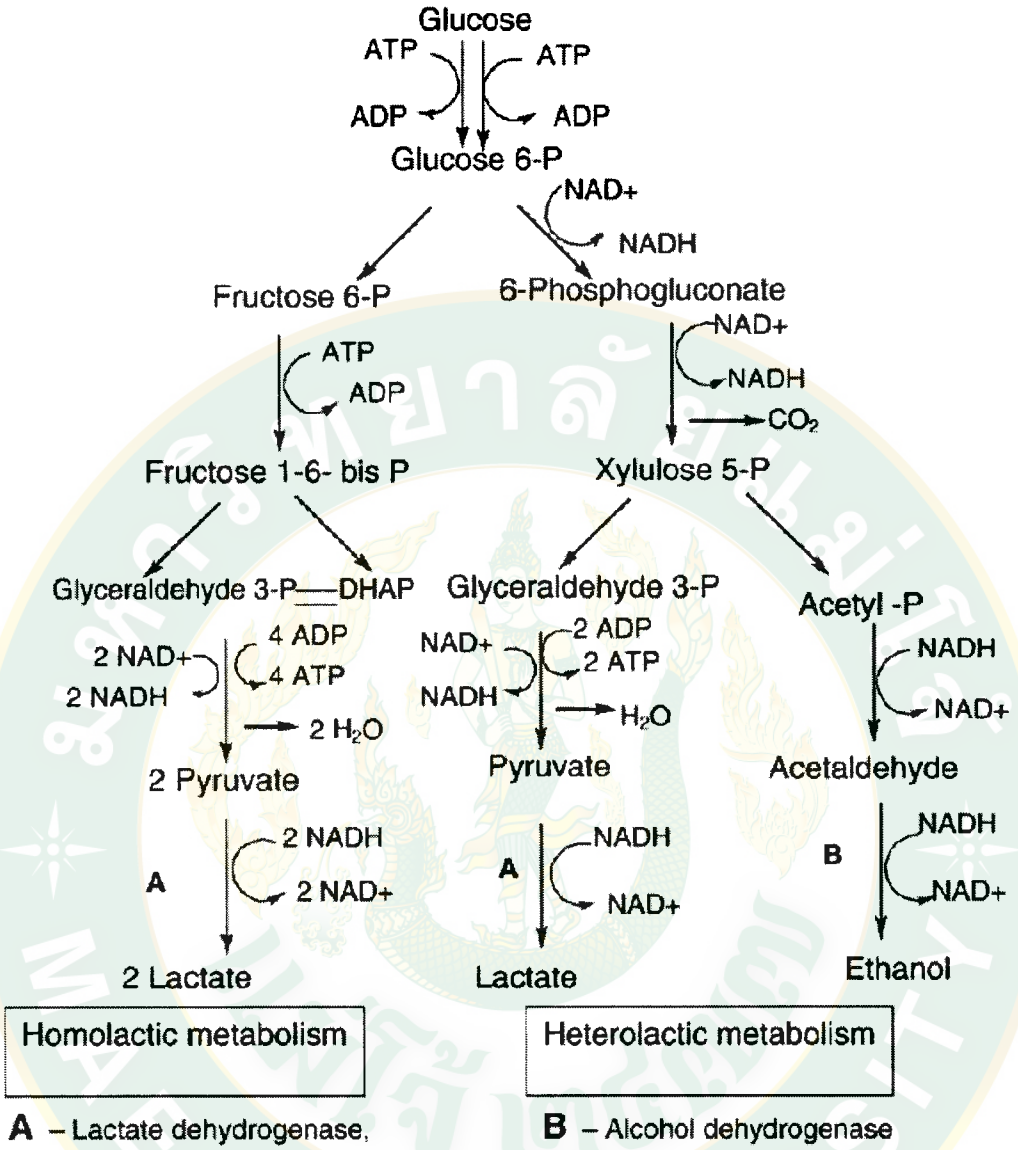

Figure 5 Major fermentation pathways of glucose;

A) homolactic fermentation (glycolysis, Embden-Meyerhof-Parnas pathway)

B) heterolactic fermentation (6-phosphogluconate/phosphoketolase pathway).

b) Heterofermentative lactic acid bacteria such as leuconostoc, weissella and heterofermentative lactobacilli, refer to the group of lactic acid bacteria that uses the 6-phosphogluconate/phosphoketolase (6-PG/PK) pathway. Heterolactic fermentation of glucose through the 6-PG/PK pathway gives 1 mole each of lactic acid, ethanol, and CO_2 and 1 ATP per

mole of glucose (Figure 5).

LAB cause rapid acidification of the raw material through the production of organic acids, mainly lactic acid. The acidification process is one of the most desirable effects of their growth. The pH may drop to as low as 4.0, low enough to inhibit the growth of most other microorganisms including the most common human pathogens, thus allowing these foods to prolong shelf-life (Reddy et al., 2008). The production of ethanol, aroma compounds, bacteriocins, exopolysaccharides, and several enzymes is of importance for enhancing product quality, shelf life, microbial safety (Leroy and Vuyst, 2004), and improved nutritional value (Holzapfel, 1997).

LAB possess the property of producing lactic acid from carbohydrates through fermentation. Carbohydrate pathway of *L. plantarum* in different medium (Plumed-Ferrer et al., 2008) is shown in Figure 6. For fermented meat product, glucose was added 0.1-0.2%. Adding the excess amount of glucose made the product too sour and unwanted growth of the microorganisms (Kröckel, 1996). The fermenting properties of *Lactobacillus* strains were in general associated with the main carbohydrate substrate of their origin such as starch fermenting strains originated from cooked rice and garlic or inulin fermenting strains from garlic and banana leaves. In Som-fak, Paludan-Müller et al. (1999) suggest that garlic may be more important than rice-starch as a carbohydrate source for fermentation by *L. plantarum* which is garlic fermenting strain. An initial presence and growth of LAB with a capacity to ferment garlic is essential for a rapid decrease in pH (Paludan-Müller et al., 1999). In addition, characteristics of carbon sources are important on substrates remaining and products formed. De Castro et al. (1998) reported that a blanching step is essential to achieve the controlled fermentation of garlic using a starter culture of *L. plantarum*, besides preventing the appearance unwanted effects (e.g. green colour). The effect of sucrose, rice, and garlic, on flavor profiles of Nham was studied in Nham with natural fermentation and Nham with *Lactobacillus sakei*. It is found that garlic gave an important effect on the flavour profiles. Nham without garlic showed no occurrence of eight sulfur-containing compounds. Nham without sucrose or cooked rice showed decreasing of alcohols and increasing of hydrocarbons and sulfur-containing compounds. Nham with natural fermentation without garlic, sugar or cooked rice took fermentation time longer than control batch. Sucrose or cooked rice had no effect on production of aldehydes, ketones, alcohols, sulfur-containing compounds

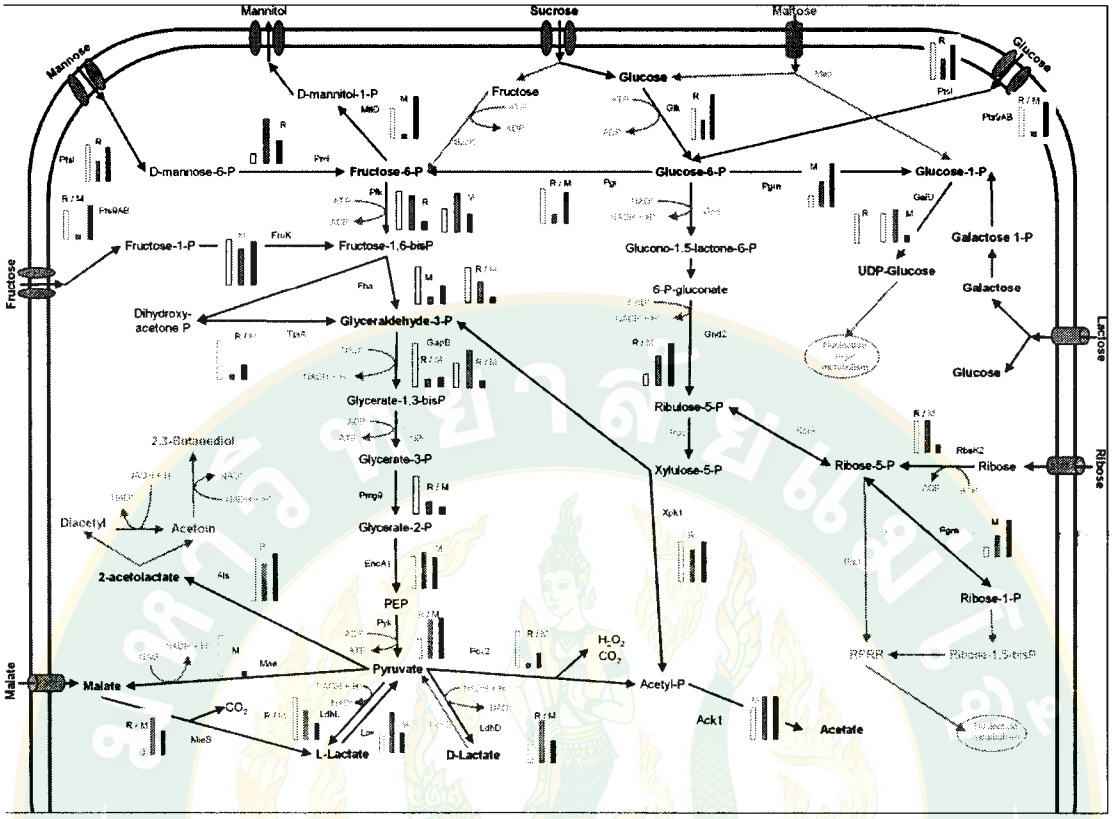

Figure 6 Carbohydrate pathways used by *L. plantarum* strains REB1 and MLBPL1. Expression of the identified enzymes that catalyze the different steps is indicated by white (MRS), gray (cucumber), and black (feed) bars. The strain and statistical significance of each protein pattern is indicated by R (REB1) or M (MLBPL1) and by black (P<0.01) or gray (P>0.01). Abbreviations: Ack1, acetate kinase; Als, acetolactate synthase; EnoA1, enolase 1; Fba, fructose bisphosphate aldolase; FruK, 1-phosphofructokinase; GalU, UTP-glucose-1-phosphate uridylyltransferase; GapB, glyceraldehyde-3-phosphate dehydrogenase; Glk, glucokinase; Gnd2, phosphogluconate dehydrogenase; Gpd, Glucose-6-phosphate 1-dehydrogenase; LdhD, D-lactate dehydrogenase; LdhL1, L-lactate dehydrogenase; Lox, lactate oxidase; LoxD, lactate oxidase (oxidoreductase); Mae, malic enzyme; Map, maltose phosphorylase; MleS, malolactic enzyme; MtlD, mannitol-1-phosphate 5-dehydrogenase; Pfk, 6-phosphofructokinase; Pgi, glucose-6-phosphate isomerase; Pgk, phosphoglycerate kinase; Pgm, phosphoglucomutase; Pmg9, phosphoglyceromutase 2; Pmi, mannose-6-phosphate isomerase; Pox2, pyruvate oxidase; Prs1, ribose-phosphate pyrophosphokinase; Pts9AB, mannose PTS, EIIAB;

PtsI, phosphoenolpyruvate-protein phosphatase; Pyk, pyruvate kinase; RbsK2, ribokinase; Rpe, ribulose-phosphate 3-epimerase; RpiA, ribose 5-phosphate isomerase A; Sack, fructokinase; TpiA, triosephosphate isomerase; Xpk1, phosphoketolase.

and hydrocarbons of *L. sakei*. However, Nham inoculated with *L. sakei* resulted generation of various flavour compounds more than Nham with natural fermentation (Khuanburi, 2005).

Lactic acid bacteria play essential role in the production of fermented meat product. *Lactobacillus* being the main species is used in the European type of fermented meat products (Hugas and Monfort, 1997). The main species found in the fermented sausages produced in Greece, Hungary and Italy was *L. plantarum*, *L. curvatus* and *L. sakei* (Rantsiou et al., 2005). The most commonly identified in French traditional fermented sausages were *L. sakei*, *L. curvatus* and *L. plantarum* (Lebert et al., 2007). *L. sakei* and *L. curvatus* are the main species found in the traditional fermented sausage produced in North of Italy (Cocolin et al., 2009). *L. sakei*, *L. plantarum* and *L. curvatus* were the dominant flora during the ripening of Sardinian sausages (Greco et al., 2005). The Salami microflora was dominated by homofermentative lactobacilli; approximately 63% of them could be identified as *L. sake*; 40% showing the traits of a racemase negative variant of this species, once referred to *L. bavaricus* (Coppola et al., 2000).

In spontaneous meat fermentation, the lactic acid bacteria derived from the raw materials or the environment are responsible for both lactic acid production resulting from carbohydrate utilization, and also a low pH value (5.9-4.6). As a consequence of this, the muscle protein coagulates, resulting in the sliceability, firmness and cohesiveness found in the final product. Ripening is also favored when pH values decrease and approach the isoelectric point of proteins. The development of curing color occurs also in acidic conditions when nitric oxide is produced from nitrite and can then react with myoglobin. Finally, the inhibition of pathogenic and spoilage bacteria is a consequence of the accumulation of lactic acid as well as acetic acid, formic acid, ethanol, ammonium, fatty acids, hydrogen peroxide, acetaldehyde, antibiotics and bacteriocins (Hugas and Monfort, 1997).

The primary contribution of LAB to flavor generation is ascribed to the production of large amounts of lactic acid and some acetic acid, although they also produce volatiles through fermentation of carbohydrates (Molly et al., 1996). They usually do not possess

strong proteolytic or lipolytic properties, although a degree of peptidase and lipase activity has been observed for some meat strains. Basic flavor results from the interaction of taste (mainly determined by lactic acid production and the pattern of peptides and free amino acids resulting from tissue-generated proteolysis) and aroma (mainly determined by volatile components derived from bacterial metabolism and lipid autoxidation) (Claeys et al., 2004). Proteolytic events that take place during the processing of meat products result in an increase in small peptides and free amino acids. The composition of these compounds contributes to the overall flavor in fermented meat products (Pereira et al., 2001). Exopeptidases from meat lactobacilli contribute, in conjunction with muscle aminopeptidases, to the generation of free amino acids, contributing to flavour (Demeyer et al., 2000). LAB isolated from Greek sausages exhibited high in vitro leucine and valine aminopeptidase activities (Papamanoli et al., 2003). *L. curvatus* and *L. homohiochii* isolated from Portuguese traditional dry fermented sausage showed the proteolytic acitivity (Pereira et al., 2001).

Lipolysis is believed to play a central role in aroma formation. Little information is available about the lipolytic activity of lactobacilli during sausage fermentation. *L. plantarum* (DSMZ 12028) is isolated from "Chourico", a traditional Portuguese dry fermented sausage, exhibit extracellular lipase (de Fátima Silva Lopes et al., 2002). However, lipases from lactobacilli often display little or no activity under conditions found in fermented sausages (Demeyer et al., 2000), although for some the production of lipase appears to be significant under conditions relevant for sausage ripening (de Fátima Silva Lopes et al., 1999).

3. The microbiology of Nham

Nham is produced by a mixed bacterial fermentation during which biochemical changes in the micro-environment promote the successive growth of microorganisms (Valyasevi and Rolle, 2002). Nham fermentation took 3-5 days and relies mainly on adventitious microorganisms which are normally found in raw materials (Khieokhachee et al., 1997). There were many reports on the microflora during the fermentation of Nham.

From the study of Tachapinyawat (1975), the total microorganisms at early phase of Nham fermentation was high. After 24 h of fermentation, some microorganisms were reduced. During 24-72 h of fermentation, both homo-and hetero-fermentative lactobacilli and

homo-fermentative cocci had rapidly growth and high lactic acid production. After 72 h of fermentation, the group of homo-fermentative lactobacilli such as *L. plantarum* had the highest growth. Most of microorganisms which cannot resist acids were reduced by acidic condition in Nham.

Nham microflora has identified lactobacilli (*L. plantarum*, *L. pentosus* and *L. sake*) and pediococci (*P. acidilactici* and *P. pentosaceus*) as the predominant microorganisms in Nham fermentation (Tanasupawat and Komagata, 1995). Other microorganisms including *Micrococcus varians*, yeast and molds were identified at early phase of fermentation. Wiriyacharee (1990) proposed that *L. plantarum* and *P. cereviseae* are important for acid production and that *M. varians* produces nitrite reductase which is important in converting nitrate to nitroso-haemoglobin, thus, imparting a pink color to the product.

Kunawasen (2000) have focused on the identification of LAB in Nham from different commercial brands. The study was used both phenotypic and Randomly Amplified Polymorphism DNA (RAPD) to confirm the identification of lactic acid bacteria strains during Nham fermentation. The results from this study have shown that the dominant genetic groups are members of lactobacilli including *L. acidophilus*, *L. cellubiose*, *L. graminis*, *L plantarum*, *L. pentosus*, *L. curvatus*, *L. sake*, *L. delbruckeii*, *L. paracasei* and *L. brevis*, while *Leuconostoc mesenteroides* and *P. pentosaceus* were found in much lower proportion.

Based on ITS-PCR database and 16S rDNA sequencing, the diversity of LAB species is clearly different when comparing microbial diversity of natural and starter cultured Nham at each sampling time of fermentation. However, both types of Nham showed a dramatic succession of LAB species during fermentation. In natural fermentation, the dominant LAB species were *Lactococcus garvieae*, *Lactococcus lactis*, *L. plantarum* and *P. pentosaceus*. In contrast, starter cultured Nham, were found to contain only 3 dominant LAB species during fermentation. Three species belonged to *Lc. garvieae*, *Lc. lactis* and *L. plantarum* (Kongtong, 2008).

In order to investigate the role of *L. plantarum* BCC 9546 during Nham fermentation, LpBCC9546 was transformed with a recombinant plasmid pRV85 to produce the recombinant strain LpG11 which is resistant to erythromycin and emit green fluorescence. LpG11 was used as starter culture for Nham fermentation. During Nham fermentation, the numbers of

LpG11 increased ten fold during the first 12 h of fermentation, reaching maximum numbers of between 10^7 and 10^8 CFU/g after 24 h, and then declining after 60 h to 10^5 CFU/g at 168 h (Laxananil et al., 2009).

As a consequence of natural fermentation, the quality and consistency of Nham cannot be controlled. Nham is generally consumed without further cooking, which is of concern as contamination of Nham with entire pathogens has been reported. Contamination with pathogens can be reduced by the use of appropriate starter culture (Petchsing and Woodburn, 1990) to achieve an acidic pH of $\leq$ 4.6 during fermentation (Paukatong and Kunawasen, 2001). Developments of starter formula for Nham have been carried out by Valyasevi et al. (2001) to improve control of microbial processes. Selected isolates from the dominant genetic groups has been selected for using as starter. Nham products were evaluated by their sensory quality and the ability of the starter culture bacteria to ferment based on different quality factors of the final product such as final pH, total acidity, color and texture. Both *L. curvatus* and *L. plantarum* BCC 9546 were found to give product with higher scores of overall acceptability based on 9 points hedonic scale sensory analysis than natural fermented Nham. *L. plantarum* BCC 9546 has been used commercially as a starter culture for Nham fermentation (Valyasevi et al., 1999). Therefore, *L. plantarum* BCC 9546 was used as a starter culture for Nham fermentation in this study.

4. Changes in qualities of Nham during fermentation

Fermentation of Nham generally takes 3-5 days at room temperature (~30 °C). Nham usually has a pH 4.4-4.8 with titratable acidity values of 0.77-1.60% (Phithakpol et al., 1995). The fermentation of Nham involved successive growth of different microorganisms dominated by lactic acid bacteria (Valyasevi et al., 2001). LAB produces organic acids from carbohydrates and causes the pH drop, which contribute to changes in physico-chemical properties of Nham.

4.1 pH

Nham pH generally decreased with increasing fermentation time. Nham inoculated with *L. curvatus* exhibited lower pH than the uninoculated Nham. The pH gradually decreased to 4.6 within 72, 48 and 36 h for the uninoculated Nham, inoculated Nham at the level

10^4 and 10^6 CFU/g, respectively (Visessanguan et al., 2006)

4.2 Color

Visessanguan et al. (2004) reported that lightness (L*) of natural Nham fermentation decreased during the first 12 h and then continuously increased to a maximum at 36 h before remaining relatively constant thereafter. Redness (a*) increased during the first 12 h of fermentation. However, no significant difference changes in redness were observed in later stage of fermentation ($P>0.05$). Besides, yellowness (b*) slightly decreased during first 24 h but a decrease in yellowness was more pronounced at 36 h. The color of Nham inoculated with *L. curvatus* during fermentation indicated an increase in both lightness and redness but a decrease in yellowness. Inoculation with *L. curvatus* accelerated both the increase in L* values and the decrease in b* values of Nham during fermentation (Visessanguan et al., 2006).

4.3 Weight loss, release water and expressible moisture

Weight loss in meat products is mainly associated with loss in water and water holding capacity (WHC) of meat. Released water is generally referred to as the water retained in the casing and at the surface. In contrast, expressible moisture is the water remaining in the sample, which can be released when pressure is applied. Weight of fermenting Nham generally decreased as the fermentation time increased. Visessanguan et al. (2006) reported that as the fermentation time proceeded, Nham inoculated with *L.curvatus* had greater weight loss than unioculated Nham ($P<0.05$). Increasing amount of released and expressible water are possibly responsible for an increase in weight loss. In addition, Nham with increasing proportion of minced pork generally resulted in higher weight losses and amount of water released during fermentation (Visessanguan et al., 2005).

4.4 Texture

Fermented Nham showed a marked increase in all TPA texture attributes, compared to non-fermented Nham. With increasing fermentation time Nham became more rigid, elastic, cohesive and less adhesive. Considerable changes in TPA were observed, particularly with in 24 h of fermentation ($P<0.05$) (Visessanguan et al., 2004). Inoculation of *L. curvatus*

resulted in faster and higher development in all TPA texture attributes, compared to Nham naturally fermented (Visessanguan et al., 2006).

4.5 Proteolysis

The initial hydrolysis of muscle proteins attributed mainly to endogenous cathepsins, and followed by the action of bacterial enzymes, which actively degrade oligopeptides to small peptides and free amino acids (Molly et al., 1997). Visessanguan et al. (2004) reported that proteolysis of both myofibrillar and sarcoplasmic proteins occurred as a result of fermentation. Degradation of myofibrillar and sarcoplasmic proteins resulted in an increase in peptides and free amino acids, which may be related to the development of flavor and aroma of Nham. Besides, the level of inoculation affected on the progressive of proteolysis. Inoculation of *L. curvatus* at the level 10^6 CFU/g showed the fastest and largest decrease in both myofibrillar and sarcoplasmic protein fractions, followed by the inoculums level at 10^4 CFU/g and uninoculated Nham, respectively. It was possible that both myofibrillar and sarcoplasmic proteins were degraded or became insoluble due to acid-induced denaturation (Visessanguan et al., 2006).

4.6 Lipolysis and lipid oxidation

Greater free fatty acids (FFA) content was observed with increasing fermentation time, indicating lipolysis of Nham lipids during fermentation. FFA content at the beginning of the process represented 0.3% of lipid content and increased to 3% at the end of fermentation (Visessanguan et al., 2006). The inoculation at 10^6 CFU/g of Nham had the highest free fatty acid, followed by 10^4 CFU/g and uninoculated Nham, respectively. Peroxides value (PV) was used as index to assess lipid oxidation of Nham. With increasing fermentation time, Nham inoculated with *L. curvatus* at the level 10^6 CFU/g exhibited the fastest and largest increase in PV, compared to the level 10^4 CFU/g and uninoculated Nham, respectively.

4.7 Biogenic amines

Biogenic amines are basic nitrogenous compounds formed mainly by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones (Silla Santos, 1996). They are compounds commonly present in living organisms in which they

are responsible for many essential functions. They can be naturally present in many foods, which contain proteins or free amino acid, such as fruits and vegetables, meat, fish, chocolate and milk but they can also be produced in high amounts by microorganisms through the activity of amino acid decarboxylases (Suzzi and Gardini, 2003). The chemical structure of biogenic amines can either be aliphatic (putrescine, cadaverine, spermine and spermidine), aromatic (tyramine and phenylethylamine), and heterocyclic (histamine and tryptamine). The most important biogenic amines occurring in foods are histamine, putrescine, cadaverine, tyramine, tryptamine, β-phenylethylamine, spermine, and spermidine. Excessive consumption of these amines can be of health concern because they are not equilibrate assumption in human organism can generate different degrees of diseases determined by their action on nervous, gastric and intestinal systems and blood pressure. Amino acid decarboxylation is the most common mode of synthesis of amines in foods, and the aromatic amines may render a food toxic (Shalaby, 1996). On the other hand, it must be taken into account that secondary amines such as putrecine and cadaverine can react with nitrite to form heterocyclic carcinogenic nitrosamines, nitrosopyrrolidine and nitrosopiperidine (Silla Santos, 1996).

For fermented sausages, high concentrations of putrescine and the presence of other amines have been attributed to microbial growth and depend on meat freshness (Suzzi and Gardini, 2003). Analysis for biogenic amines is being proposed as a quality index for fresh meat. (Vinci and Antonelli, 2002). Fermentation of foods leads to the formation of biogenic amines during the aging or ripening process (McCabe-Sellers et al., 2006). There are many reports about biogenic amine in fermented sausages. Maijala and Eerola (1993) detected increased concentrations of histamine and tyramine during sausage fermentation. Turkish dry fermented sausage, Sucuk, is the most popular meat product in Turkey. The most important biogenic amine in 30 samples of Sucuk obtained from retailed market were tyramine (range 2.4-676 mg kg^{-1}), followed by putrescine varied from not detected to 364 mg kg^{-1} Histamine content was under 50 mg kg^{-1} in 80% of the samples while it was over 100 mg kg^{-1} in only one sample. Tryptamine was detected in 16 of 30 samples in the range of 1.2-82.3 mg kg^{-1} (Gençcelep et al., 2008). Aminogenesis in traditional fermented sausages produced in Europe was studied. Tyramine was the major amine, follow by putrescine and cadaverine, although the occurrence of di-amines was much more variable (Latorre-Moratalla et al., 2008). Total level of biogenic amines in "Salami

Italiani alla cacciatore PDO" ranged from 71 to 586 mg kg^{-1}. The amine recovered in higher concentrations was tyramine 372 mg kg^{-1}, followed by histamine (165 mg kg^{-1}) (Coïsson et al., 2004).

During fermentation of Nham, cadaverine, tyramine and putrescine were main biogenic amines detected while the other amines were not detectable. These amines were mostly formed with the increased time of fermentation (Limsuwan, 2004). Moreover, Nham incubated at either 30 or 40 °C contained higher amounts of all biogenic amines detected (P<0.05). During storage, lesser changes in biogenic amines in Nham were observed at -20 °C and 4 °C in comparison with those observed at 30 °C. However, there are the studies have been attempted in order to reduce biogenic amine formation. It has been pointed out that starter cultures result in formation of smaller amounts of biogenic amines than when natural microflora is responsible of fermentation (Silla Santos, 1996). The use of a modified atmosphere pack (MAP) with carbon dioxide has been shown lowest amines content when compared with vacuum pack (VP) and air at 4 °C during storage (Özogul and Özogul, 2006). Addition of starter culture with high concentration of additives (nitrite, nitrate, α-tocopherol, ascorbic acid, potassium sorbate, potassium pyrophosphate and di-potassium hydrogenphosphate) decreased the formation of biogenic amine (Bozkurt and Erkmen, 2002). The use of decarboxylase negative lactic acid bacteria as starter cultures, which produces a rapid decrease on the pH of the meat mixture, and the use of a sugar concentration (mainly glucose) in a range of 0.5–1%, are two important factors to be considered in order to reduce the levels of biogenic amines in Chorizo dry sausage (González-Fernández et al., 2003). Starter culture and nitrite in sucuk manufacture hindered formation of putrescine, cadaverine and tyramine. The addition of sodium nitrite (75 ppm) with starter cultures was enough to reduce biogenic amines formation in sucuk (Gençcelep et al., 2007).

5. Post-acidification

Nham is one of the most famous Thai traditional fermented meat products which is allowed to ferment approximately 48-72 h or until pH dropped to 4.6. Many producers favor to keep Nham at room temperature, which can cause over-fermentation or post-acidification leading to unacceptable characteristics of product. There are a few studies have been attempted in order to

stop the post-acidifcation in various types of fermented foods. Yoghurt fermented by *Lactobacillus delbrueckii* subsp. *bulgaricus* mutants with reduced membrane-bound H^+ ATPase activity can reduce post-acidification and enhance viability of *Bifidobacterium breve* during refrigerated storage. Consumer preference indicated that yoghurt fermented using the mutant strain had the desired sour taste and is acceptable for consumption (Ongol et al., 2007). The short shelf-life of Som-fug, mainly caused by over-fermentation, is a drawback of this product. The growth of LAB and the changes in Som-fug properties still occur during storage (Valyasevi and Rolle, 2002). Riebroy et al. (2007) reported that Som-fug produced from bigeye snapper could be extended by using irradiation at 2 kGy in combination with refrigerated storage. Gamma irradiation could retard microbial growth in Som-fug leading to the retardation of spoilage and over-fermentation.

Post-acidification or over-fermentation is the most pressing problem that affect on physical and chemical properties of fermented food. For Nham, excessive acid production results in quality losses which can be noticeable by water release, undesirable pale appearance, and off-flavor. Over-production of lactic acid is considered to be chiefly responsible for a product with the state of over-acidity, which is not accepted. The over-fermented Nham cause changes in texture and sensory leading to unacceptable characteristics from consumers. During fermentation, indigeneous lactobacilli continue to grow and diversity at the end of fermentation was high evidenced by 5 species of *Lactobacillus* had been found. The use of ITS-PCR and rRNA analysis indicated that 6 types of lactic acid bacteria have been involved in over-fermentation. Chaicherdsakul et al. (2006) reported that *L. plantarum* was the dominant species in over-fermented Nham therefore, minimizing the numbers of *L. plantarum* might be one alternative in prolonging the shelf-life of Nham when stored at room temperature.

6. Principal component analysis (PCA)

Principal component analysis is a data compression method based on the correlation among variables. Its aim is to group correlated variables, and replace them by new sets called principal components, PCs. PCs are completely uncorrelated and they are built as a simple linear combination of the original variables. It should be stressed that PCs contain most of the data set variability, but in a much lower-dimensional space. The first principal component, PC1,

is defined as the direction of maximum variance of the whole data set. PC2 is the direction that describes the maximum variance in the orthogonal subspace to PC1. The subsequent components are taken orthogonally to the ones previously chosen and describe the maximum of the remaining variance. When redundancy is removed, only the first few principal components are required to describe most of the information contained in the original data set (Ferreira et al., 2000; Malinowski, 1991; Sharaf et al., 1986). Therefore, PCA method has been numerously used to characterize meat (Qiao et al., 2007; De la Fuente et al., 2009; Costa et al., 2009; Kwasiborski et al., 2009) and meat product such as: Spanish dry-cured sausage (Ruiz Pérez-Chacho et al., 2005), Italian traditional fermented sausage (Casaburi et al., 2008), Portuguese meat product (Patarata et al., 2008) and cooked ham (Casiraghi et al., 2007). In these studies reported about the parameters related to their physico-chemical properties. In the present study an attempt has been taken to collect parameters associated with successive or failed fermentation of Nham produced by limitation of carbohydrate sources.

CHAPTER 3

MATERIALS AND METHODS

1. Chemical reagents and media

Acetic acid (CH_3COOH), agar, formic acid (CH_2O_2), fructose, D(+)-glucose-monohydrate, isooctane, maltose monohydrate, meat extract, perchloric acid ($HClO_4$), di-potassium hydrogen phosphate (K_2HPO_4), pyridine (C_5H_5N), sodium acetate (CH_3COONa), sodium hydroxide (NaOH), sulfuric acid (H_2SO_4) and yeast extract were purchased from Merck (Darmstadt, Germany). Ethyl alcohol (C_2H_5OH), lead (II) acetate [$Pb(CH_3COO)_2.3H_2O$], magnesium sulfate heptahydrate ($MgSO_4.7H_2O$), manganese sulfate monohydrate ($MnSO_4.H_2O$), sodium oxalate $(COONa)_2$, and sucrose ($C_{12}H_{22}O_{11}$) were purchased from Carlo Erba Reagenti (Lambadia, Italy). Di-ammonium hydrogen citrate [$(NH_4)_2HC_6H_5O_7$] and Tween 80 were purchased from BDH Labolatory Supplies (Poole, UK). Calcium carbonate ($CaCO_3$), DL-lactic acid ($C_3H_6O_3$) and L(+)-rhamnose monohydrate ($C_6H_{12}O_5.H_2O$) were purchased from Fluka Biochemika (Switzerland). Acetonitrile (ACN) and hydrochloric acid (HCl) were purchased from Fisher (Loughborough, UK). Chlorotrimethylsilane (C_3H_9ClSi), dansyl chloride, 1,7-diaminoheptane, hexamethyldisilasane ($C_6H_{19}NSi_2$), hydroxylamine hydrochloride ($NH_2OH.HCl$), and methyl-α-D-glucopyranoside ($C_7H_{14}O_6$) were purchased from Sigma-Aldrich (MO, USA). Proteose Peptone No.3 was purchased from Difco (Detriot, USA).

2. Nham starter

L. plantarum BCC 9546 used in this work was obtained from the BIOTEC-Culture Collection, National Center for Biotechnology and Genetic Engineering (BIOTEC), Thailand. The culture was maintained at -80°C in De Man Rogasa and Sharpe (MRS) broth (Appendix A) containing 40% (v/v) glycerol.

3. Inoculum preparation

A loopful of stock culture of *L. plantarum* BCC 9546 was streaked on MRS agar and incubated at 30°C for 48 h. A single colony was transferred to MRS broth and incubated at 30 °C for 12 h. When cell concentration in MRS broth was reached approximately 10^9 CFU/ml, cells

were harvested by centrifugation at 4°C at 10,300 *x g* for 15 min using a refrigerated centrifuge (Eppendorf model 5810R, Germany), washed twice with 0.1% peptone water and subsequently suspended in the same solution. Dilution of the starter culture was made to obtain required cell concentration before using for inoculum.

4. Experimental procedure

4.1 Nham preparation

Unless otherwise stated, Nham were prepared according to the standard Nham formulation of The Department of Livestock, Thailand (Table 1). Lean meat obtained from local retailer was trimmed of all visible fat and connective tissue. After trimming, the meat was minced through a 2 mm plate. Pork skin was trimmed of all visible fat and brought to a boil in water to cook and ensure removal of pig hair from the follicle. After scalding, the de-fatted skin was finely shredded. During mixing of the ingredients, *L. plantarum* BCC 9546 was added at the level 10^4 CFU/g. Samples of the pork sausage were extruded through the stuffing horn into polyethylene casing with a diameter of 3.0 cm (approximately 200 g each) and sealed tightly. Samples were incubated at 30 °C and 50 ± 2% relative humidity in an incubator (Memmert, BE 600, Germany).

To estimate the amounts of total glucose and fructose required for complete fermentation of Nham inoculated with *L. plantarum* BCC 9546, 6 batches of Nham were prepared according to the standard Nham formulation. The samples were collected to analyse pH, sugar and lactic acid content at 0 h (raw mix) and 12-h or 24-h intervals. The average amount of T_G, T_F and T_{G+F} utilized during fermentation to reach pH 4.6 were estimated by subtraction of Initial T_G, T_F, and T_{G+F} with those determined from Nham with pH 4.6, respectively.

To study the effect of various carbohydrate sources (Figure 7), 4 separated experiments were carried out with a slight modification to obtain the initial T_{G+F} at 12.7±2.4 g kg^{-1}, compared to those formulated with standard formulation (SF-1 to SF-4). For Experiment I (Nham IF-1 to IF-5), sucrose was omitted and the amounts of cooked rice and garlic were varied from 0.6 to 3.7 and 0.8 to 6.3, respectively. In the experiment, the amounts of T_G and T_F were roughly estimated based on the average sugar composition data obtained from raw materials used in each study. For Experiment II (Nham IF-7 to IF-11), Nham were produced to study the effect of cooked rice and sucrose on biogenic amine production. In the experiment, garlic was added at

the optimal amount previously determined from the Experiment I, whereas the amounts of cooked rice and sucrose were varied from 0 to 1.3 and 0.1 to 0.5, respectively. To study the effect of cooked rice and garlic, Nham IF-12 to IF-14 in Experiment III were formulated with varying amounts of garlic and cooked rice in which the initial amounts of total fructose (T_F) in Nham were varied from 8.5 to 11.0 g kg^{-1}. To study the effect of cooked rice, Nham IF-13 and IF-15 in Experiment IV were formulated with the presence and absence of cooked rice. Lastly, to investigate the changes in Nham quality during fermentation and post-acidification, Nham IF-16 was produced by an optimal controlled fermentation strategy in which the T_{G+F}, T_G and T_F were limited to ~12.7, ~3 and ~8 g kg^{-1}, respectively.

4.2 Sample collection

Nham ingredients including minced pork, cooked pork rind, cooked rice, garlic and chili were collected and subjected to free and total sugar analysis using oxime-TMS procedure (Valyasevi et al., 2003) as the method described in 5.2.3 and 5.2.4. Nham samples were randomly sampled for pH and sugar analyses at 12-h or 24-h intervals up to 7 days, except 14 days for Nham SF-4 and IF-16. The average amounts of total and free sugars utilized during fermentation to reach pH 4.6 were determined. Total LAB count, pH, organic acid and biogenic amines were determined as the methods described in 5.1.1, 5.2.1, 5.2.2, and 5.2.4, respectively.

4.3 Sample preparation for analysis

In order to prepare the sample for analysis, after removing the outer casing, samples were thoroughly cut up and ground in a meat grinder (Osterizer, USA) until homogeneous sample was obtained.

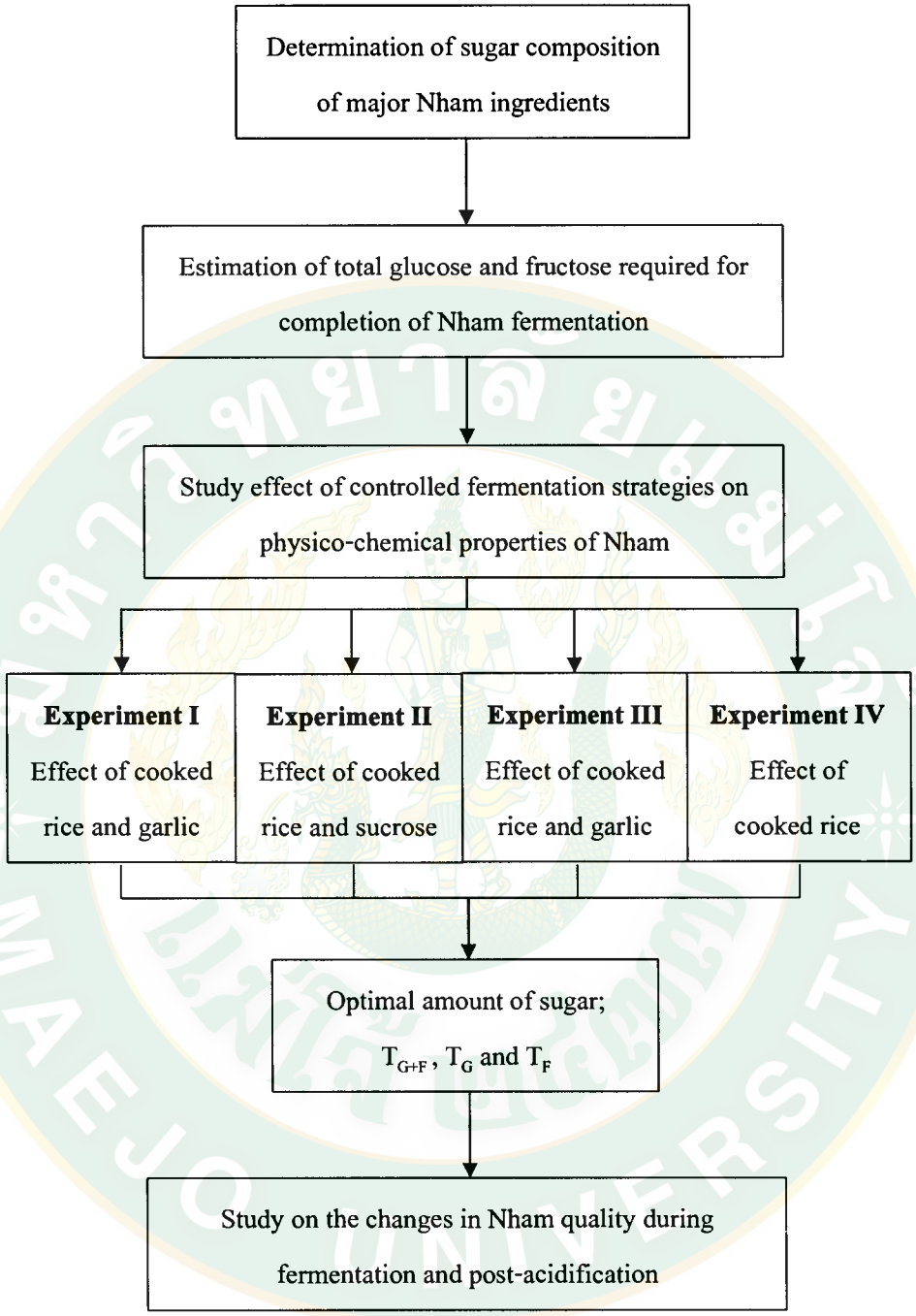

Figure 7 Experimental plan of the study

Table 1 Standard Nham formulation used in the study

Nham ingredients	(%)
Ground pork	52.1
Pork rind	34.7
Cooked rice	4.3
Fresh garlic	4.3
Sucrose	0.3
Curing salt	1.9
Sodium tripolyphosphate	0.2
Monosodium glutamate	0.2
Erythorbate	0.2
Chilli	1.7

5. Analytical procedures

5.1 Microbiological analyse

5.1.1 Total LAB count

Nham samples (25 g) were aseptically suspended in 225 ml of 0.1% peptone water and macerated for 60 s. by using a stomacher (Seward: model 400, England). The homogenate was 10-fold serially diluted with the same solution. The aliquot (0.1 ml) of three proper dilutions were spread on MRS agar containing 0.5% (w/v) calcium carbonate (Appendix A). Each dilution was performed in duplicate. The plates were incubated at 30 °C for 48 h. The numbers of colonies surrounded by clear zone were counted and expressed as total number of lactic acid bacteria (as colony-forming unit, CFU) per gram sample.

5.2 Chemical analyses

5.2.1 pH measurement

The ultimate pH (pH_U) and post-acidification pH values of Nham were determined by using a pH meter (SevenEasy: Mettler-Toledo, Switzerland). The pH measurement was done in triplicates in 3 separate samples.

5.2.2 Determination of organic acids

Organic acids in Nham were extracted according to the method of Visessanguan et al. (2004). Formic acid (50 mg/ml) 1 ml was added to coarsely ground samples (5 g) and extracted with 44 ml of deionized water using an Ultra Turrax homogeniser at 8,000 rpm for 30 s. The homogenate was centrifuged at 4 °C at 6,800 x *g* for 10 min by using a centrifuge (eppendorf model 5416, Germany). The supernatant (500 µl) was taken into microcentrifuge tube. Then, the mixture was deproteinised with 1 ml of 0.5 N perchloric acid. The mixture was stood for 5 min at room temperature (~25 °C) and centrifuged at 4 °C at 15,300 x *g* for 10 min to remove the precipitated proteins. The supernatant was collected and filtered through a 0.45 µm membrane filter prior to analysis by high performance liquid chromatography (HPLC). The HPLC system included an Aminex HPX-87H ion exclusion column (300 mm x 7.8 mm i.d.). The column was heated to 65 °C. A Waters Separation Module 2690 was operated to give a flow rate of 0.6 ml/min of the solvent, 0.02 N H_2SO_4. A sample, 20 µl, was injected and a photo diode array (Model Waters 996), set at the wavelength of 210 nm, was used as the detector. Data were processed and analysed using Millennium 32 software. The organic acid concentration was expressed as a percentage of each organic acid in Nham (w/w).

5.2.3 Determination of free sugars

Free sugar was determined according to the method modified by Valyasevi et al. (2003). Ten grams of Nham was coarsely ground by using a blender (Osterizer, USA). Sample was added with 200 µl of 50 mg/ml rhamnose solution and extracted with 125 ml of 75% ethanol by using an Ultra Turrax homogeniser at 9,500 rpm for 2 min. The homogenate was filtered through filter paper (Whatman No.1). The resulting sample (100 ml) was evaporated to 5 ml by using an evaporator system (BÜCHI, Switzerland). Sample was added with 0.5 ml of lead (II) acetate solution to precipitate protein and then, adjusted volume to 10 ml with distilled water and mixed thoroughly. The mixture was left at room temperature for 15 min and then centrifuged at 2,200 x *g* for 15 min. The supernatant was added with sodium oxalate to precipitated Pb^{2+} and centrifuged at 2,200 x *g* for 15 min. The supernatant (1 ml) was added with 20 µl of 50 mg/ml methyl-α-D-glucopyranoside before freeze-drying until the dry extract was obtained. To prepare Oxime-TMS ether derivatives, 1 ml of 2.5% (w/v) hydroxylamine hydrochloride in anhydrous

pyridine was added to the dry extract then, mixed thoroughly and sonicated for 3 min. The extract was heated at 75 °C for 30 min by using a heating block (Stuart Sciencetific, UK). The heated extract was centrifuged at 4 °C at 15,300 x *g* for 15 min. The supernatant (0.7 ml) was pipetted to vial and dried with N_2 in heating block at 55 °C. Then, 0.5 ml of anhydrous pyridine was added to dried extract and mixed with a vortex mixer for 1 min. The extract was added with 200 µl of chlorotrimethylsilane and 300 µl of hexamethyldisilasane, respectively and mixed for 1 min. The derivatives were stood for 1 h at room temperature and centrifuged at 4 °C at 15,300 x *g* for 15 min. The resulting supernatant (0.7 ml) was dried with N_2 in heating block at 55 °C. The dried derivatives were added with 0.7 ml of isooctane, mixed for 2 min and centrifuged at 15,300 x *g* for 15 min. The supernatant (1 ml) was injected to gas chromatography (GC) which consists of DB-1 column (30 m x 0.25 mm i. d.). Heating profile of the column was heated to 170 °C for 35 min then heated to 280 °C for 19 min with rate of heating 10 °C/min. Helium was used as carrier gas at flow rate 1.5 ml/min. Flame ionization detector (FID) was used as the detector.

5.2.4 Determination of total sugars

Nham samples (5 g) were hydrolysed with 1 M HCl at 80 °C for 6 h. The resulting hydrolysate was cooled immediately, adjusted pH to 7.0 and then centrifuged at 4 °C at 27,200 x *g* for 15 min. The supernatant was transferred to 50 ml volumetric flask. The precipitate was washed with 15 ml of distilled water and then centrifuged at 4 °C at 27,200 x *g* for 15 min. The resulting supernatant from precipitate was collected and combined with previous supernatant. Combined supernatant was adjusted volume to 50 ml. Five ml of supernatant was added with 0.5 ml of lead (II) acetate solution and left to room temperature for 15 min and then centrifuged at 2,200 x *g* for 15 min. The supernatant was added with sodium oxalate to precipitated Pb^{2+} and centrifuged at 2,200 x *g* for 15 min. The supernatant (1 ml) was added with 20 µl of 50 mg/ml methyl-α-D-glucopyranoside before freeze-drying until the dry extract was obtained. Oxime-TMS ether derivatives were prepared and analysed with gas chromatography as previous described in free sugar determination.

Table 2 Gradient mobile phases for biogenic amines analysis

Time (min)	Flow rate (ml/min)	0.1% Acetic acid	0.1% Acetic acid in acetonitrile
0	1	50	50
25	1	10	90
35	1	50	50
40	1	50	50

5.2.5 Determination of biogenic amines

Biogenic amines in Nham were extracted according to the method of Eerola et al. (1993). 1,7-Diaminoheptane (1 mg/ml) 500 µl was added to ground Nham samples (5 g) and extracted with 24.5 ml of perchloric acid (0.4 N) then, macerated by using stomacher at 200 rpm for 8 min. The homogenate was centrifuged at 4 °C 12,800 x *g* for 15 min. The supernatant (300 µl) was prepared to dansyl derivatives by react with dansyl chloride and filtered through a 0.45 µm membrane filter prior to analysis by high performance liquid chromatography. The HPLC system included a Sulfire C18 column (200 mm x 4.6 mm i.d.). A Waters Separation Module 2690 was operated to give a gradient flow rate showed in Table 2. A sample (20 µl) was injected and a photo diode array detector (Model Waters 996), set at the wavelength of 254 nm, and was used as the detector. Data were processed and analysed using Millennium 32 software. The concentration of biogenic amines in Nham was expressed as mg kg^{-1} based on wet weight basis.

5.3 Physical analyses

5.3.1 Color measurement

The surface color of Nham samples were measured by using a tristimulus colorimeter (Minolta Colour Meter CR300; Minolta Camera Ltd., Osaka, Japan). Before each measurement, the apparatus was standardized against a white tile (L = 90.7, a = -0.9 and b = -0.1). Color parameters (L*: lightness, a*: redness and b*: yellowness) were measured in the CIE Lab mode. The variation of each measurement was compensated by recording the average of three reading taken on the round surface of sample.

5.3.2 Weight loss

Weight loss was determined as described by Nakao et al. (1991). Nham with casing (200 g) were accurately weighted before fermentation using a balance (Model AG 204: Mettler Toledo, Switzerland). During fermentation process, Nham was taken and then reweighed. Difference in weight of Nham before and after fermentation was referred to weight loss.

5.3.3 Released water

Drip was used to describe exudate. Drip loss was measured immediately upon sample collection. The percentage of water released from samples was measured immediately upon sample collection according to the method of Nakao et al. (1991). Sample with casing was weighed (A). After removing the sample from the casings, water released on the surface was absorbed using filter paper and sample was weighed (B). The empty casing was weighed (C). The percentage of released water will be calculated according to the following equation:

$$\text{Released water (\%)} = 100 \times \{(A-B)-C\}/(A-C)$$

5.3.4 Expressible moisture

The percentage of expressible moisture from Nham was measured by the method of Funami et al. (1998) with a slight modification. The expressible moisture was measured as the weight loss resulting from the compression of sample. Samples were cut into a cylinder form (1.5 cm height x 3.0 cm diameter), placed between double layers of filter papers (Whatman No. 4) and subjected to compression using a texture analyzer with cylindrical aluminium probe (50 mm diameter). The measurement was performed with crosshead speed of 3 mm/s to 70% strain for 60 s. The expressible moisture content (g/100 g) was calculated as the ratio of the apparent expressible moisture to the total moisture content of the Nham according to the following equation:

$$\%\text{Expressible moisture} = 100 \times \{(\text{apparent expressible moisture}) / (\text{moisture content (\%) in Nham})\}$$

5.3.5 Texture Profile Analysis (TPA)

TPA measurements were carried out using a TA-XT2i texture analyser (Stable

Micro System, UK) with cylindrical aluminium probe (50 mm diameter). The samples were cut into cylinders (30 mm high x 30 mm diameter) and placed on the instrument's base. The tests were performed with two compression cycles. TPA parameters were measured at room temperature with the following testing conditions: crosshead speed 5.0 mm/s, 50% strain, surface sensing force 99.0 g, threshold 30.0 g, and time interval between first and second stroke 1 s. The Texture Expert version 1.0 software was used to collect and process the data. TPA analyses were defined and calculated as previously described by Bourne (1976). Hardness, springiness, cohesiveness, adhesiveness fracturability and resilience were calculated from the force-time curves generated for each sample.

5.4 Sensory evaluation

Sensory evaluation was carried out by thirty untrained panelists. The panelists were recruited from staff of BIOTEC who consumed Nham regularly. Nham sample were cut and halved into 1 cm thick pieces. A semicircle of Nham per sample was served uncooked in a small cup at room temperature. Samples were coded with three digit random number and presented at the same time in randomized number. The panelists were asked to assess samples for their appearance as well as their general liking on a nine-point scale (1 = dislike extremely, 9 = like extremely) (Appendix B). The ratings of each attribute were converted to numerical scores for further statistical analysis. Treatments receiving overall acceptance score ≥ 5 were considered to be acceptable for human consumption and score < 5 unacceptable and rejected. The maximum shelf life for each treatment was defined as the last sampling day where the treatment received a score of 5 or above.

5.5 Statistical analysis

All statistical analysis methods in this study were used the statistical package SPSS 15.0 software for Windows (SPSS Inc., Chicago, IL). One-way ANOVA was carried out to determined significant difference of each formulation and treatment study at 95% confident interval ($P \leq 0.05$). Mean comparisons were run by Duncan's multiple range test.

To simplify and understand the structure of a correlation within group of chemical analysis, all 14 chemical parameters changes during Nham fermentation were analyzed

by principal component analysis (PCA) extracted by Varimax method with rotated component. The PCA is primarily used for data reduction or structure detection. The purpose of data reduction is to remove redundant (highly correlated) variables from the data file, perhaps replacing the entire data file with a smaller number of uncorrelated variables. The principal components method of extraction begins by finding a linear combination of variables (a component) that accounts for as much of the remaining variation as possible and is uncorrelated with the previous component, continuing in this way until there are as many components as original variables. Usually, a few components will account for most of the variation, and these components can be used to replace the original variables. This method is most often used to reduce the number of variables in the data file.

CHAPTER 4

RESULTS AND DISCUSSION

1. Sugar composition of major Nham ingredients

Glucose (G) and fructose (F) either in free or bound forms were the major sugars found in all Nham ingredients tested, except cooked pork rind (Table 3). Minced pork contributed glucose which was mainly found in free form in the range between 0.8-1.4 mg g^{-1}. In post-mortem meat, glucose was probably formed from glycogen which is the principal storage form of glucose in muscle via glycogenolysis pathway (Pösö and Puolanne, 2005; Scheffler and Gerrard, 2007). Compared to Wood (1985), the values obtained were in agreement with those found in fresh post-rigor pork (1.26 mg g^{-1}). Garlic mainly contributed fructose and glucose which also were mainly present in bound form. Total fructose (T_F) and total glucose (T_G) were in the ranges between 116 to 168 mg g^{-1} and 10 to 17 mg g^{-1}, respectively. Sharma et al. (2006) reported that garlic contained approximately 30% of fructo-oligasaccharide in the form of inulin. Losso and Nakai (1997) found that garlic contained a high concentration of fructose, ranging from 125 to 235 mg g^{-1} on a wet weight basis and the fructose:glucose ratio presented in garlic was about 15:1. Cooked rice contributed mainly glucose in bound form. Amylose and amylopectin were the major storage components in rice which are composed of glucose monomer (Mukerjea et al., 2007). Chili mainly contributed glucose and sucrose in free form. Although, cellulose is one of the most abundant polysaccharides in plants but bound glucose could not be detected in this study. It is well established that fermentable carbohydrates have influence on development of the typical organoleptic characteristics of the fermented sausages (Bacus, 1984). During fermentation, lactic acid bacteria especially *L. plantarum* is able to convert glucose, fructose, sucrose, and mannose (their primary energy source) to lactic acid, which is the main component responsible for the pH decrease (Plumed-Ferrer et al., 2008). In addition, this acidification has a preservative effect due to inhibition of pathogenic and spoilage bacteria little resistant to low pH (Bover-Cid et al., 2001b).

Table 3 The sugar composition[a] of the important Nham ingredients

Nham ingredients	Moisture content (%)	T_G (mg g^{-1})	T_F (mg g^{-1})	F_G (mg g^{-1})	F_F (mg g^{-1})	F_S (mg g^{-1})	F_M (mg g^{-1})
Ground pork (n^b=4)	67.72 ± 2.18	1.01 ± 0.36	ND[c]	1.10 ± 0.27	ND	ND	ND
Cooked pork rind (n=4)	66.46 ± 1.36	ND	ND	ND	ND	ND	ND
Garlic (n=13)	65.34 ± 2.35	13.66 ± 3.68	141.52 ± 26.50	ND	0.57 ± 0.45	12.01 ± 3.03	ND
Cooked rice (n=4)	62.10 ± 0.62	290.14 ± 23.99	ND	ND	ND	ND	ND
Chilli (n=4)	76.02 ± 1.82	ND	ND	2.61 ± 2.00	ND	3.66 ± 1.42	ND

[a] Means ± SD. T_G, T_F, F_G, F_F, F_S and F_M represent the amounts of total glucose, total fructose, free glucose, free fructose, free sucrose, and free maltose, respectively.

[b] "n" represent the number of replication.

[c] ND: not detectable.

2. Estimation of total glucose and fructose required for completion the fermentation of Nham

2.1 Sugar composition of Nham

Estimations of the amounts of sugars based on sugar composition in Table 3 and the actual values determined from each batch of Nham prepared from different occasions are shown in the Table 4. Estimated T_{G+F}, T_G, T_F and F_S were considered to be similar to their actual values found in Nham. However, slight differences in F_G, F_F and F_M between estimated and actual values were observed. With the smaller amount of these sugars, their effects on the total sugar composition and utilization would be less significant. Glucose, fructose, sucrose and maltose were the sugars found in Nham. These sugars could be served as carbohydrate substrates for LAB during Nham fermentation. Valyasevi et al. (2003) reported that glucose, fructose and sucrose were preferentially utilized at the higher rate than maltose during Nham fermentation. This was probably explained by the fact that glucose, fructose and sucrose were translocated and phosphorylated in a single step at lower expense of ATP than maltose (Neves et al., 2005). Based on standard formulation (Table 1) commonly used in Nham processing, it could be estimated that glucose and fructose initially found in Nham were present in the bound form (Table 4). In Nham, approximately 82% of T_G and 78% of T_F were derived from cooked rice and garlic, respectively.

Table 4 Estimation of total and free sugar[a] in Nham

Nham ingredients	(%)	Estimated sugar ($g\ kg^{-1}$)						
		T_{G+F}	T_G	T_F	F_G	F_F	F_S	F_M
Ground pork	52.1	-	0.53	ND[b]	0.57	ND	ND	ND
Cooked pork rind	34.7	-	ND	ND	ND	ND	ND	ND
Garlic	4.3	-	0.59	6.14	ND	0.02	0.52	ND
Cooked rice	4.3	-	12.59	ND	ND	ND	ND	ND
Sucrose	0.3	-	1.70	1.70	ND	ND	3.40	ND
Chilli	1.7	-	ND	ND	0.05	ND	0.06	ND
Estimated total and free sugar ($g\ kg^{-1}$)[c]	-	23.25	15.41	7.84	0.62	0.02	3.98	ND
Actual total and free sugar ($g\ kg^{-1}$)[d] (n=6)	-	21.61±3.96	13.16±3.24	8.45±2.74	1.25±0.49	0.16±0.11	4.54±1.23	0.99±0.29

[a] T_G, T_F, F_G, F_F, F_S and F_M represent the amounts of total glucose, total fructose, free glucose, free fructose, free sucrose, and free maltose, respectively. T_{G+F} represent the summation of total glucose and total fructose.

[b] ND: not detectable.

[c] Estimated total and free sugars are calculated from data shown in Table 3.

[d] Actual total and free sugars were obtained from the experimental determinations from 6 batches of Nham that prepared from different occasions.

While simple sugars including glucose, fructose, and sucrose were present only 4%, 0.3% and 17%, respectively. The results showed that cooked rice and garlic were the important source of fermentable sugars for lactic acid production by which these complex carbohydrates must be degraded to more simple sugars before uptake and utilized by the cells.

2.2 Sugar utilization during fermentation of Nham inoculated with *L. plantarum* BCC 9546

Table 5 depicts the average amounts of total glucose and total fructose present and utilized in 6 batches of Nham that inoculated with *L. plantarum* BCC 9546 after fermentation at 30 °C to pH 4.6. From the results, T_{G+F} required for acidification of Nham to pH 4.6 was estimated to be $12.70 \pm 2.40\ g\ kg^{-1}$. The value obtained in this study was quite similar to the

amount of dextrose required to achieve the final product pH in other fermented sausage. For example, to achieve final product pH of Lebanon Bologna at 4.4-4.6 at 100 F, it is recommended to add 10-15 g kg^{-1} of dextrose. Based on the amount of T_{G+F} utilized in Nham, only 61-62 % of the T_G and T_F were utilized in which the excess glucose and fructose were in the bound forms. It is noted that total glucose was depleted at higher rate and extent than total fructose during Nham fermentation. Generally, glucose is a preferred energy source over fructose and other carbohydrate sources by many microorganisms (Fisher, 1987; Pastan and Adhya, 1976; Posma and Lengeler, 1985). Passos et al. (1994) reported that at high concentrations, glucose was degraded faster than fructose in cucumber juice fermentation by *L. plantarum* MOP3. In homofermentative LAB, such as *L. plantarum*, glucose and fructose are transported into cells via the phosphoenolpyruvate (PEP)-dependent sugar phosphotransferase system (PTS) (Romano et al., 1979; Thompson, 1987) and metabolized via glycolysis (Embden-Meyerhof pathway) to produce primarily lactic acid (Thompson, 1987). The metabolism of the two sugars differs only in the sugar transport step and the subsequent 1 or two steps of glycolysis. Glucose is transported into cells via the glucose-PTS and mannose-PTS (Thompson, 1987). The transport process is coupled to the phosphorylation of glucose to glucose-6-phosphate, which in turn converts to fructose-6-phosphate. Fructose is transported into cells via mannose-PTS and fructose-PTS, producing fructose-6-phosphate and fructose-1-phosphate, respectively (Thompson, 1987). The study on *Lactococcus lactis* (Thompson and Thomas, 1977) suggested that the mannose-PTS catalyses the transport and phosphorylation of sugars in the order: glucose > 2-deoxy-D-glucose > mannose > glucosamine > fructose. Therefore, the transport of glucose is much faster than the transport of fructose via mannose-PTS.

2.3 Changes in lactic acid and pH of Nham during post-acidification

As a result of lactic acid accumulation through post-acidification period, the continuous decreases in pH were typically observed in Nham processed with standard formulation (Figure 8). The pH of Nham could be lowered to 4.2 or lower during prolonged incubation at 30 °C up to 168 h. In agreement with changes in pH, lactic acid was produced rapidly during 48 to96 h and increased slowly thereafter. The remaining carbohydrates both complex and simple sugars due to the excess amounts of cooked rice, garlic and sucrose after the completion of Nham

Table 5 Sugar utilization and lactic acid production[a] during fermentation of Nham

Characteristics	Nham inoculated with *L. plantarum* BCC 9546 at 10^4 CFU/g (n=6)
Initial T_G (g kg^{-1})	13.16 ± 3.24
Utilized T_G (g kg^{-1})	7.71 ± 1.21
Utilized T_G (%)	61.1 ± 16.2
Rate of T_G utilization (g kg^{-1} h^{-1})	0.16 ± 0.04
Initial T_F (g kg^{-1})	8.45 ± 2.74
Utilized T_F (g kg^{-1})	5.00 ± 1.69
Utilized T_F (%)	62.0 ± 24.3
Rate of T_F utilization (g kg^{-1} h^{-1})	0.10 ± 0.02
Initial T_{G+F} (g kg^{-1})	21.61 ± 3.96
Utilized T_{G+F} (g kg^{-1})	12.70 ± 2.40
Utilized T_{G+F} (%)	61.0 ± 18.3
Rate of T_{G+F} utilization (g kg^{-1} h^{-1})	0.26 ± 0.04
Lactic acid production (g%)	2.14 ± 0.49
Fermentation time (h)	50 ± 11

[a]Means ± SD from 6 batches of Nham that prepared from different occasions. Initial T_G, T_F and T_{G+F} represent the amounts of total glucose, total fructose, and the summation of total glucose and total fructose in Nham raw mix, respectively.

Utilized T_G, T_F and T_{G+F} were calculated by subtraction of the initial T_G, T_F, and T_{G+F} with those determined from Nham with pH 4.6, respectively.

Rate of utilization was calculated by divided utilized T_G, T_F and T_{G+F} with time of fermentation required to reach pH 4.6.

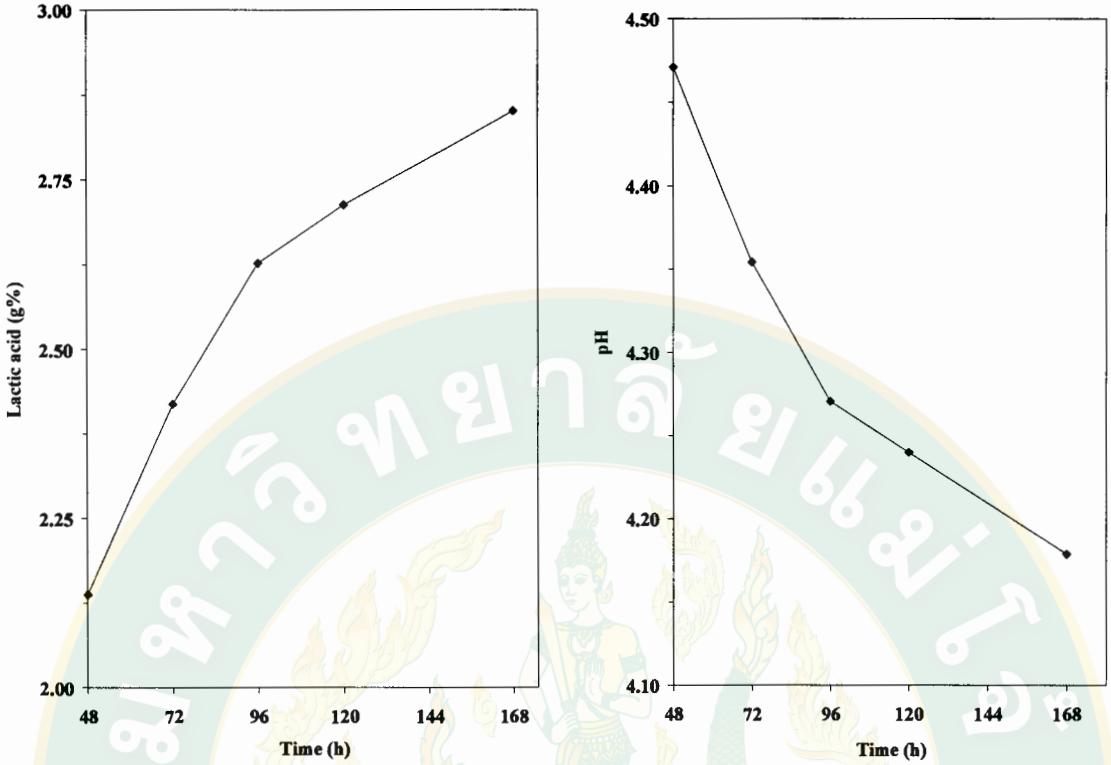

Figure 8 Typical changes in lactic acid and pH during post-acidification of Nham with standard formulation (n=6).

fermentation at pH 4.6 should primarily serve as carbohydrate sources for unwanted lactic acid production during post-acidification. Chaicherdsakul et al. (2006) reported that total organic acid at the over-fermentation period increased continuously with correlation to the decreases of total glucose, fructose and pH of Nham during storage at room temperature (~30°C).

3. Evaluation of various carbohydrate controlling strategies on post-acidification of Nham

Nham with varying amounts of cooked rice, garlic, and sucrose were prepared to study the effect of these carbohydrate sources on fermentation characteristics and some important physico-chemical properties that are indications of quality and safety of Nham. Equal amounts of minced pork, cooked pork rind, seasonings and other ingredients were added to each batch of Nham. Based on the total Nham weight, T_G and T_F were limited and varied at different levels in which the T_{G+F} in all formulations was controlled at 12.70±2.40 g kg^{-1} (Table 6). Nham samples were incubated at 30 °C up to 168 h. Changes in pH of Nham during fermentation, sugar

utilization, LAB count, lactic acid and biogenic amines were determined.

3.1 Effect of varying levels of cooked rice and garlic

3.1.1 Sugar utilization

In this study, initial T_G and T_F of Nham were limited by omitting sucrose and varying the levels of cooked rice and garlic added. Varying levels of cooked rice and garlic added affected not only on the initial amounts of total glucose and total fructose present in Nham but also the utilization of these substrates during fermentation (Table 6). Compared to Nham prepared with standard formulation (SF-1), limitation of carbohydrate substrates by varying amount of cooked rice and garlic resulted in lower initial amounts of T_G, T_F, and T_{G+F} ($P<0.05$). An increase in amount of cooked rice added resulted in Nham with high T_G, whereas an increase in amount of garlic added resulted in Nham with high T_F. Concerning the sugar utilization, limitation of T_{G+F} resulted in higher percentage utilization of T_{G+F} ($P<0.05$). However, the extent of utilized T_G and T_F varied largely depending on the initial amount of T_G and T_F. In Nham IF-1, IF-2, and IF-3 that contained T_G lower than 5 g kg^{-1}, the utilization of T_F was much higher than T_G ($P<0.05$). In addition, T_G was still present in Nham, although the initial T_G was at the lowest level added in this study. In contrast to Nham IF-4 and IF-5 that contained T_F lower than 3 g kg^{-1}, the utilization of T_G was higher but T_F was found to be completely exhausted. The results indicated that T_F was more preferred substrate in fermentation of Nham inoculated with *L. plantarum* BCC 9546. This is probably due to the capacity to ferment garlic of *L. plantarum* BCC 9546 used in this study (Valyasevi et al., 2003). Purification of inulinase which is enzyme used for inulin degradation from *L. plantarum* has been reported (Takahashi, 1975). Therefore, the ability to ferment garlic was paralleled by a capacity to ferment inulin (Paludan-Müller et al., 1999). In addition, IF-2 was likely to be the most appropriate formulation in which about 82% of carbohydrate substrates were utilized.

3.1.2 pH of Nham

Varying levels of cooked rice and garlic resulted in marked differences in the ultimate pH (pH_U) of Nham and their changes during post-acidification ($P<0.05$) (Figure 9). Only Nham IF-1 and IF-2 exhibited pH_U lower than 4.6 within 48 h of fermentation. According to

Table 6 Effect of cooked rice and garlic on sugar utilization[a] at 48 h of fermentation

Nham ingredients (%)	Nham formulations					
	SF-1	IF-1	IF-2	IF-3	IF-4	IF-5
Ground pork	52.1	53.3	53.8	54.1	54.4	54.7
Pork rind	34.7	35.5	35.8	36.1	36.3	36.4
Cooked rice	4.3	0.6	1.7	2.5	3.2	3.7
Fresh garlic	4.3	6.3	4.5	3.0	1.8	0.8
Sucrose	0.3	-	-	-	-	-
Initial T_G (g kg^{-1})	14.17 ± 0.49d	2.96 ± 0.22a	3.62 ± 0.58ab	4.98 ± 0.67b	9.26 ± 1.27c	9.71 ± 0.55c
Utilized T_G (g kg^{-1})	5.07 ± 0.49b	2.43 ± 0.22a	1.59 ± 0.58a	1.58 ± 0.67a	6.79 ± 1.27c	6.85 ± 0.55c
Utilized T_G (%)	35.7 ± 2.2ab	82.0 ± 1.3d	43.2 ± 9.1b	31.1 ± 9.2a	73.1 ± 3.7cd	70.5 ± 1.7c
Initial T_F (g kg^{-1})	10.19 ± 0.39e	11.08 ± 0.53f	8.48 ± 0.40d	5.48 ± 0.28c	3.09 ± 0.07b	1.29 ± 0.04a
Utilized T_F (g kg^{-1})	4.37 ± 0.39c	8.19 ± 0.53e	7.97 ± 0.40e	5.48 ± 0.28d	3.09 ± 0.07b	1.29 ± 0.04a
Utilized T_F (%)	42.8 ± 2.2a	73.9 ± 1.2b	94.0 ± 0.3c	100.0 ± 0.0d	100.0 ± 0.0d	100.0 ± 0.0d
Initial T_{G+F} (g kg^{-1})	24.36 ± 0.62d	14.04 ± 0.69c	11.86 ± 0.56ab	10.30 ± 0.75a	12.34 ± 1.34b	10.99 ± 0.58ab
Utilized T_{G+F} (g kg^{-1})	9.44 ± 0.62bc	10.63 ± 0.69c	9.68 ± 0.56bc	6.90 ± 0.76a	9.88 ± 1.35bc	8.12 ± 0.59ab
Utilized T_{G+F} (%)	38.7 ± 1.6a	75.6 ± 1.2c	81.6 ± 0.9d	66.9 ± 2.4b	79.9 ± 2.2d	73.9 ± 1.4c
Fermentation time (h)	48	48	48	48	48	48

[a] Means ± SD from three determinations. Different letters (a, b, c) in the same row indicate significant differences ($P<0.05$).

Initial T_G, T_F and T_{G+F} represent the amounts of total glucose, total fructose, and the summation of total glucose and total fructose in Nham raw mix, respectively.

Utilized T_G, T_F and T_{G+F} were calculated by subtraction of the initial T_G, T_F, and T_{G+F} with those determined from Nham at 48 h of fermentation, respectively.

Nham standard TIS 1219-2546 (2003) issued by the Thai Industrial Standards Institute, Ministry of Industry, Nham is recommended to be fermented until pH ≤ 4.6 before consumption. Since Nham is normally consumed without cooking, the occurrence of pathogens such as *Salmonella* spp., *Staphylococcus aureus*, and *Listeria monocytogenes* was found specially in Nham with pH higher than 4.6 (Paukatong and Kunawasen, 2001). Recently, the study of Chokesajjawatee et al. (2009) showed that pH is a key risk factor for *S. aureus* exposure from Nham consumption. The risk of *S. aureus* exposure was almost four-fold higher for consumption of Nham with pH > 4.6 than Nham with pH of ≤ 4.6.

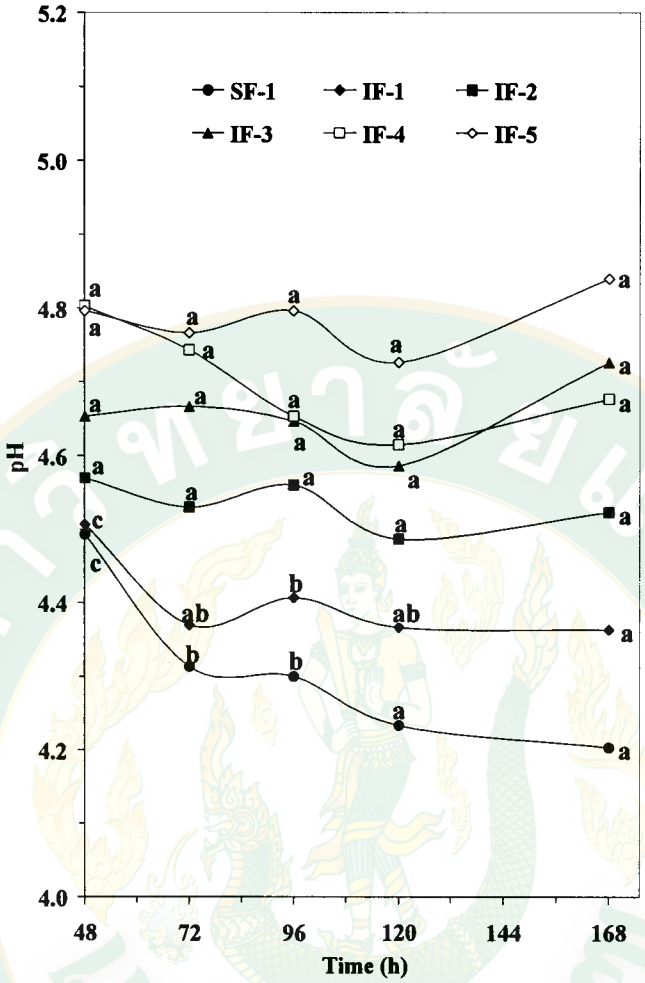

Figure 9 Effect of cooked rice and garlic on changes in pH during post-acidification. Different letters (a, b, c) indicate significant differences within each formulation (P<0.05).

Considering with the sugar utilization, the results obviously suggested a significant role of garlic on the ultimate pH values of Nham, especially when garlic fermenting LAB strain like *L. plantarum* BCC 9546 was used as a starter (Valyasevi et al., 2003). The amount of garlic added might be more important than cooked rice to determine whether the fermentation would succeed to pH 4.6 or fail. Although the higher utilization of T_G was observed particularly when T_F was totally depleted, pH of Nham could not be lowered below 4.6 within 48 h of fermentation. Compared to Nham prepared with the standard formulation in which carbohydrate sources were not limited, Nham with varying levels of cooked rice and garlic exhibited much lesser changes in post-acidification pH. Therefore, IF-2 was found to be the most

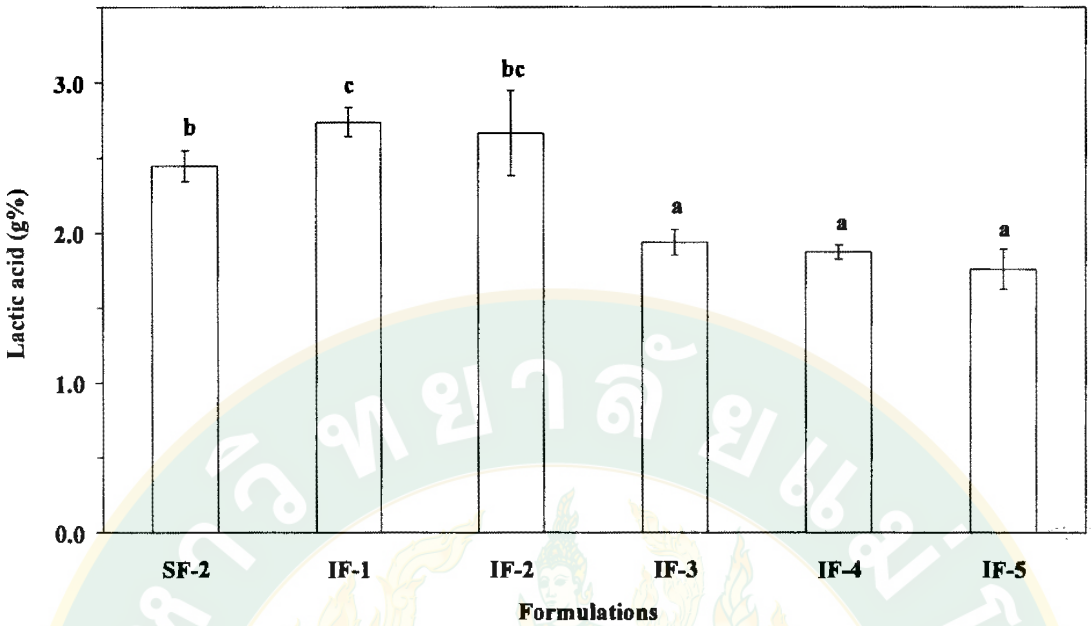

Figure 10 Effect of cooked rice and garlic on lactic acid production at 48 h of fermentation. Different letters (a, b, c) indicate significant differences between formulations (P<0.05).

appropriate formulation among all formulations tested in this study and the optimal initial T_F should be about 8 g kg^{-1}.

3.1.3 Lactic acid production

Nham containing high T_F or high amount of garlic had higher production of lactic acid (Figure 10). Lactic acid was the major organic acid formed during fermentation of Nham (Visessanguan et al., 2004). In agreement with the ultimate pH values, to have pH_U below 4.6 the content of lactic acid formed in Nham should exceed 2.5 g%.

3.1.4 LAB count

Incorporating varying levels of cooked rice and garlic had no effect on the counts of LAB in Nham during post-acidification (P>0.05). The number of lactic acid bacteria of all formulations remained constant at ~8 log CFU/g during 48-96 h of fermentation and slightly decreased with the increasing incubation time (Figure 11). Similar result was reported by

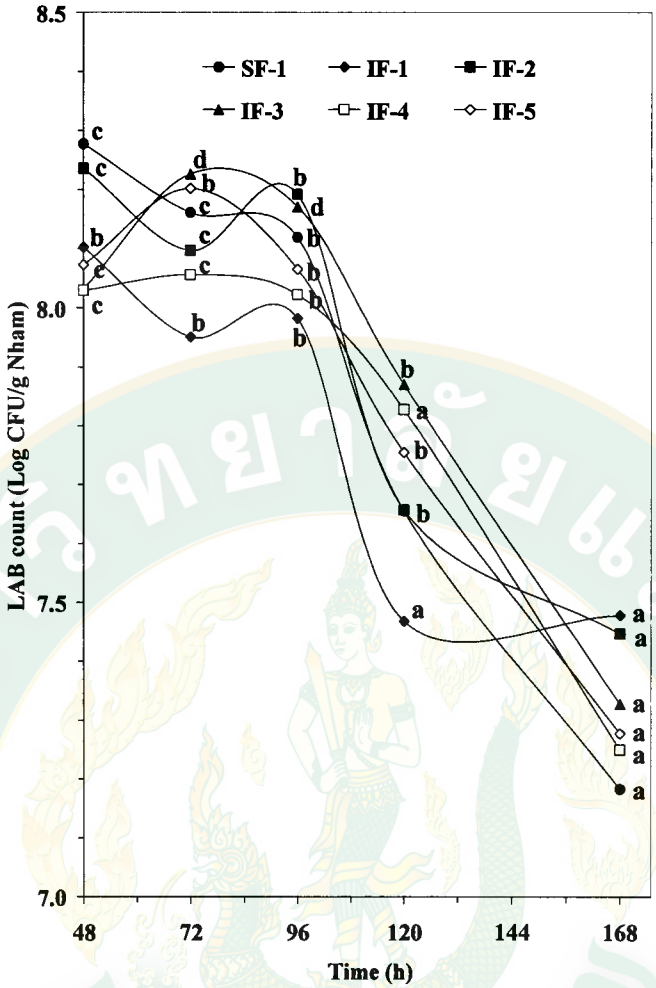

Figure 11 Effect of cooked rice and garlic on LAB count. Different letters (a, b, c) indicate significant differences within each formulation (P<0.05).

Chaicherdsakul et al. (2006) that the LAB population decreased after 96th h of fermentation, possibly due to the inhibition by higher amount of acid formed.

3.1.5 Biogenic amines

Nham containing lower proportion of garlic had higher amount of total biogenic amines (Figure 12). Among Nham with varying levels of cooked rice and garlic, Nham IF-1 showed the lowest amount of total biogenic amines (P<0.05). No marked differences in total biogenic amines was observed between Nham IF-1 and control (P>0.05). As shown by Nham IF-2, IF-3, IF-4, and IF-5, Nham containing T_F lower than 11.08 g kg^{-1}, exhibited higher production of total biogenic amines. The production of biogenic amines is a characteristic of several groups

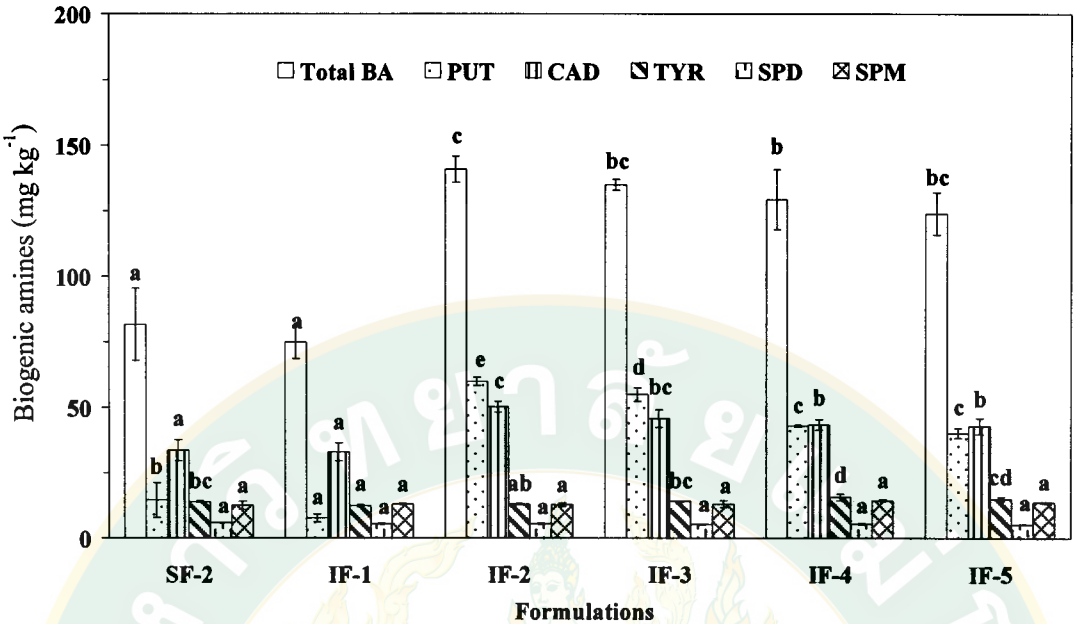

Figure 12 Effect of cooked rice and garlic on biogenic amines production at 48 h of fermentation. Different letters (a, b, c) indicate significant differences between formulations (P<0.05). Total BA: total biogenic amines, PUT: putrescine, CAD: cadaverine, TYR: tyramine, SPD: spermidine and SPM: spermine.

of microorganisms such as *Enterobacteriaceae*, lactic acid bacteria and *Micrococcaceae* (Suzzi and Gardini, 2003). The results indicated that garlic may play an important role on the inhibition of microorganisms with amino acid decarboxylase activity. In agreement with the study of Mah et al. (2009), the garlic extract was effective in inhibiting the bacterial growth, and thereby suppressing the total biogenic amines production in Myeolchi-joet. The growth or decarboxylase activity of amines-produced microorganisms might be suppressed by antimicrobial components, such as allicin, in garlic. Some authors have reported that the main responsible factor for low levels of biogenic amines is the low pH reached during the manufacture process of dry sausages (González-Fernández et al., 2003; Bover-Cid et al., 2001a; Maijala et al., 1993). Putrescine and cadaverine were found to be the major biogenic amines in Nham. Suzzi and Gardini (2003) and Bover-Cid et al. (2001a) reported that *Enterobacteriaceae* play an important role on putrescine and cadaverine formation. Nham with the proportion of garlic lower than 6.3% contained higher amount of putrescine and cadaverine (P<0.05). Decrease in garlic proportion resulted in higher

production of tyramine. However, spermidine and spermine was not significant differences among all formulations of Nham (P>0.05). Hernandez-Jover et al. (1997) emphasized that spermidine and spermine were only type of amine that are always detected in meat and meat products. These amines can naturally occur in fresh beef and they are present in significant levels in the meat used for fermented sausage production. These results showed that biogenic amines production in Nham depended on T_{G+F}, garlic content and rate of pH drop.

Garlic plays a major role as carbohydrate source for LAB in Nham, specially that inoculated with *Lactobacillus plantarum* BCC 9546. These results indicated that at least 4.5% of garlic which is equivalent to T_F of ~ 8 g kg^{-1} was prerequisite for the completion of Nham fermentation. As a consequence, pH changes and lactic acid production could be minimized during post-acidification. In this study, Nham IF-2 seemed to be the most appropriate formulation though the high amount of biogenic amines were observed. Bover-Cid et al. (2001c) reported that the omission of sugars in the sausage formulation would not be recommended because this procedure leads to sausages with higher amine contents. In order to reduce biogenic amines production, effect of sucrose and cooked rice on biogenic amines production of Nham produced by controlled fermentation strategy was studied and Nham IF-2 was used as control in this experiment.

3.2 Effect of varying levels of sucrose and cooked rice

3.2.1 Sugar utilization

Higher amount of sucrose added had no effect on T_{G+F} utilization (Table 7). There are no marked differences were observed among all Nham formulations (P<0.05). Although a decrease in cooked rice content resulted in lower amount of T_G, it was not exhausted in all formulations except Nham IF-11. An increase in sucrose content yielded higher initial T_F, however it was found to be lower than 8 g kg^{-1} which was the optimal initial T_F. As a result, TF was completely exhausted in all formulations of Nham tested. Since similar phenomenon was also observed with the control that processed with the same formulation as IF-2, this could be explained by the variation of T_F contained in garlic used in this experiment. Though, the same amount of garlic was added, the fermentation was apparently limited by the amount of fermentable sugars due to the variation of sugar composition in garlic.

Table 7 Effect of sucrose and cooked rice on sugar utilization[a] at 48 h of fermentation

Nham ingredients (%)	Nham formulations					
	IF-6	IF-7	IF-8	IF-9	IF-10	IF-11
Ground pork	53.8	54.0	54.1	54.2	54.3	54.5
Pork rind	35.8	36.0	36.1	36.1	36.2	36.3
Cooked rice	1.7	1.3	1.0	0.6	0.3	-
Fresh garlic	4.5	4.5	4.5	4.5	4.5	4.5
Sucrose	-	0.1	0.2	0.3	0.4	0.5
Initial T_G (g kg^{-1})	5.43 ± 1.84d	4.86 ± 0.42cd	5.23 ± 0.27d	4.41 ± 0.09c	3.40 ± 0.03a	3.62 ± 0.07b
Utilized T_G (g kg^{-1})	3.82 ± 1.84b	4.27 ± 0.42b	4.42 ± 0.27b	4.32 ± 0.09b	3.38 ± 0.03a	3.62 ± 0.07a
Utilized T_G (%)	83.9 ± 5.4a	87.8 ± 1.1a	84.5 ± 0.8a	97.8 ± 0.1b	99.6 ± 0.0c	100.0 ± 0.0d
Initial T_F (g kg^{-1})	5.46 ± 0.54a	6.25 ± 0.48a	6.04 ± 0.48a	7.32 ± 0.32b	7.47 ± 0.20b	7.40 ± 0.28b
Utilized T_F (g kg^{-1})	5.46 ± 0.54a	6.25 ± 0.48b	6.04 ± 0.48ab	7.32 ± 0.32c	7.47 ± 0.20c	7.40 ± 0.28c
Utilized T_F (%)	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
Initial T_{G+F} (g kg^{-1})	9.72 ± 1.63a	11.11 ± 0.68a	11.27 ± 0.75a	11.73 ± 0.41a	10.87 ± 0.32a	11.02 ± 0.20a
Utilized T_{G+F} (g kg^{-1})	9.03 ± 1.63a	10.52 ± 0.68a	10.46 ± 0.75a	11.16 ± 0.41a	10.85 ± 0.32a	11.02 ± 0.20a
Utilized T_{G+F} (%)	92.8 ± 1.2a	94.7 ± 0.3ab	92.8 ± 0.5a	95.2 ± 0.2b	99.8 ± 0.0c	100.0 ± 0.0d
Fermentation time (h)	48	48	48	48	48	48

[a] Means ± SD from three determinations. Different letters (a, b, c) in the same row indicate significant differences (P<0.05).

Initial T_G, T_F and T_{G+F} represent the amounts of total glucose, total fructose, and the summation of total glucose and total fructose in Nham raw mix, respectively.

Utilized T_G, T_F and T_{G+F} were calculated by subtraction of the initial T_G, T_F, and T_{G+F} with those determined from Nham at 48 h of fermentation, respectively.

3.2.2 pH of Nham

Addition of sucrose had no effect on pH_U and post-acidification pH of Nham (Figure 13). All formulations of Nham exhibited pH higher than 4.6 which is not acceptable due to the safety for consumption and showed an increase in pH during post-acidification period which is a sign of spoilage. Therefore, the fermentation was considered fail as acid production in all Nham formulations studied was limited by an inadequate initial T_F especially from garlic.

3.2.3 Lactic acid production

Higher amount of sucrose had no significant effect on lactic acid production

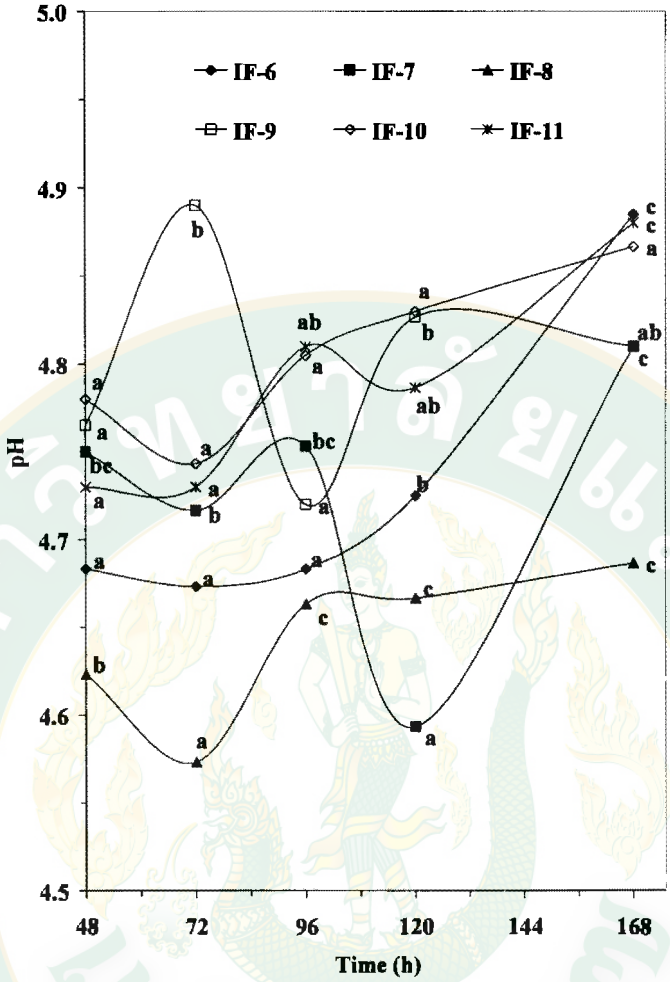

Figure 13 Effect of sucrose and cooked rice on changes in pH during post-acidification. Different letters (a, b, c) indicate significant differences within each formulation ($P<0.05$).

(Figure 14). However, lactic acid content was inadequate to decrease pH below 4.6 in all formulations tested.

3.2.4 Biogenic amines

In this study, incorporating varying levels of sucrose had no effect on biogenic amines formation (Figure 15). Due to the insufficient initial T_F, all formulations of Nham contained high amount of total biogenic amines (458.57-618.09 mg kg^{-1}). Formation of biogenic amines in food generally occurs due to decarboxylation of amines are also of concern in relation

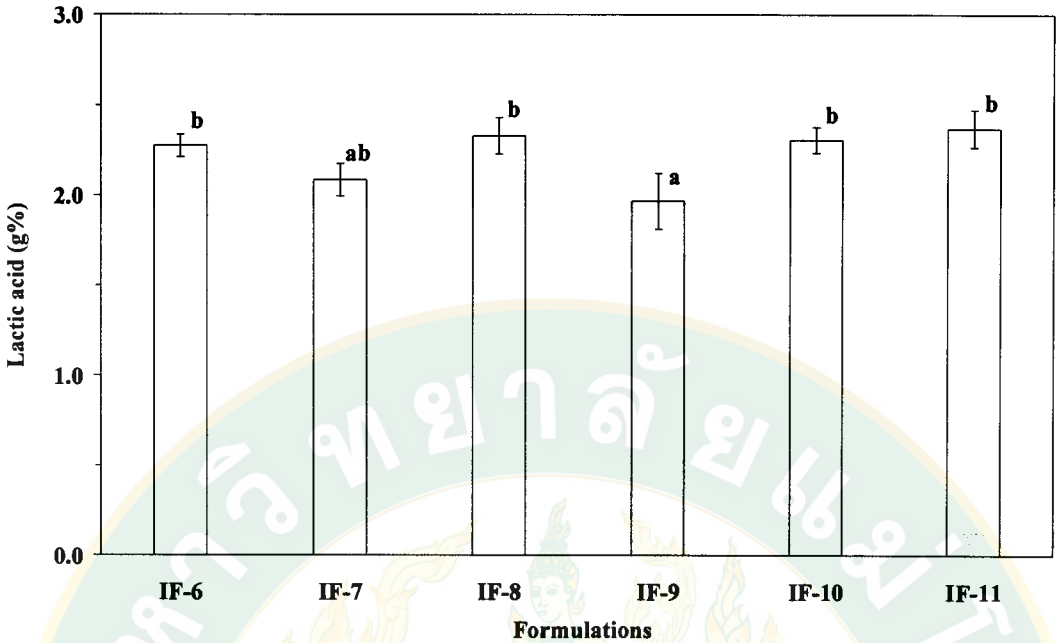

Figure 14 Effect of sucrose and cooked rice on lactic acid production at 48 h of fermentation. Different letters (a, b, c) indicate significant differences between formulations (P<0.05).

to food spoilage because they result from decarboxylase activity of spoilage microflora during the storage of food (Vidal-Carou et al., 1990). The results indicated that addition of sucrose which is simple sugar could not reduce biogenic amines formation in Nham. These results disagreed with and González-Fernández et al. (2006). Bover-Cid et al. (2001c) suggested that sugar omission (glucose and lactose) was not recommended because it might increase biogenic amines accumulation during the manufacture and storage of slightly fermented sausages. González-Fernández et al. (2006) reported that the use of sugar concentration (mainly glucose) in a range of 0.5-1% was the important factor to be considered in order to reduce the level of biogenic amines in Chorizo dry sausage. The production of biogenic amines is an extremely complex phenomenon, dependant on several variables, such as the growth kinetics of the microorganisms, their proteolytic and decarboxylase activities (Suzzi and Gardini, 2003), type of starter culture, level of sugar and good quality of raw materials (González-Fernández et al., 2006). The values of spermidine and spermine were in the range 0.54-2.90 mg kg^{-1} and 18.19-23.90 mg kg^{-1}.The results indicated effect of pH on growth inhibition of microorganisms. In this case, meat pH was not

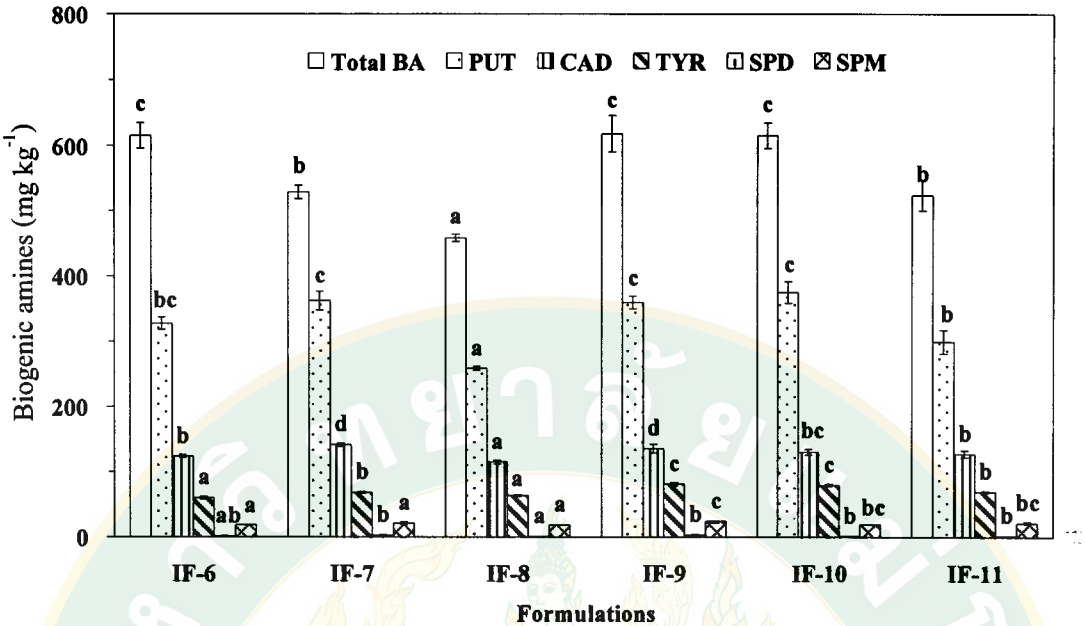

Figure 15 Effect of sucrose and cooked rice on biogenic amines production at 48 h of fermentation. Different letters (a, b, c) indicate significant differences between formulations (P<0.05). (Total BA: total biogenic amines, PUT: putrescine, CAD: cadaverine, TYR: tyramine, SPD: spermidine and SPM: spermine.

low enough to prevent the growth of spoilage microorganisms. Since this is considered failed, so the protective effect of sucrose could not be achieved. However, the significant role of fructose in garlic could be emphasized. Therefore, to accurate estimation of sugar in Nham in controlled fermentation strategy, the amount of garlic added should be adjusted based on the actual T_F in garlic added.

3.3 Effect of varying levels of cooked rice and garlic

3.3.1 Sugar utilization

Increase in amount of garlic resulted in higher T_{G+F} and T_F utilization (Table 8). Nham with higher proportion of garlic showed more T_{G+F} and T_F utilization (P<0.05). It was possibly due to an ability to ferment inulin of this starter. However, no significant differences were observed in T_G utilization (P>0.05). The amount of cooked rice and garlic had no effect on fermentation time of Nham.

Table 8 Effect of cooked rice and garlic on sugar utilization[a] at 48 h of fermentation

Nham ingredients (%)	Nham formulations		
	IF-12	IF-13	IF-14
Ground pork	51.3	51.8	52.4
Pork rind	34.2	34.6	34.9
Cooked rice	0.54	0.70	0.86
Fresh garlic	9.8	8.8	7.7
Sucrose	-	-	-
Initial T_G (g kg^{-1})	3.38 ± 0.16a	3.72 ± 0.15a	3.80 ± 0.02a
Utilized T_G (g kg^{-1})	3.00 ± 0.16a	3.17 ± 0.15a	3.12 ± 0.02a
Utilized T_G (%)	88.7 ± 0.5b	90.7 ± 0.4c	82.0 ± 0.9a
Initial T_F (g kg^{-1})	12.83 ± 0.63b	11.54 ± 0.57b	8.90 ± 0.88a
Utilized T_F (g kg^{-1})	11.78 ± 0.63b	10.60 ± 0.57b	7.78 ± 0.88a
Utilized T_F (%)	91.8 ± 0.4a	91.8 ± 0.4a	92.7 ± 0.1a
Initial T_{G+F} (g kg^{-1})	16.11 ± 0.63b	15.04 ± 0.59b	12.70 ± 1.08a
Utilized T_{G+F} (g kg^{-1})	15.03 ± 0.63b	13.97 ± 0.59b	10.99 ± 1.08a
Utilized T_{G+F} (%)	93.3 ± 0.3b	94.6 ± 0.2c	91.0 ± 0.0a
Fermentation time (h)	48	48	48

[a]Means ± SD from three determinations. Different letters (a, b, c) in the same row indicate significant differences ($P<0.05$).

Initial T_G, T_F and T_{G+F} represent the amounts of total glucose, total fructose, and the summation of total glucose and total fructose in Nham raw mix, respectively.

Utilized T_G, T_F and T_{G+F} were calculated by subtraction of the initial T_G, T_F, and T_{G+F} with those determined from Nham at 48 h of fermentation, respectively.

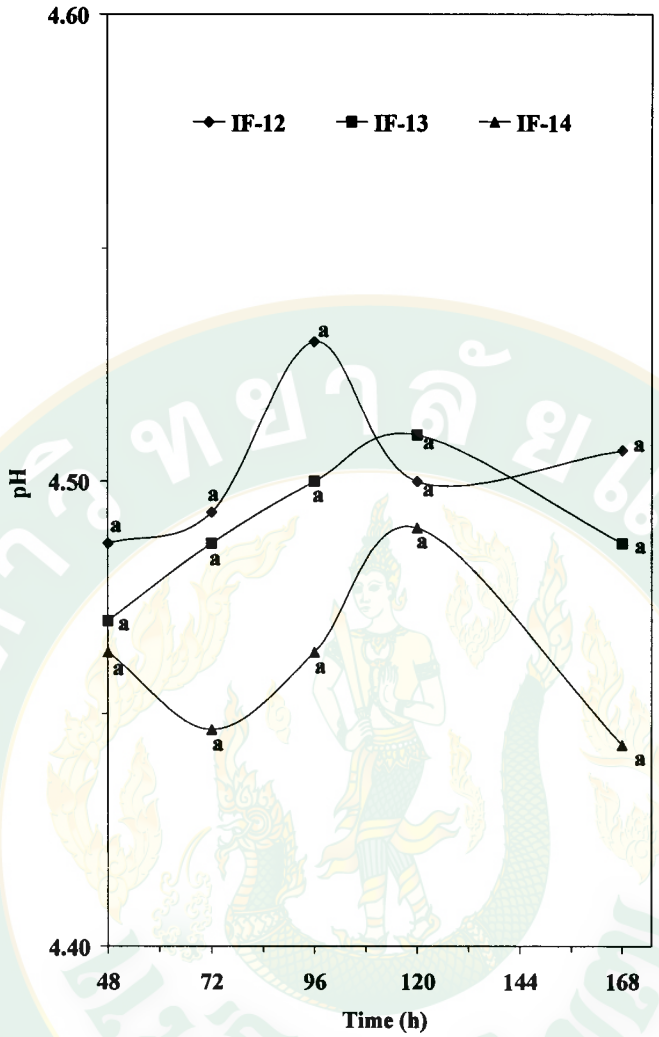

Figure 16 Effect of cooked rice and garlic on changes in pH during post-acidification. Different letters (a, b, c) indicate significant differences within each formulation (P<0.05).

3.3.2 pH of Nham

Higher proportion of garlic had no effect on pH_U and post-acidification pH of Nham (Figure 16). No marked differences in pH_U of Nham were observed (P>0.05). As post-acidification proceeded, no significant differences in post-acidification pH of Nham were observed (P>0.05). The pH_U of all formulations had lower than 4.6 and still below 4.6 through post-acidification period. It indicated an adequate amount of fructose from garlic gave the satisfied pH_U (≤4.6) and less changes in post-acidification pH.

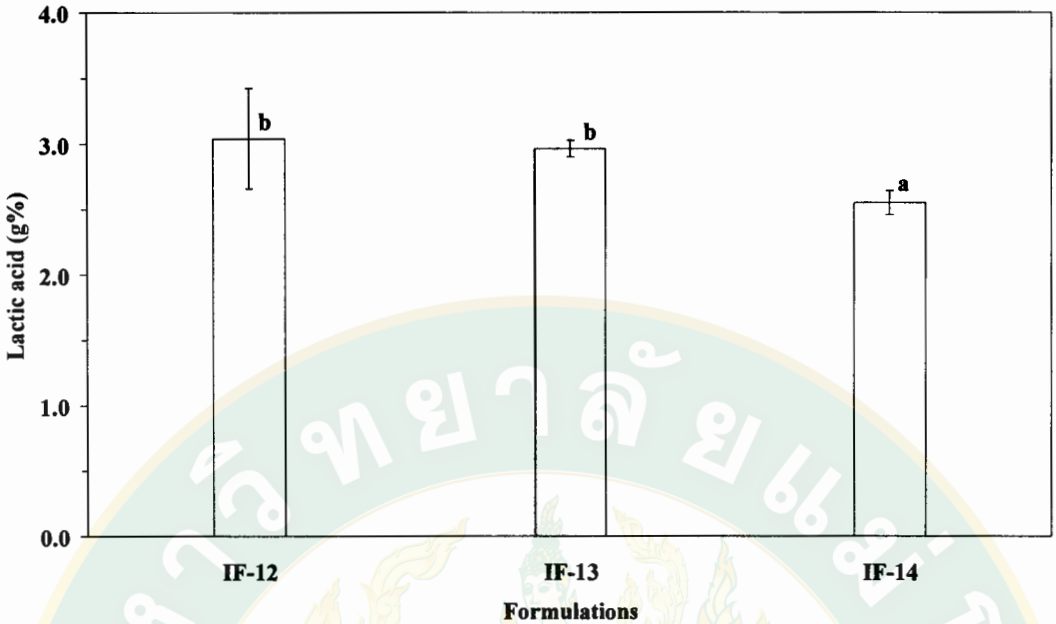

Figure 17 Effect cooked rice and garlic on lactic acid production at 48 h of fermentation. Different letters (a, b, c) indicate significant differences between formulations (P<0.05).

3.3.3 Lactic acid production

Nham with higher proportion of garlic exhibited higher lactic acid production (P<0.05) (Figure 17). This results was similar to Paludan-Müller et al. (2002), in Som-fak with garlic, a maximum level of 2.8% (w/w) lactic acid was produced after 4 days, whereas in Som-fak without garlic, no more than 1.8% (w/w) lactic acid was produced during the fermentation period. Although Nham with higher proportion of garlic exhibited higher lactic acid production but their pH_U was not significant differences. It might be due to buffering capacity of protein in Nham. Opaswatcharanon (2004) reported that ground pork and cook pork rind which are the major ingredients in Nham contained large amount of protein. The charge group on proteins can be very important for the ability to resist changes in pH of Nham.

3.3.4 Biogenic amines

Higher proportion of garlic resulted in higher the total biogenic amines especially, tyramine (Figure 18). Nham IF-12 had the highest amount of total biogenic amines

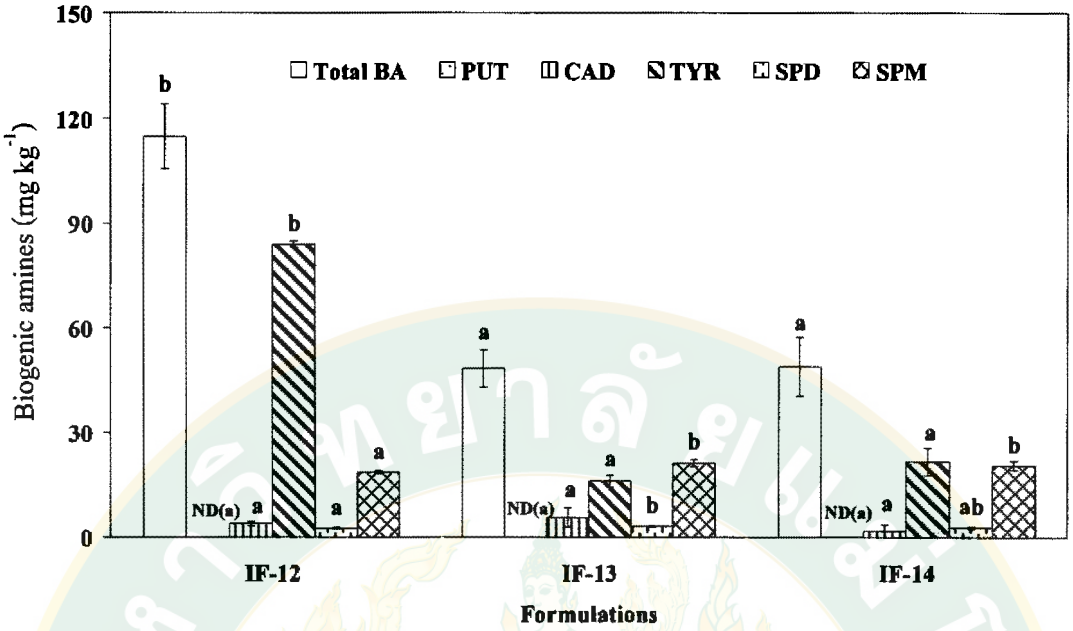

Figure 18 Effect of cooked rice and garlic on biogenic amines production at 48 h of fermentation. Different letters (a, b, c) indicate significant differences between formulations (P<0.05). (Total BA: total biogenic amines, PUT: putrescine, CAD: cadaverine, TYR: tyramine, SPD: spermidine and SPM: spermine).

(P<0.05). Tyramine was the most abundant biogenic amines found in this formulation. Strains of lactobacilli belonging to the species *L. buchneri*, *L. alimentarius*, *L. plantarum*, *L. curvatus*, *L. farciminis*, *L. bavaricus*, *L. homohiochii*, *L. reuteri* and *L. sakei* were amine-positive and tyramine is quantitatively the most important biogenic amines produced (Masson et al., 1996; Montel et al., 1999; Bover-Cid et al., 2001a; Pereira et al., 2001; De las Rivas et al., 2008). With the highest lactic acid content in Nham IF-12, the function of biogenic amine production in microorganisms is believed to be a protective mechanism developed to maintain the intracellular pH homoeostasis when growing in acid conditions. Indeed, the decarboxylase enzymes are induced by the presence of the specific precursor amino acid as well as acid environment. The decarboxylation reaction consumes a proton, which results in an increased pH in the cytoplasm. Decarboxylase enzymes function in cooperation with an amino acid/amine antiporter system, which moves the amine to the outside of the cell in exchange for an extracellular amino acid. (Molenaar et al., 1993; Bearson et al., 1997). However, cadaverine was detected in small amount

and putrescine was not detectable in all formulations of Nham. In agreement with Mah et al. (2009), garlic extract caused a considerable delay in the formation of putrescine and cadaverine during early ripening of Myeolchi-joet. Spermidine was detected in small amount (1.79 to 5.61 mg kg^{-1}). Spermine was found in the range 18.55 to 21.19 mg kg^{-1}.

3.4 Effect of cooked rice

3.4.1 Sugar utilization

In previous section (3.3), cooked rice was added into Nham with controlled fermentation strategy in very small amount. Therefore, Nham IF-13 and IF-15 were prepared to study effect of cooked rice on changes in physico-chemical properties during post acidification. Omission of cooked rice resulted in lower amount of T_{G+F} and T_G utilization (Table 9). Nham IF-15 exhibited lower T_{G+F} and T_G utilization ($P<0.05$). It was possibly due to cooked rice contributed glucose as the major sugar. Therefore, omission of cooked rice had effect on initial amount of T_{G+F} and T_G. With lower amount of T_G, T_G was exhausted during fermentation of Nham.

3.4.2 pH of Nham

Omission of cooked rice resulted in higher pH_U and post-acidification pH of Nham (Figure 19). Nham IF-15 had higher pH_U than 4.6 ($P<0.05$). Therefore, the fermentation was considered to be failed. Although T_F was sufficient to complete fermentation but it could not decrease pH lower than 4.6. It has been suggested that the main role of rice was to reduce the high buffering capacity of the fish in order to obtain a rapid decrease in pH of fermented fish product (Owen and Mendoza, 1985). During post-acidification, a larger extent in post-acidification pH was observed in Nham IF-15 ($P<0.05$). Therefore, cooked rice is assumed to be another essential carbohydrate substrate for fermentation by LAB which has to be added when produced Nham with controlled fermentation strategy. From this study, the optimal initial T_G was about 3 g kg^{-1}.

Table 9 Effect of cooked rice on sugar utilization[a] at 48 h of fermentation

Nham ingredients (%)	Nham formulations	
	IF-13	IF-15
Ground pork	51.8	54.5
Pork rind	34.6	36.3
Cooked rice	0.70	-
Fresh garlic	8.8	8.8
Sucrose	-	-
Initial T_G (g kg^{-1})	3.50 ± 0.14b	2.14 ± 0.07a
Utilized T_G (g kg^{-1})	3.17 ± 0.14b	2.14 ± 0.07a
Utilized T_G (%)	90.7 ± 0.4a	100.0 ± 0.0b
Initial T_F (g kg^{-1})	11.54 ± 0.57a	10.88 ± 0.58a
Utilized T_F (g kg^{-1})	10.60 ± 0.57a	10.35 ± 0.58a
Utilized T_F (%)	91.8 ± 0.4a	95.0 ± 0.3b
InitialT_{G+F} (g kg^{-1})	15.04 ± 0.59b	13.05 ± 0.65a
Utilized T_{G+F} (g kg^{-1})	13.97 ± 0.59b	12.51 ± 0.65a
Utilized T_{G+F} (%)	94.6 ± 0.2a	95.9 ± 0.2b
Fermentation time (h)	48	48

[a]Means ± SD from three determinations.

Different letters (a, b, c) in the same row indicate significant differences (P<0.05).

Initial T_G, T_F and T_{G+F} represent the amounts of total glucose, total fructose, and the summation of total glucose and total fructose in Nham raw mix, respectively.

Utilized T_G, T_F and T_{G+F} were calculated by subtraction of the initial T_G, T_F, and T_{G+F} with those determined from Nham at 48 h of fermentation, respectively.

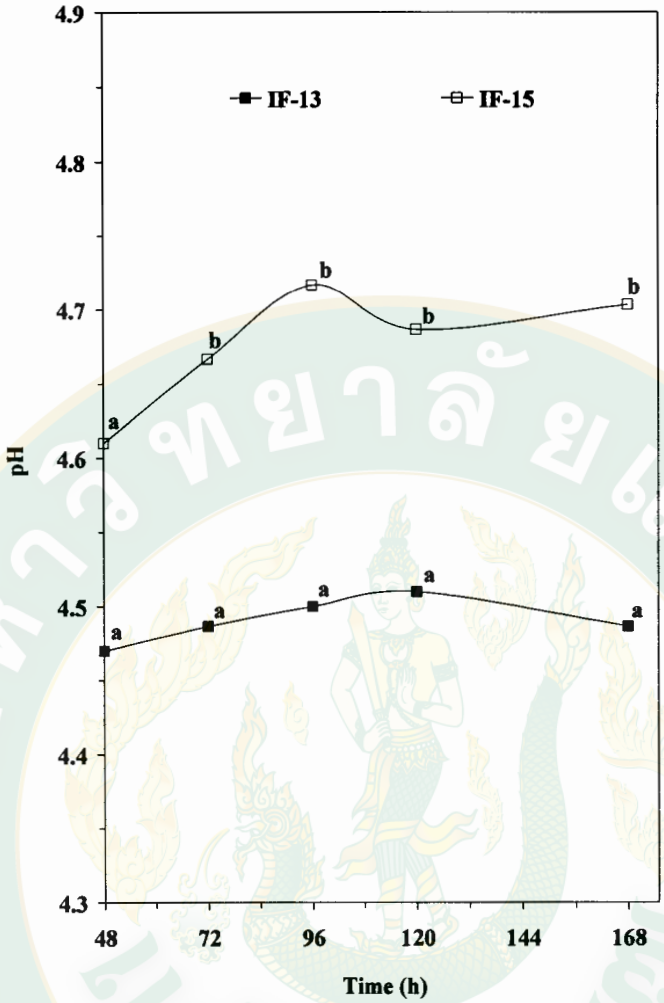

Figure 19 Effect of cooked rice on changes in pH during post-acidification. Different letters (a, b, c) indicate significant differences within each formulation ($P<0.05$).

3.4.3 Lactic acid production

The omission of cooked rice resulted in lower production of lactic acid (Figure 20). Nham IF-15 exhibited lower lactic acid content than with cooked rice ($P<0.05$). It was possibly due to lower amount of carbohydrate substrate in Nham IF-15.

3.4.4 Biogenic amines

Omission of cooked rice resulted in higher biogenic amines production (Figure 21). Nham processed without cooked rice exhibited higher total biogenic amines production than

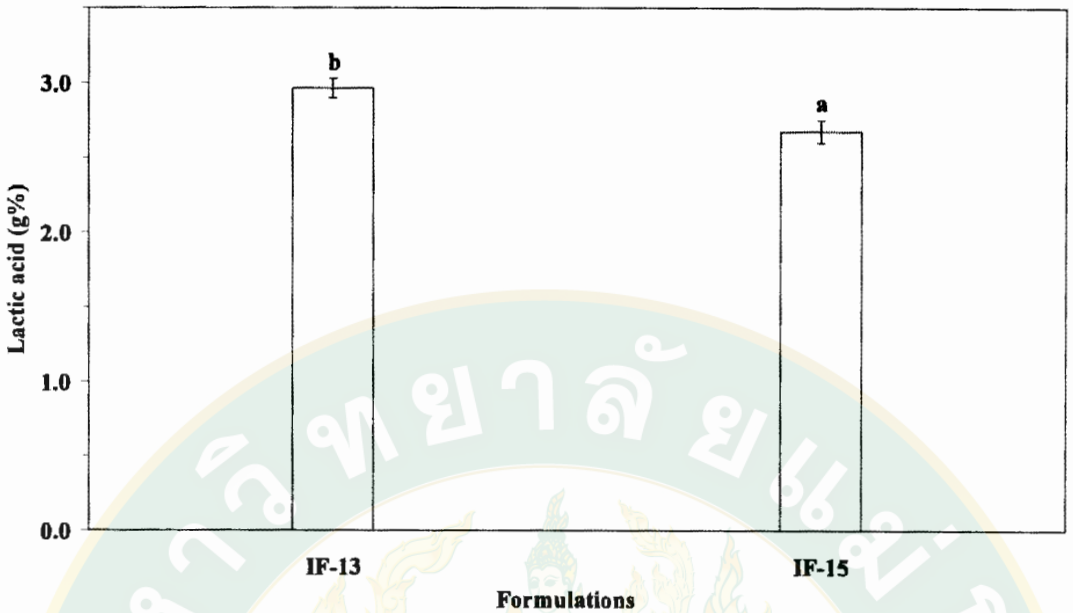

Figure 20 Effect of cooked rice on lactic acid production at 48 h of fermentation. Different letters (a, b, c) indicate significant differences between formulation (P<0.05).

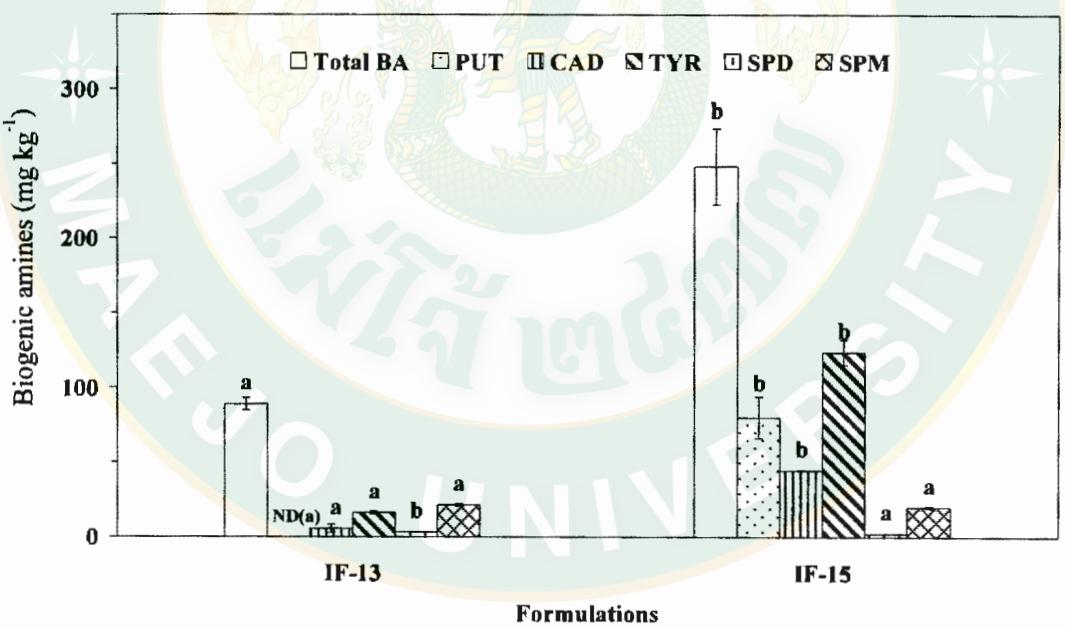

Figure 21 Effect of cooked rice on biogenic amines production at 48 h of fermentation. Different letters (a, b, c) indicate significant differences between formulation (P<0.05). (Total BA: total biogenic amines, PUT: putrescine, CAD: cadaverine, TYR: tyramine, SPD: spermidine and SPM: spermine.

that processed with cooked rice ($P<0.05$). Tyramine was found to be the major amine detected in Nham with no cooked rice added, followed by putrescine and cadaverine, respectively. Spermidine and spermine were in the range 2.07-3.12 mg kg^{-1} and 19.80-21.19 mg kg^{-1}, respectively.

4. Chemical parameters related to pH_U and changes in post-acidification pH (pH_{PA}) of Nham

To simplify and understand the correlation structure among chemical parameters, obtained data were analyzed by factor analysis by principle component analysis (PCA). The data consisted of 14 variables measured in 18 Nham formulations as shown in Table 10.

Fourteen variables explained differences in pH_U and changes in pH_{PA} of Nham during post-acidification were reduced into 4 components. The 35.54% of the total variance was explained by PC1, 26.34% by the PC2 and so on (Table 11). Generally, a cumulative explained variance percentage above 50% is considered satisfactory for the extracted factors with the first PC accounting for the major part of the total variance (Mataragus et al., 2007). Therefore, the first two principle components (PCs) were only extracted which explained the 61.88% of total variance. PC1 contained 35.54% of the total variance, and the loading indicated that there were significant contributions of total fructose, garlic content, lactic acid content, free fructose, with positive loadings and pH_U with negative loading. The latter variable was inversely correlated to the four former ones. The second PC described 26.34% of the total variance and presented high positive loadings for cooked rice content, total glucose, total glucose and fructose variables whereas pH_{PA} variable presented high negative loading in PC2.

The score and loading plots for PC1 versus PC2 are presented in Figure 22 and 23. The PC2 scores discriminated Nham produced by using standard formulation (SF, positive scores) from those processed with improved formulations with carbohydrate limitation strategies (IF, negative scores) (Figure 21). Nham produced by standard formulation was characterized by higher cooked rice content, T_G, T_{G+F} and lower pH_U and pH_{PA} (Figure 22). The results indicated that high amount of cooked rice in Nham produced by standard formulation which contributed to total glucose had effect on the increases in total glucose and fructose and resulting in lower pH_U

Table 10 The data set[a] of variables measured in all formulations of Nham

Nham Formulations	Cooked rice (CC)	Garlic (GC)	Sucrose (SC)	T_{G-F} (TGF)	T_G (TG)	T_F (TF)	F_G (FG)	F_F (FF)	F_S (FS)	pH_U (pHU)	pH_{PA}[b] (pHPA)	Lactic acid (LA)	Acetic acid (AA)	Total biogenic amines (BA)
SF-1	4.3 ± 0.0	4.3 ± 0.0	0.3 ± 0.0	24.37 ± 0.62	14.17 ± 0.49	10.20 ± 0.39	1.59 ± 0.24	0.23 ± 0.03	3.72 ± 0.12	4.49 ± 0.03	-2.3E-03 ± 2.53E-04	2.45 ± 0.11	0.057 ± 0.006	81.62 ± 13.81
SF-2	4.3 ± 0.0	4.3 ± 0.0	0.3 ± 0.0	21.76 ± 1.24	12.38 ± 2.02	7.92 ± 0.80	0.96 ± 0.07	0.16 ± 0.01	3.12 ± 0.08	4.53 ± 0.02	-7.6E-04 ± 1.11E-04	2.61 ± 0.03	0.090 ± 0.010	483.10 ± 10.06
SF-3	4.3 ± 0.0	4.3 ± 0.0	0.3 ± 0.0	17.17 ± 0.04	10.57 ± 0.10	6.60 ± 0.14	1.63 ± 0.02	0.34 ± 0.03	3.08 ± 0.28	4.52 ± 0.03	-1.2E-03 ± 8.40E-04	2.65 ± 0.01	0.062 ± 0.002	342.65 ± 6.38
IF-1	0.6 ± 0.0	6.3 ± 0.0	0.0 ± 0.0	14.05 ± 0.70	2.97 ± 0.22	11.08 ± 0.52	0.96 ± 0.03	0.15 ± 0.01	0.31 ± 0.02	4.51 ± 0.02	-7.2E-04 ± 2.45E-04	2.74 ± 0.11	0.040 ± 0.004	74.93 ± 6.37
IF-2	1.7 ± 0.0	4.5 ± 0.0	0.0 ± 0.0	11.86 ± 0.40	3.62 ± 0.41	8.48 ± 0.40	0.99 ± 0.04	0.11 ± 0.01	0.22 ± 0.01	4.57 ± 0.04	-4.1E-04 ± 7.84E-04	2.67 ± 0.24	0.045 ± 0.004	140.88 ± 4.98
IF-3	2.5 ± 0.0	3.0 ± 0.0	0.0 ± 0.0	10.31 ± 0.54	4.98 ± 0.48	5.48 ± 0.28	1.00 ± 0.04	0.07 ± 0.00	0.15 ± 0.01	4.65 ± 0.10	5.1E-04 ± 9.83E-04	1.94 ± 0.11	0.042 ± 0.001	134.89 ± 2.03
IF-4	3.2 ± 0.0	1.8 ± 0.0	0.0 ± 0.0	12.34 ± 0.95	9.26 ± 0.90	3.09 ± 0.05	1.01 ± 0.08	0.06 ± 0.00	0.09 ± 0.01	4.77 ± 0.10	-4.4E-04 ± 1.22E-03	1.87 ± 0.05	0.044 ± 0.002	129.24 ± 11.47
IF-5	3.7 ± 0.0	0.8 ± 0.0	0.0 ± 0.0	10.99 ± 0.41	9.71 ± 0.39	1.29 ± 0.04	1.71 ± 0.08	0.06 ± 0.01	0.05 ± 0.00	4.90 ± 0.13	1.8E-03 ± 3.75E-04	1.76 ± 0.11	0.049 ± 0.006	123.89 ± 8.13
IF-6	1.7 ± 0.0	4.5 ± 0.0	0.0 ± 0.0	9.72 ± 1.15	5.43 ± 1.84	5.46 ± 0.55	1.06 ± 0.05	0.11 ± 0.01	0.36 ± 0.01	4.68 ± 0.02	1.2E-03 ± 6.86E-04	2.36 ± 0.09	0.079 ± 0.006	615.12 ± 19.29
IF-7	1.3 ± 0.0	4.5 ± 0.0	0.1 ± 0.0	11.11 ± 0.68	4.86 ± 0.42	6.25 ± 0.48	0.89 ± 0.06	0.11 ± 0.01	1.19 ± 0.03	4.75 ± 0.00	3.2E-04 ± 1.16E-03	2.18 ± 0.07	0.077 ± 0.001	528.73 ± 7.33
IF-8	1.0 ± 0.0	4.5 ± 0.0	0.2 ± 0.0	11.27 ± 0.75	5.23 ± 0.27	6.04 ± 0.48	1.13 ± 0.03	0.12 ± 0.00	2.08 ± 0.04	4.62 ± 0.01	7.5E-04 ± 4.52E-04	2.17 ± 0.10	0.062 ± 0.019	458.57 ± 5.68
IF-9	0.6 ± 0.0	4.5 ± 0.0	0.3 ± 0.0	11.58 ± 0.39	4.28 ± 0.24	7.30 ± 0.23	0.82 ± 0.06	0.14 ± 0.01	3.07 ± 0.07	4.77 ± 0.02	7.7E-04 ± 2.50E-04	1.97 ± 0.08	0.094 ± 0.024	618.09 ± 19.65
IF-10	0.3 ± 0.0	4.5 ± 0.0	0.4 ± 0.0	11.21 ± 0.40	7.47 ± 0.20	3.40 ± 0.03	0.62 ± 0.02	0.14 ± 0.01	3.99 ± 0.03	4.78 ± 0.02	1.1E-03 ± 5.11E-04	2.24 ± 0.03	0.081 ± 0.005	615.12 ± 19.29
IF-11	0.0 ± 0.0	4.5 ± 0.0	0.5 ± 0.0	11.02 ± 0.20	3.62 ± 0.08	7.40 ± 0.28	0.72 ± 0.02	0.17 ± 0.01	4.47 ± 0.19	4.73 ± 0.02	1.2E-03 ± 5.70E-04	2.46 ± 0.03	0.099 ± 0.011	524.01 ± 23.39
IF-12	0.5 ± 0.0	9.8 ± 0.0	0.0 ± 0.0	16.11 ± 0.45	3.38 ± 0.16	12.83 ± 0.45	1.17 ± 0.07	0.43 ± 0.02	0.76 ± 0.06	4.49 ± 0.05	1.2E-04 ± 2.64E-04	3.04 ± 0.38	0.101 ± 0.006	114.80 ± 9.26
IF-13	0.7 ± 0.0	8.8 ± 0.0	0.0 ± 0.0	15.04 ± 0.59	3.50 ± 0.14	11.55 ± 0.57	1.50 ± 0.05	0.54 ± 0.09	0.62 ± 0.10	4.47 ± 0.00	1.5E-04 ± 3.28E-04	2.96 ± 0.04	0.152 ± 0.004	48.31 ± 5.35
IF-14	0.9 ± 0.0	7.7 ± 0.0	0.0 ± 0.0	12.70 ± 1.08	3.80 ± 0.20	8.89 ± 0.88	1.58 ± 0.20	0.41 ± 0.04	0.59 ± 0.07	4.46 ± 0.04	-1.2E-04 ± 5.79E-04	2.55 ± 0.09	0.000 ± 0.000	48.87 ± 8.43
IF-15	0.0 ± 0.0	8.8 ± 0.0	0.0 ± 0.0	13.05 ± 0.46	2.14 ± 0.06	10.88 ± 0.42	0.87 ± 0.10	0.44 ± 0.05	0.56 ± 0.09	4.61 ± 0.04	6.6E-04 ± 2.33E-04	2.67 ± 0.05	0.074 ± 0.005	272.69 ± 17.56

[a]Means ± SD from three determinations.

[b]pH_{PA} is calculated from equation: (pH at the end of fermentation - pH at the end of post-acidification)/(time at the end of post-acidification - time at the end of fermentation). Positive values (+) indicate an increase in pH_{PA}; Negative values (-) indicate a decrease in pH_{PA}.

SF refers to standard formulation. IF refers to improved formulation by controlled fermentation strategy; IF-1 - IF-5: Nham with varying amount of cooked rice and garlic (lot No. 1), IF-6 - IF-11: Nham with varying amount of cooked rice and sucrose, IF-12 - IF-14: Nham with varying amount of cooked rice and garlic (lot No. 2) and IF-15: Nham without cooked rice.

Table 11 Loadings for the principle components and their respective variances

Variables	PC1	PC2	PC3
Total fructose (TF)	0.949	0.031	-0.039
Garlic content (GC)	0.936	-0.273	-0.058
Lactic acid content (LA)	0.932	0.066	0.030
Free fructose (FF)	0.867	0.023	-0.089
pH_U	-0.835	-0.381	0.165
Cooked rice content (CC)	-0.376	0.869	-0.097
Total glucose (TG)	-0.366	0.863	0.232
Total glucose and fructose (TGF)	0.429	0.835	0.219
pH_{PA}	-0.379	-0.793	0.043
Free glucose (FG)	0.132	0.600	-0.470
Sucrose content (SC)	-0.120	0.191	0.925
Free sucrose (FS)	-0.004	0.255	0.922
Total biogenic amines (BA)	-0.327	-0.343	0.762
Acetic acid (AA)	0.372	-0.235	0.532
% of total variance	35.54	26.34	21.04
Cumulative % variance	35.54	61.88	82.92

Positive value (+) indicated positive correlation between variables and PCs.

Negative value (-) indicated negative correlation between variables and PCs.

and higher changes in pH_{PA}. PC1 scores discriminated Nham successfully fermented ($pH_U \leq 4.6$) (positive scores) from those failed fermentation ($pH_U > 4.6$) (negative scores) (Figure 21). Nham with successive fermentation was characterized by higher amount of garlic added, T_F, free fructose, lactic acid, and lower pH_U (see loading in Figure 22).

The results showed that high amount of garlic in Nham produced by controlled fermentation strategy which contributed to bound fructose, had effect on the increases in total fructose, free fructose, resulting in higher lactic acid content, lower pH_U and lesser changes in pH_{PA}. Therefore, addition of garlic with high amount of bound fructose into Nham produced by controlled fermentation strategy was essential for a success of fermentation and less changes in pH_{PA}. Although, IF-15 was Nham with failed fermentation but it was classified into the group of successful fermentation. It might be due to its marginal pH_U value (pH_U=4.61).

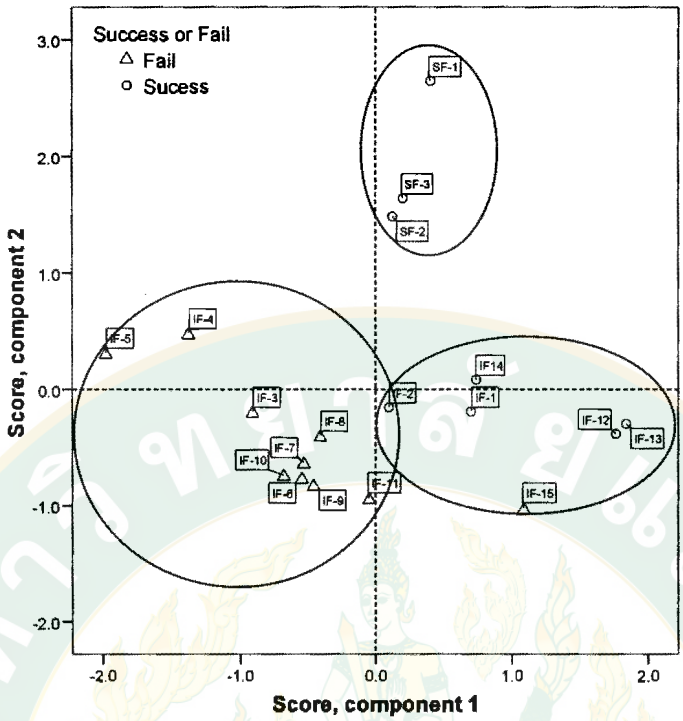

Figure 22 Score plot of all formulations of Nham on the PC1-PC2 space.

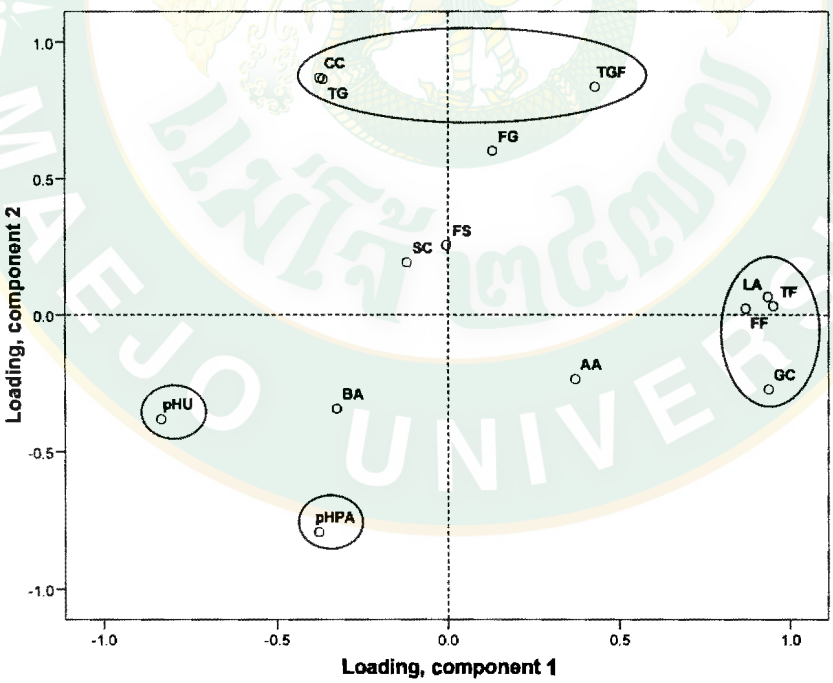

Figure 23 Loading plot of the variables on the PC1-PC2 space.

5. Study on the changes in Nham quality during fermentation and post-acidification

Nham produced by controlled fermentation strategies which contained the optimal amount of T_{G+F}, T_G and T_F (~12.7, ~3 and ~8 g kg^{-1}) was prepared (IF-16) to study changes in physico-chemical properties and acceptability for consumption, compared with that produced by standard formulation (SF-4) up to 14 days.

5.1 Effect on sugar utilization

Controlled fermentation strategy resulted in lower amount of initial and utilized T_{G+F} (Table 12). However, amount of T_{G+F} of Nham produced by standard formulation was about 15 g kg^{-1} which was in the range of T_{G+F} required for completion of Nham fermentation (10 to 15 g kg^{-1}). This was possibly due to variation of T_F in garlic. Based on T_F had to be added at 8 g kg^{-1}, Nham produced by controlled fermentation strategy was added with higher amount of garlic and lower amount of cooked rice than standard formulation. Therefore, amount of initial T_G found in Nham produced by controlled fermentation strategy was lower than standard formulation about two folds whereas initial T_F was higher than standard formulation. T_G in Nham produced by controlled fermentation strategy was utilized lower than standard formulation around two folds. T_F in Nham produced by standard formulation could not be detected at the complete fermentation time. It was shown that T_F in Nham produced by standard formulation was not sufficient and glucose was utilized as secondary carbohydrate source. This evidence resulted in the delay complete fermentation time. Fermentation of Nham produced by standard formulation and controlled fermentation strategy took 42 and 36 h, respectively. It was shown that Nham produced by standard formulations showed the complete fermentation time longer than controlled fermentation strategy.

5.2 Effect on pH_U and post-acidification pH

Controlled fermentation strategy resulted in faster, lower pH_U and lesser changes in post-acidification pH when compared with standard formulation ($P<0.05$) (Table 13). Nham produced by controlled fermentation strategy exhibited faster and lower pH_U than standard formulation (36 and 42 h, respectively) ($P<0.05$). A rapid decrease in pH of Nham with

Table 12 Effect of controlled fermentation strategy on sugar utilization[a]

Nham ingredients (%)	Nham formulations	
	SF-4	IF-16
Ground pork	52.1	52
Pork rind	34.7	34.7
Cooked rice	4.3	0.8
Fresh garlic	4.3	8.3
Sucrose	0.3	-
Initial T_G (g kg^{-1})	8.75 ± 0.36b	4.14 ± 0.33a
Utilized T_G (g kg^{-1})	7.11 ± 0.36b	3.73 ± 0.33a
Utilized T_G (%)	81.2 ± 0.8a	90.2 ± 0.7b
Initial T_F (g kg^{-1})	6.06 ± 0.52a	8.31 ± 0.01b
Utilized T_F (g kg^{-1})	6.06 ± 0.52a	7.63 ± 0.01b
Utilized T_F (%)	100.0 ± 0.0b	91.8 ± 0.0a
Initial T_{G+F} (g kg^{-1})	14.81 ± 0.80b	12.26 ± 0.03a
Utilized T_{G+F} (g kg^{-1})	13.17 ± 0.80b	11.17 ± 0.03a
Utilized T_{G+F} (%)	88.9 ± 0.6a	91.2 ± 0.0b
Fermentation time (h)	42	36

[a]Means ± SD from three determinations. Different letters (a, b, c) in the same row indicate significant differences ($P<0.05$).

Initial T_G, T_F and T_{G+F} represent the amounts of total glucose, total fructose, and the summation of total glucose and total fructose in Nham raw mix, respectively.

Utilized T_G, T_F and T_{G+F} were calculated by subtraction of the initial T_G, T_F, and T_{G+F} with those determined from Nham with pH 4.6, respectively.

Table 13 Effect of controlled fermentation strategy on changes in pH[a] of Nham during post-acidification

Time (h)	pH	
	SF-4	IF-16
at pH_U	4.57 ± 0.02bB	4.51 ± 0.03aB
48	4.50 ± 0.01bB	4.45 ± 0.01aAB
72	4.56 ± 0.02bB	4.45 ± 0.06aAB
96	4.54 ± 0.06bB	4.51 ± 0.01aB
120	4.41 ± 0.05aA	4.43 ± 0.01aAB
168	4.52 ± 0.03bB	4.43 ± 0.02aA
240	4.41 ± 0.04aA	4.44 ± 0.04aA
336	4.75 ± 0.01bC	4.48 ± 0.01aAB

[a]Means ± SD from three determinations.

The small letters in the same row indicate significant differences ($P<0.05$).

The capital letters in the same column indicate significant differences ($P<0.05$).

controlled fermentation strategy during fermentation was possibly due to higher amount of garlic substrate and the ability to degrade inulin of *L. plantarum* BCC 9546 (Valyasevi et al., 2003). An association of garlic content and *L. plantarum* that had a capacity to ferment garlic was found in this study in accordance with Paludan-Müller et al. (1999, 2002). During post-acidification period, Nham produced by controlled fermentation strategy gave lesser changes in post-acidification pH and still lower than 4.6. While the post-acidification pH of Nham produced by standard formulation gradually decreased and finally increased to 4.75. An increase in post-acidification pH indicated the spoilage of Nham. Demeyer et al. (1979) and Bover-Cid et al. (1999) reported that pH increase during the last period of storage was probably due to the liberation of ammonical compounds.

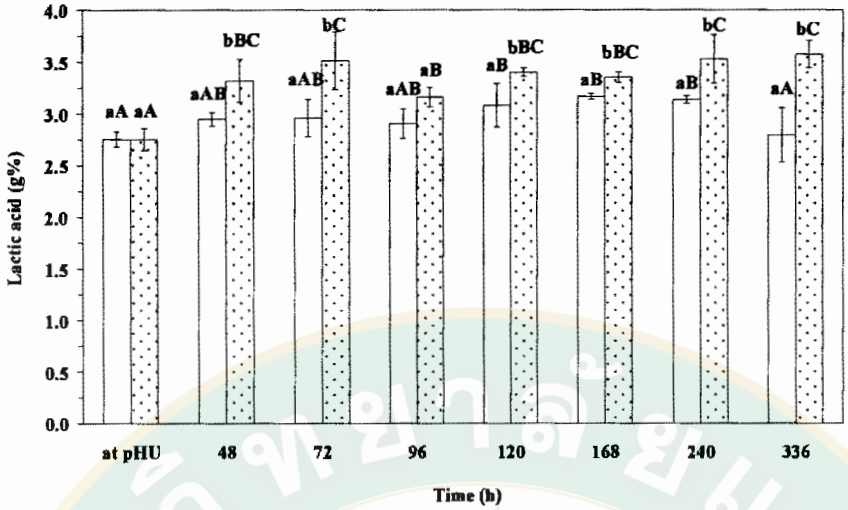

Figure 24 Changes in lactic acid content of Nham produced by standard formulation (□) and controlled fermentation strategy (⊡) during post-acidification. The small letters indicate significant differences between formulations at the same post-acidification time (P<0.05). The capital letters indicate significant differences within each formulation (P<0.05).

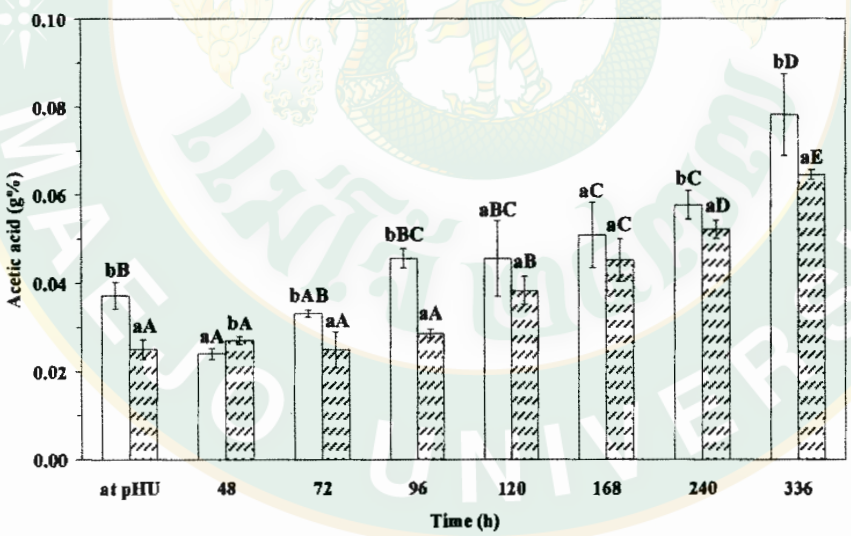

Figure 25 Changes in acetic acid content of Nham produced by standard formulation (□) and controlled fermentation strategy (⊡) during post-acidification. The small letters indicate significant differences between formulations at the same post-acidification time (P<0.05). The capital letters indicate significant differences within each formulation (P<0.05).

5.3 Effect on acid production

Controlled fermentation strategy resulted in higher production of lactic acid than standard formulation (Figure 24). At the end of fermentation, no significant differences in lactic acid content were observed between Nham produced by controlled fermentation strategy and standard formulation ($P>0.05$). When post-acidification proceeded, Nham produced by controlled fermentation strategy exhibited higher extent in lactic acid production than standard formulation. Garlic might be directly involved in the fermentation process of Nham with supplying the garlic fermenting starter. In Som-fak with garlic, a maximum level of 2.8% (w/w) lactic acid was produced after 4 days, whereas in Som-fak without garlic, no more than 1.8% (w/w) lactic acid was produced during the fermentation period (Paludan-Müller et al., 2002). Controlled fermentation strategy resulted in lower production of acetic acid content. Acetic acid continually increased when post-acidification proceeded (Figure 25). At the end of fermentation, Nham produced by standard formulation exhibited higher acetic acid content when compared with controlled fermentation strategy ($P<0.05$). When post-acidification increased, the extent of acetic acid in Nham produced by standard formulation had greater than controlled fermentation strategy ($P<0.05$). *L. plantarum* BCC 9546 was the homofermentative lactic acid bacteria which gave only lactic acid by carbohydrate fermentation. Therefore, acetic acid in Nham might be produced from undesirable bacteria. Due to the rate of pH drop, Nham produced by standard formulation had more undesirable bacteria which resulted in higher production of acetic acid. In addition, acetic acid formed during post-acidification period might affect on decreases in post-acidification pH of Nham produced by standard formulation.

5.4 Effect on biogenic amines production

Controlled fermentation strategy could reduce biogenic amines production and their accumulation in Nham (Figure 26). At the end of fermentation, Nham produced by controlled fermentation strategy exhibited lower biogenic amines than standard formulation ($P<0.05$). It was possibly due to higher proportion of garlic as a source of carbohydrate. An adequate amount of garlic results in rapid decrease in pH and consequently, amine-produced microorganisms were inhibited. Some authors have reported that the main responsible factor for low levels of biogenic amines is the low pH reached during the manufacture process of dry

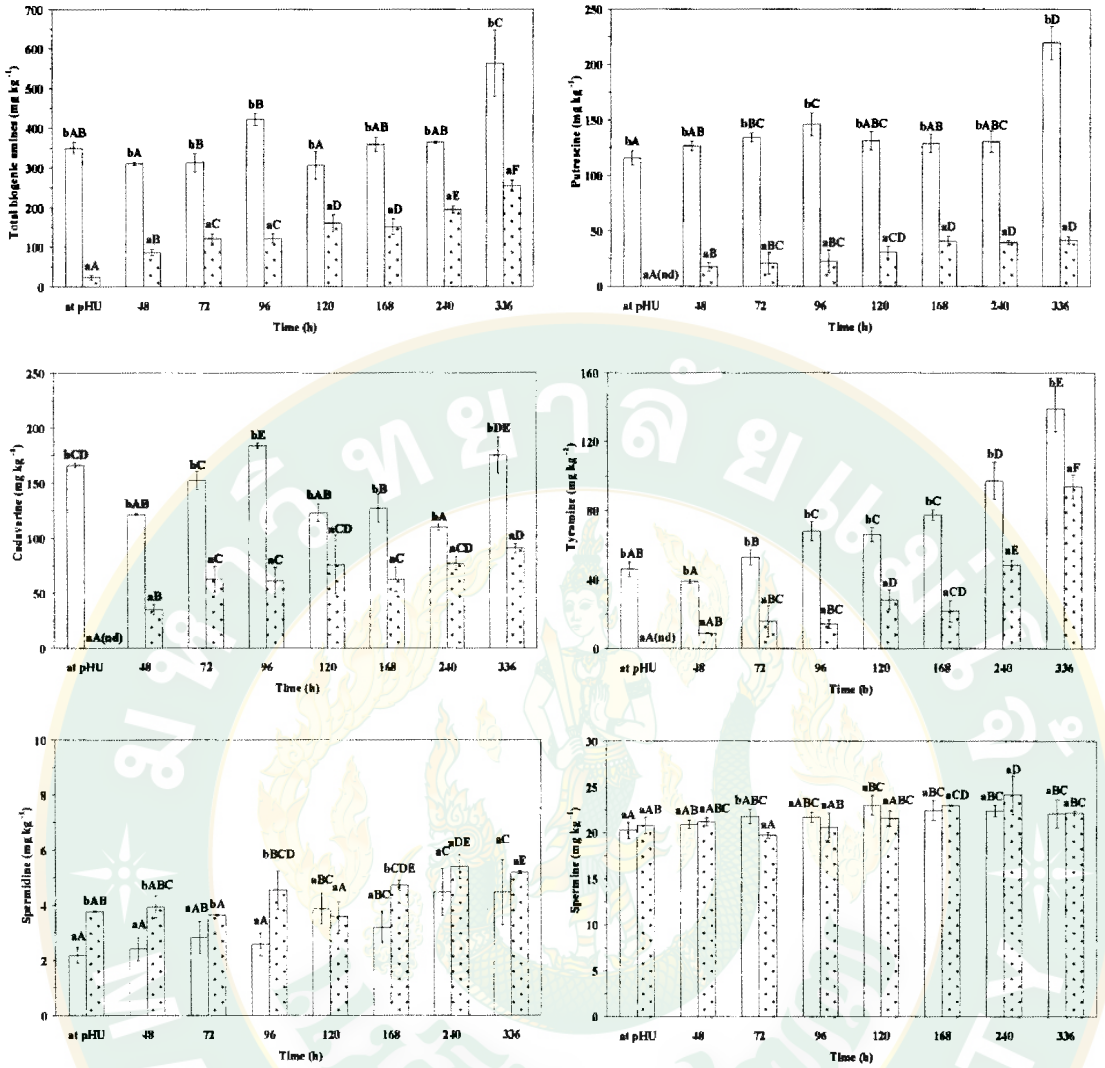

Figure 26 Changes in biogenic amines of Nham produced by standard formulation (□) and controlled fermentation strategy (⊡) during post-acidification. The small letters indicate significant differences between formulations at the same post-acidification time (P<0.05). The capital letters indicate significant differences within each formulation (P<0.05).

sausages (González-Fernández et al., 2003; Maijala et al., 1993). In addition, the growth or decarboxylase activity of amines-produced microorganisms might be suppressed by antimicrobial components, such as allicin, in garlic. Mah et al. (2009) reported that the total biogenic amines production in Myoelchi-joet sample treated with garlic extract was reduced by 8.7%. Although, Nham produced by standard formulation had an excess amount of carbohydrate sources which

resulted in a decrease in pH below 4.6 but a large amount of biogenic amines was observed. It might be due to the slower pH drop when compared with Nham produced by controlled fermentation strategy. In this study, putrescine, cadaverine and tyramine were found to be the main biogenic amines in Nham. At the end of fermentation, putrescine, cadaverine and tyramine in Nham produced by controlled fermentation strategy were not detected. While Nham produced by standard formulation exhibited high amount of putrescine (115.59 mg kg^{-1}), cadaverine (166.23 mg kg^{-1}) and tyramine (45.96 mg kg^{-1}). These amines were the major biogenic amines formed in Nham and other fermented sausages (Limsuwan, 2004; Bover-Cid et al., 2001a; González-Fernández et al., 2003; Gençcelep et al., 2008). These amines showed the corresponding free amino acid (FAA) precursors such as ornitine, lysine and tyrosine (Virgili et al., 2007). When post-acidification period increased, Nham produced by standard formulation had greater extent in total biogenic amines production than controlled fermentation strategy ($P<0.05$). Similar to other studies, biogenic amines continuously increased during storage of fermented sausages (Limsuwan, 2004; Kurt and Zorba, 2009). The generation of biogenic amines during aging is influenced by proteolytic, decarboxylase enzyme activity for free amino acid and degradation to biogenic amines (Virgili et al., 2007). Putrescine in Nham produced by standard formulation showed a large increase at 336 h. Nham produced by standard formulation had higher amount of cadaverine than controlled fermentation strategy. Tyramine in Nham produced by standard formulation continually increased and had the highest content at 336 h of post-acidification. High levels of histamine, putrescine and cadaverine formed indicated the spoilage of fish and meat (Brink et al., 1990). The value of spermidine (2.19-5.40 mg kg^{-1}) was low in both formulations. Amount of spermine was slightly increased in both samples ($P<0.05$). Small changes in contents of these amines are usually found during sausage fermentation because these amines are naturally present in raw materials and they are not formed by microbial decarboxylation of amino acids (Hernandez-Jover et al., 1997).

5.5 Effect on weight loss, released water and expressible moisture

Nham produced by controlled fermentation strategy had lower weight loss and released water. Weight loss, released water and expressible moisture were continually increased when post-acidification proceeded (Table 14). Weight loss in meat products is mainly associated

with loss in water and water-holding capacity (WHC) of meat. Increasing amounts of released and expressible water are possibly responsible for an increase in weight loss. Increases in the amount of expressible water and released water were presumably caused by proteolysis and denaturation of Nham proteins during fermentation (Visessanguan et al., 2004). Proteolytic activity during sausage ripening can be mainly attributed to the endogenous proteases while bacteria seem to be a minor role during sausages ripening (Molly et al., 1997; Fernendez et al., 2000; Visessanguan et al., 2004). In this study, the same amount of ground meat was added into both formulations, thus endogenous proteases activity might not difference. Controlled fermentation strategy resulted in lower pH than standard formulation which could inhibit growth of proteolytic microorganisms. The results indicated that the main responsible factor for the development of released water and expressible moisture in Nham produced by standard formulation is the action of bacterial enzyme, which actively degrade oligopeptides to small peptides and free amino acids (Molly et al., 1997). In both formulations exhibited the increases in weight loss, released water and expressible moisture during post-acidification. Nham produced by controlled fermentation strategy gradually increased in weight loss whereas Nham produced by standard formulation had a great increase at 72 h of post-acidification. Nham produced by controlled fermentation strategy showed lesser extent of released water than standard formulation. Controlled fermentation strategy had no effect on expressible moisture of Nham. Nevertheless, both formulations showed the gradual increases in expressible moisture during post-acidification.

5.6 Effect on Nham color

L*, a* and b* values of Nham samples during post-acidification were depicted in Table 15. Nham produced by controlled fermentation strategy had no effect on lightness, redness and yellowness of Nham. No significant differences in L*, a* and b* values were observed between Nham produced by controlled fermentation strategy and standard formulation ($P>0.05$). An increase in post-acidification period had no effect on lightness, redness and yellowness of Nham.

Table 14 Effect of controlled fermentation strategy on weight loss[a], released water and expressible moisture of Nham during post-acidification

Time (h)	Weight loss (g/100g)		Released water (g/100g)		Expressible moisture (g/100g)	
	SF-5	IF-16	SF-5	IF-16	SF-5	IF-16
at pH_U	1.08 ± 0.88bA	0.14 ± 0.01aA	1.40 ± 0.11bA	0.71 ± 0.09aA	1.72 ± 0.19bBC	1.35 ± 0.12aA
48	1.30 ± 0.97bA	0.20 ± 0.00aABC	1.13 ± 0.43aA	0.80 ± 0.04aA	1.63 ± 0.04aAB	1.64 ± 0.20aBC
72	2.97 ± 0.26bB	0.28 ± 0.00aABC	1.25 ± 0.51aA	0.95 ± 0.23aA	1.46 ± 0.04aA	1.39 ± 0.19aAB
96	3.11 ± 0.29bB	0.40 ± 0.10aBCD	1.45 ± 0.58aA	1.60 ± 0.17aB	1.74 ± 0.20aBC	1.67 ± 0.08aC
120	3.17 ± 0.29bB	0.48 ± 0.14aCDE	1.71 ± 0.69aA	1.82 ± 0.13aB	1.87 ± 0.02aCDE	1.78 ± 0.17aC
168	3.25 ± 0.28bB	0.61 ± 0.12aDEF	3.12 ± 0.42aB	3.07 ± 0.10aC	1.94 ± 0.06aDE	1.88 ± 0.18aC
240	3.29 ± 0.27bB	0.67 ± 0.11aEF	4.80 ± 0.37bC	3.80 ± 0.08aD	1.82 ± 0.07aBCD	1.90 ± 0.08aC
336	3.36 ± 0.27bB	0.73 ± 0.11aF	8.40 ± 0.02bD	4.58 ± 0.35aE	2.03 ± 0.02aE	1.90 ± 0.12aC

[a]Means ± SD from three determinations.

The small letters in the same row indicate significant difference ($P<0.05$).

The capital letters in the same column indicate significant difference ($P<0.05$).

Table 15 Effect of controlled fermentation strategy on color (L*, a*, b*) of Nham during post-acidification

Time (h)	L* (lightness)		a* (redness)		b* (yellowness)	
	SF-5	IF-16	SF-5	IF-16	SF-5	IF-16
at pH_U	56.24 ± 1.15aA	56.09 ± 1.01aA	8.00 ± 0.50aA	7.09 ± 0.76aA	5.32 ± 0.35aA	5.31 ± 0.50aA
48	54.97 ± 1.40aA	55.89 ± 0.86aA	7.82 ± 0.43aA	7.36 ± 0.73aA	5.69 ± 0.27aA	5.87 ± 0.56aA
72	56.20 ± 1.61aA	55.57 ± 0.39aA	7.66 ± 0.60aA	7.23 ± 0.67aA	5.74 ± 0.38aA	5.59 ± 0.46aA
96	54.86 ± 1.87aA	56.01 ± 1.14aA	7.97 ± 0.01bA	7.20 ± 0.32aA	5.15 ± 0.10aA	5.75 ± 0.41aA
120	56.32 ± 1.82aA	55.75 ± 1.24aA	7.53 ± 0.36aA	8.03 ± 0.30aA	5.05 ± 0.71aA	6.36 ± 0.56aA
168	55.13 ± 0.55aA	55.22 ± 1.33aA	7.36 ± 0.18aA	8.21 ± 0.71aA	5.33 ± 0.82aA	5.50 ± 0.48aA
240	56.12 ± 0.99aA	56.02 ± 0.53aA	8.81 ± 0.45aA	8.43 ± 0.33aA	5.03 ± 0.71aA	5.48 ± 0.36aA
336	53.31 ± 1.85aA	55.06 ± 2.61aA	8.01 ± 0.17aA	8.13 ± 0.43aA	4.85 ± 0.05aA	6.04 ± 0.60bA

[a]Means ± SD from three determinations.

The small letters in the same row indicate significant difference ($P<0.05$).

The capital letters in the same column indicate significant difference ($P<0.05$).

5.7 Effect on textural characteristics of Nham

Controlled fermentation strategy resulted in an increase in rigidity of Nham. However, both formulations became less rigid, adhesive, cohesive and elastic when post-acidification period increased (Figure 27). At the end of fermentation, Nham produced by controlled fermentation strategy exhibited higher hardness than standard formulation ($P<0.05$). A decrease in pH affected the conformational changes of muscle protein and their functional properties. It was most likely that acid-induced gelation of these proteins was mainly responsible for the formation of Nham texture (Visessanguan et al., 2006). Due to faster fermentation rate of Nham produced by controlled fermentation strategy, the fermentation rate might affect hardness of Nham. No significant differences in other TPA texture attributes were observed ($P>0.05$). Substantial decreases in all TPA texture attributes of both formulations were observed during post-acidification ($P<0.05$). This evidence was caused by endogenous protease in meat and proteolytic enzyme of bacteria.

5.8 Sensory evaluation

Controlled fermentation strategy had no effect on sensory scores (color, flavor, sourness, texture and overall acceptance) (Figure 28). No significant differences in sensory scores were observed during post acidification. Due to the pH and safety for consumption of Nham during post-acidification, Nham produced by standard formulation was served as uncooked from to 240 h, whereas Nham produced by controlled fermentation strategy was served from 36 h to 336 h. Nham samples were considered to be acceptable for human consumption until the overall acceptance score reached 5. Acceptability of Nham produced by controlled fermentation strategy was longer than standard formulation about 72 h or 3 days. Most of consumers gave the overall acceptance scores below 5 and rejected for consumption because they could detect putrid, unusual smell (off-flavor) and texture softened which were characterized to spoil of Nham.

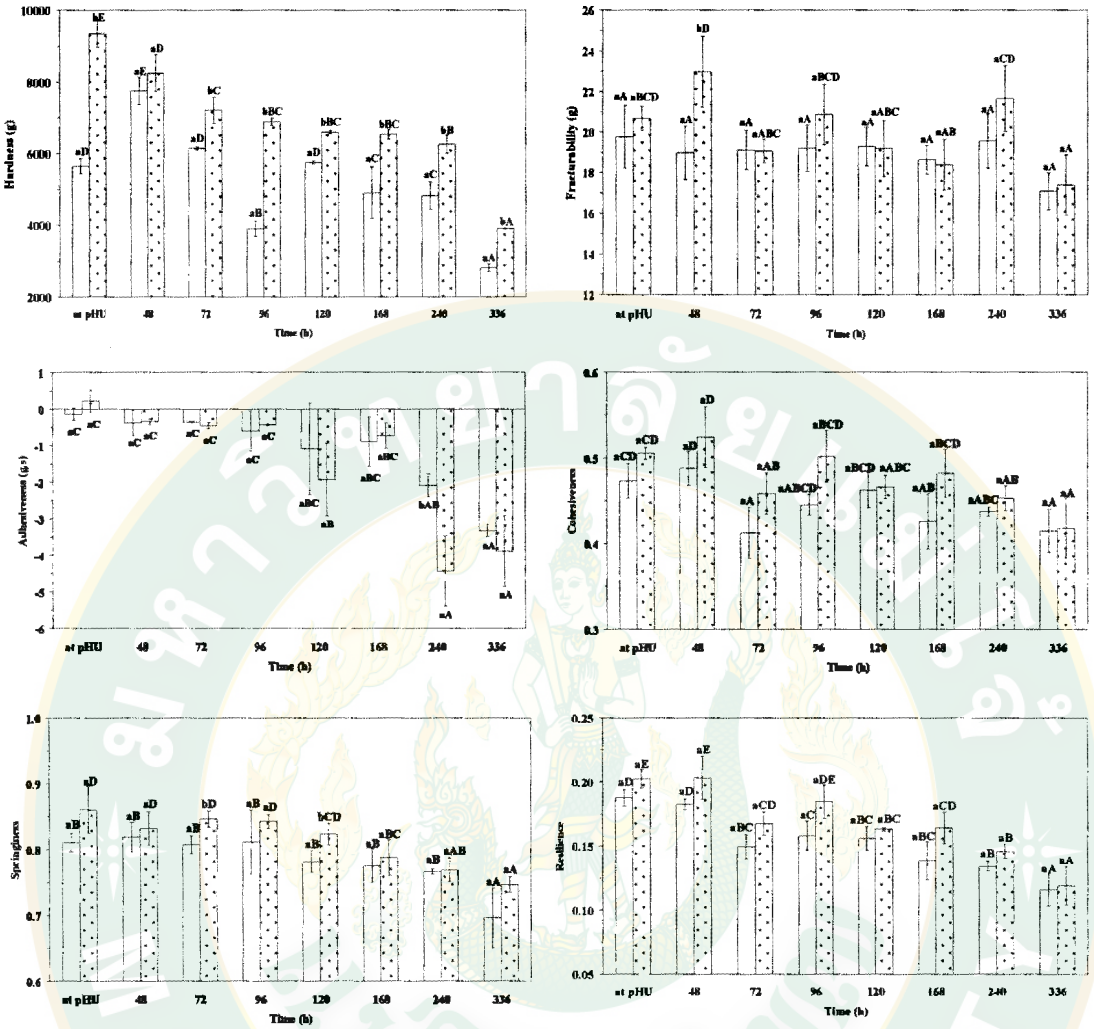

Figure 27 Changes in TPA texture attributes of Nham produced by standard formulation (□) and controlled fermentation strategy (⊡) during post-acidification. The small letters indicate significant differences between formulations at the same post-acidification time (P<0.05). The capital letters indicate significant differences within each formulation (P<0.05).

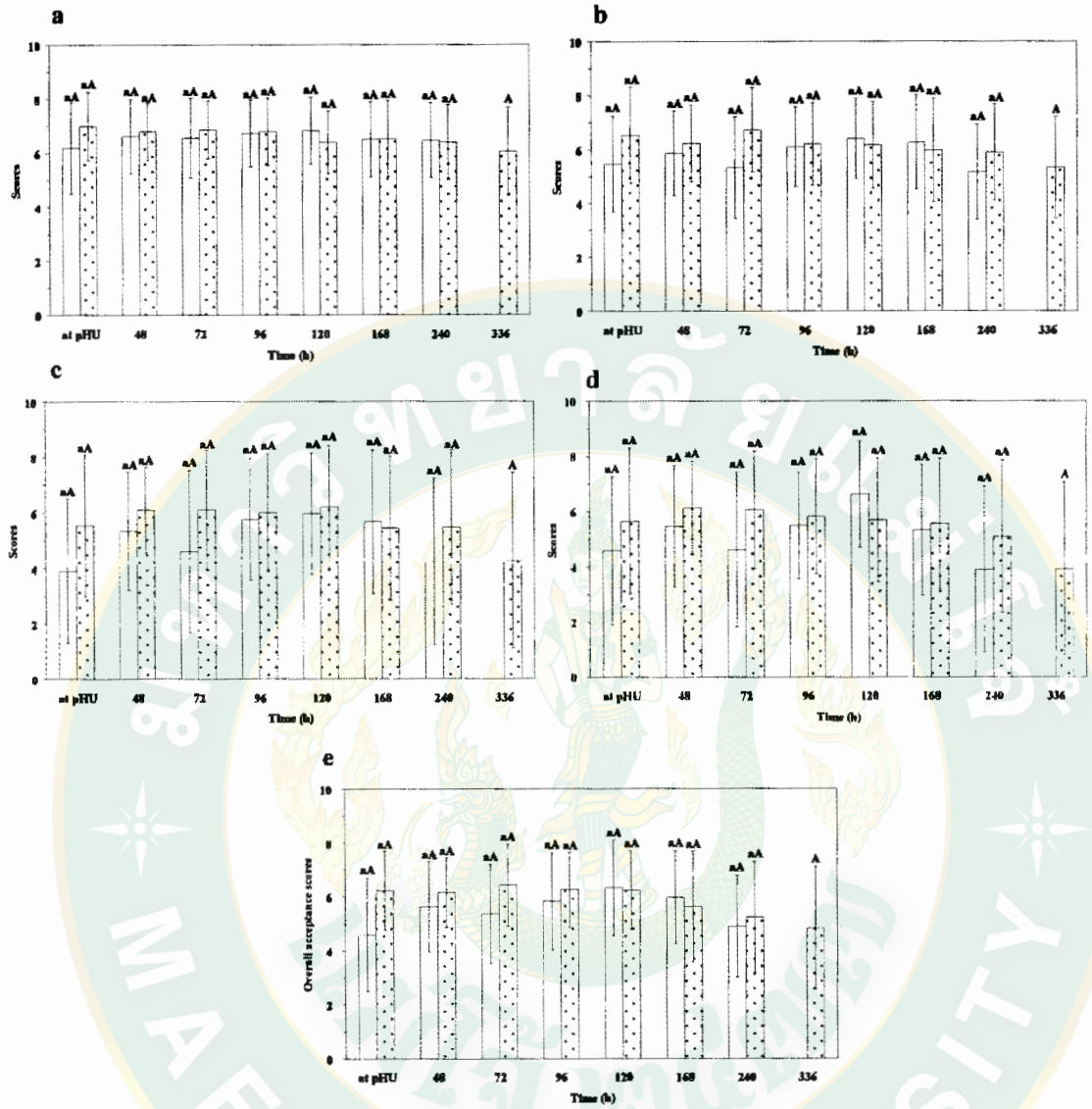

Figure 28 Changes in color (a), flavor (b), sourness (c), texture (d) and overall acceptance (e) scores of Nham produced by standard formulation (□) and controlled fermentation strategy (⊡).

CHAPTER 5

CONCLUSIONS

1. Glucose (G) and fructose (F) either in free or bound forms were the major sugars found in all Nham ingredients tested, except cooked pork rind (Table 3). Minced pork contributed to glucose which was mainly found in free form in the range between 0.8-1.4 mg g^{-1}. Garlic mainly contributed to fructose and glucose which also were mainly present in bound form. Total fructose (T_F) and total glucose (T_G) were in the ranges between 116 to 168 mg g^{-1} and 10 to 17 mg g^{-1}, respectively. Cooked rice contributed mainly glucose in bound form in the ranges between 266 to 314 mg g^{-1}.

2. Based on standard formulation commonly used in Nham processing, it could be estimated that glucose and fructose initially found in Nham were present in the bound form. In Nham, approximately 82% of T_G and 78% of T_F were derived from cooked rice and garlic, respectively. While simple sugars including glucose, fructose, and sucrose were present only 4%, 0.3% and 17%, respectively. The results showed that cooked rice and garlic were the important source of fermentable sugars for lactic acid production by which these complex carbohydrates must be degraded to more simple sugars before uptake and utilized by the cells.

3. The average amount of total glucose and total fructose (T_{G+F}) utilized from 6 batches of Nham that inoculated with *L. plantarum* BCC9546 after fermentation at 30 °C to pH 4.6 was estimated to be 12.70 ± 2.40 g kg^{-1}. Based on the amount of T_{G+F} utilized in Nham, only 61-62 % of the T_G and T_F were utilized in which the excess glucose and fructose were in the bound forms. Through post-acidification period, the continuous decreases in pH were typically observed in Nham processed with standard formulation. The pH of Nham could be lowered to 4.2 or lower during prolonged incubation at 30 °C up to 168 h. To minimize pH changes and excessive lactic acid production during post-acidification, T_G and T_F were limited and varied at different levels in which the T_{G+F} in all formulations was controlled at 12.70±2.40 g kg^{-1}.

4. Varying levels of cooked rice and garlic added affected not only on the initial amounts of total glucose and total fructose present in Nham but also the utilization of these substrates during fermentation. Compared to Nham prepared with standard formulation (SF), limitation of carbohydrate substrates by varying amount of cooked rice and garlic resulted in lower initial amounts of T_G, T_F, and T_{G+F}. An increase in amount of cooked rice added resulted in Nham with high T_G, whereas an increase in amount of garlic added resulted in Nham with high T_F. Concerning the sugar utilization, limitation of T_{G+F} resulted in higher percentage utilization of T_{G+F}. However, the extent of utilized T_G and T_F varied largely depending on the initial amount of T_G and T_F. The results indicated that T_F was more preferred substrate in fermentation of Nham inoculated with *L. plantarum* BCC9546.

5. Garlic plays a major role as carbohydrate source for LAB in Nham, specially that inoculated with *Lactobacillus plantarum* BCC9546. Using cooked rice and garlic at appropriate ratio and level could minimize pH changes and excessive lactic acid production during post-acidification. The amount of garlic added might be more important than cooked rice to determine whether the fermentation would succeed to pH 4.6 or fail. Compared to Nham prepared with the standard formulation in which carbohydrate sources were not limited, Nham with varying levels of cooked rice and garlic exhibited much lesser changes in post-acidification pH. The optimal initial T_F should be about 8 g kg^{-1}. Nham containing high T_F or high amount of garlic had higher production of lactic acid. In agreement with the ultimate pH values, to have pH_U below 4.6 the content of lactic acid formed in Nham should exceed 2.5 g%. Decrease in garlic proportion resulted in higher production of tyramine. These results showed that biogenic amines production in Nham depended on T_{G+F}, garlic content and rate of pH drop.

6. Incorporating varying levels of sucrose had no effect on biogenic amines formation. Due to the insufficient initial T_F and failed fermentation, all formulations of Nham contained high amount of total biogenic amines. Therefore, to accurate estimation of sugar in Nham in controlled fermentation strategy, the amount of garlic added should be adjusted based on the actual T_F in garlic added.

7. Omission of cooked rice resulted in lower amount of T_{G+F} and T_G. With lower amount of T_G, T_G was exhausted during fermentation of Nham. The omission of cooked rice resulted in lower production of lactic acid and higher biogenic amines production. Therefore, the fermentation was considered to be failed. Although T_F was sufficient to complete fermentation but it could not decrease pH lower than 4.6. Therefore, cooked rice is assumed to be another essential carbohydrate substrate for fermentation by LAB which has to be added when produced Nham with controlled fermentation strategy. From this study, the optimal initial T_G was about 3 g kg^{-1}.

8. To simplify and understand the correlation structure among chemical parameters, obtained data were analyzed by factor analysis by principle component analysis (PCA). The data consisted of 17 variables measured in 21 Nham formulations. Seventeen variables explained differences in pH_U and changes in pH_{PA} of Nham during post-acidification were reduced into 2 components (PC1 and PC2) with the total variance explained of 63.1%. PC1 discriminated Nham successfully fermented ($pH_U \leq 4.6$) (positive scores) from those failed fermentation ($pH_U > 4.6$) (negative scores) by higher amount of garlic added, T_F, free fructose, lactic acid, and lower pH_U. The PC2 discriminated Nham produced by using standard formulation (SF, positive scores) from those processed with improved formulations with carbohydrate limitation strategies (IF, negative scores). Nham produced by standard formulation was characterized by higher T_{G+F}, T_G, cooked rice, bound glucose, and lower pH_U and pH_{PA}. The results indicated that high amount of cooked rice in Nham produced by standard formulation which contributed to bound glucose, had effect on the increases in total glucose and fructose and total glucose, resulting in lower pH_U and higher changes in pH_{PA}.

9. Nham produced by controlled fermentation strategy exhibited faster and lower pH_U than standard formulation (36 and 42 h, respectively). A rapid decrease in pH of Nham with controlled fermentation strategy during fermentation was possibly due to higher amount of garlic substrate and the ability to degrade inulin of *L. plantarum* BCC9546. During post-acidification period, Nham produced by controlled fermentation strategy gave lesser changes in post-acidification pH and still lower than 4.6.

10. Controlled fermentation strategy could reduce biogenic amines production and their accumulation in Nham. At the end of fermentation, Nham produced by controlled fermentation strategy exhibited lower biogenic amines than standard formulation. At the end of fermentation, putrescine, cadaverine and tyramine in Nham produced by controlled fermentation strategy were not detected. While Nham produced by standard formulation exhibited high amount of putrescine (115.59 mg/kg), cadaverine (166.23 mg/kg) and tyramine (45.96 mg/kg). When post-acidification period increased, Nham produced by standard formulation had greater extent in total biogenic amines production than controlled fermentation strategy.

11. Nham produced by controlled fermentation strategy had lower weight loss and released water but had no effect on expressible moisture, color properties of Nham during post-acidification. At the end of fermentation, Nham produced by controlled fermentation strategy exhibited higher hardness than standard formulation. Controlled fermentation strategy had no effect on sensory scores (color, flavor, sourness, texture and overall acceptance). Acceptability of Nham produced by controlled fermentation strategy was longer shelf-life than standard formulation about 72 h or 3 days.

BIBLIOGRAPHY

Ahn, D. U. and K. C. Nam. 2004. Effects of ascorbic acid and antioxidants on color, lipid oxidation and volatiles of irradiated ground beef. **Radiation Physics and Chemistry** 71: 149-154.

Axelsson L. 2004. Lactic acid bacteria: classification and physiology. In S. Salminen, A. von Wright and A. Ouwehand. (eds.). **Lactic acid bacteria: microbiological and functional aspects.** 3rd ed. New York: Marcel Dekker, Inc. 655 p.

Bacus, J. N. 1984. **Utilization of microorganisms in meat processing.** England: Research Studies Press, Ltd. 170 p.

Barringer, S. A., J. Abu-Ali and H. -J. Chung. 2005. Electrostatic powder coating of sodium erythorbate and GDL to improve color and decrease microbial counts on meat. **Innovative Food Science and Emerging Technologies** 6: 189-193.

Baumgartner S., T. G. Dax, W. Praznik and H. Falk. 2000. Characterisation of high-molecular weight fructan isolated from garlic (*Allium sativum* L.). **Carbohydrate Research** 328: 177-183.

Bearson, S., B. Bearson and J. Foster. 1997. Acid stress responses in enterobacteria. **FEMS Microbiology Letters** 147(2): 173-180.

Berge, P., P. Ertbjerg, L. M. Larsen, T. Astruc, X. Vignon and A. J. Møller. 2001. Tenderization of beef by lactic acid injected at different time post mortem. **Meat Science** 57: 347-357.

Bernbom, N., Y. -Y. Ng, C. Paludan-Müller and L. Gram. 2009. Survival and growth of *Salmonella* and *Vibrio* in Som-fak, a Thai low-salt garlic containing fermented fish product. **International Journal of Food Microbiology** 134: 223-229.

Bourne, M. C. 1976. Interpretation of force curves from instrumental texture measurements. In J. M. deMan, P. W. Voisey, V. F. Rasper and D. W. Stanley. (eds.). **Rheology and texture in food quality**. Westport: CT: AVI Publ. Co. 274 p.

Bover-Cid, S., M. Izquierdo-Pulido and M. C. Vidal-Carou. 1999. Effect of proteolytic starter cultures of *Staphylococcus* spp. on biogenic amine formation during the ripening of dry fermented sausages. **International Journal of Food Microbiology** 46: 95-104.

Bover-Cid, S., M. Hugas, M. Izquierdo-Pulido and M. C. Vidal-Carou. 2001a. Amino acid

decarboxylase activity of bacteria isolated from fermented pork sausages. **International Journal of Food Microbiology** 66: 185-189.

Bover-Cid, S., M. J. Miguélez-Arrizado and M. C. Vidal-Carou. 2001b. Biogenic amine accumulation in ripened sausages affected by the addition of sodium sulphite. **Meat Science** 59: 391-396.

Bover-Cid, S., M. Izquierdo-Pulido and M. C. Vidal-Carou. 2001c. Changes in biogenic amine and polyamine content in slightly fermented sausages manufactured with and without sugar. **Meat Science** 57: 215-221.

Bozkurt H. and O. Erkmen. 2002. Effect of starter cultures and additives on the quality of Turkish style sausage (sucuk). **Meat Science** 61: 149-156.

Brink, B. ten, C. Damink, H. M. L. J. Joosten and J. H. J. Huis in't Veld. 1990. Occurrence and formation of biologically active amines in foods. **International Journal of Food Microbiology** 11: 73-84.

Casaburi, A., R. D. Monaco, S. Cavella, F. Toldrá, D. Ercolini and F. Vallani. 2008. Proteolytic and lipolytic starter cultures and their effect on traditional fermented sausages ripening and sensory traits. **Food Microbiology** 25: 335-347.

Casiraghi, E., C. Alamprese and C. Pompei. 2007. Cooked ham classification on the basis of brine injection level and pork breeding country. **LWT** 40: 164-169.

Chaicherdsakul, T., W. Phoonsawat, and V. Plengvidhya. 2006. Analysis of chemical and microbiological changes during over-fermentation of Nham by using biochemical and molecular biology methods. **Assumption University Journal** 9: 217-221.

Chokesajjawatee, N., S. Pornaem, Y. -G. Zo, S. Khamdee, P. Luxanalil, S. Wanasen and R. Valyasevi. 2009. Incidence of *Staphylococcus aureus* and associated risk factors in Nham, a Thai fermented pork product. **Food Microbiology** 26: 547-551.

Claeys, E., S. De Smet, A. Balcaen, K. Raes and D. Demeyer. 2004. Quantification of fresh meat peptides by SDS-PAGE in relation to ageing time and taste intensity. **Meat Science** 67: 281-288.

Coppola, S., G. Mauriello, M. Aponte, G. Moschetti and F. Villani. 2000. Microbial succession during ripening of Naples-type salami, a Southern Italian fermented sausage. **Meat Science** 56: 321-329

Cocolin, L., P. Dolci, K. Rantsiou, R. Urso, C. Cantoni and G. Comi. 2009. Lactic acid bacteria ecology of three traditional fermented sausages produced in the North of Italy as determined by molecular method. **Meat Science** 82: 125-132.

Coïsson, J. D., C. Cerutti, F. Travaglia and M. Arlorio. 2004. Production of biogenic amines in "Salami Italiani alla cacciatore PDO". **Meat Science** 67: 343-349.

Corzo-Martínez, M., N. Corzo and M. Villamiel. 2007. Biological properties of onions and garlic. **Trends in Food Science and Technology** 18: 609-625.

Costa, P., L. C. Roseiro, V. Alves, C. R. Calkins, R. J. B. Bessa snd C. Santos. 2009. Nutrient and fiber type relationships in Barrosã muscle. **Livestock Science** 125: 50-59.

Costa-Corredor, A., X. Serra, J. Arnau and P. Gou. 2009. Reduction of NaCl content in restructured dry-cured hams: Post-resting temperature and drying level effects on physicochemical and sensory parameters. **Meat Science** In press, Corrected proof.

De Castro, A., A. Montaño, A. H. Sánchez. and L. Rejano. 1998. Lactic acid fermentation and storage of blanched garlic. **International Journal of Food Microboilogy** 39: 205-211.

De Fátima Silva Lopes, M., A. L. Leitão, M. Regalla, J. J. F. Marques, M. J. T. Carrondo and M. T. B. Crespo. 1999. Processing of extracellular lipase of *Lactobacillus plantarum* involvement of a metalloprotease. **FEMS Microbiology Letters** 00: 483-487.

De Fátima Silva Lopes, M., A. L. Leitão, M. Regalla, J. J. F. Marques, M. J. T. Carrondo and M. T. B. Crespo. 2002. Characterisation of a highly thermostable extracellular lipase from *Lactobacillus plantarum*. **International Journal of Food Microbiology** 76: 107-115.

De la Fuente, J., M. T. Diaz, I. Álvarez, M. A. Oliver, M. Font i Furnols, C. Sañudo, M. M. Campo, F. Montossi, G. R. Nute and V. Cañeque. 2009. Fatty acid and vitamin E composition of intramuscular fat in cattle rared in different production systems. **Meat Science** 82: 331-337.

De las Rivas B., C. Ruiz-Capillas, A. V. Carrascova, J. A. Curiel, F. Jerménez-Colmenero and R. Muñoz. 2008. Biogenic amines production by Gram-positive bacteria isolated from Spanish dry-cured "chorizo" sausage treated with high pressure and kept in chilled storage. **Meat Science** 80: 272-277.

Demeyer, D. J., P. Vandekerchkove and R. Mormans. 1979. Compounds determining pH in dry

sausage. **Meat Science** 3: 161-167.

Demeyer, D., M. Raemaekers, A. Rizzo, A. Holck, A. De Smedt, B. ten Brink, B. Hagen, C. Montel, E. Zanardi, , E. Murbrekk, F. Leroy, F. Vandendriessche, K. Lorentsen, K. Venema, L. Sunesen, L. Stahnke, L. De Vuyst, R. Talon, R. Chizzolini and S. Eerola. 2000. Control of bioflavor and safety in fermented sausages: first results of a European project. **Food Research International** 33: 171-180.

Dineen, N. M., J. P. Kerry, P. B. Lynch, D. J. Buckley, P. A. Morrissey and D. K. Arendt. 2000. Reduced nitrite levels and dietary α-tocopheryl acetate supplementation: effect on color and oxidative stability of cooked hams. **Meat Science** 55: 475-482.

Dušek, M., F. KvasniČka, L. Lukáškovš and J. Krátká. 2003. Isotachophoretic determination of added phosphate in meat products. **Meat Science** 65: 765-769.

Eerola, S., R. Hinkkanen, E. Lindfors and T. Hirvi. 1993. Liquid chromatographic determination of biogenic amines in dry sausages. **Journal of AOAC** International 76(3): 575-577.

Ferreira, M. M. C., M. A. Morgano, S. C. D. Queiroz and D. M. B. Mantovani. 2000. Relationships of the minerals and fatty acid contents in processed turkey meat products. **Food Chemistry** 69: 259-265.

Fernendez, M., J. A. Ordóñez, J. M. Bruna, B. Herranz and L. de la Hoz. 2000. Accelerated ripening of dry fermented sausages. **Trends in Food Science and Technology** 11: 201-209.

Fisher, S. 1987. Catabolite repression in *Bacillus subtilis* and *Streptomyces*. In: Reizer, J., A. Peterkofsky (eds.). **Sugar transport and metabolism in grampositive bacteria.** New York: John Wiley & Sons. 385 p.

Flores, J. and Toldrá, F. 1993. Curing. In R. Macrae, R. K. Robinson and M. J. Sadler (eds.). **Encyclopaedia of food science, food technology and nutrition.** New York: Academic Press Inc. 6000 p.

Flores, M., M. P. Gianelli, M. Peréz-Juan and F. Toldrá. 2007. Headspace concentration of selected dry-cured aroma compounds in model system as affected by curing agents. **Food Chemistry** 102: 488-493.

Funami, T., H. Yada. and Y. Nakao. 1998. Thermal and rheological properties of curdlan gel in minced pork gel. **Food Hydrocolloids** 12: 55-64.

Gençcelep, H., G. Kaban, M. I. Aksu, F. Öz and M. Kaya. 2008. Determination of biogenic amine in sucuk. **Food control** 19: 868-872.

Gençcelep, H., G. Kaban and M. Kaya. 2007. Effect of starter cultures and nitrite levels on formation of biogenic amines in sucuk. **Meat Science** 77: 424-430.

González-Fernández, C., E. M. Santos, I. Jaime and J. Rovira. 2003. Influence of starter cultures and sugar concentrations on biogenic amine contents in *chorizo* dry sausage. **Food Microbiology** 20: 275-284.

González-Fernández, C., E. M. Santos, J. Rovira and I. Jaime. 2006. The effect of sugar concentration and starter culture on instrumental and sensory textural properties of Chorizo-Spanish dry-cured sausage. **Meat Science** 74: 467-475.

Gonzalez, B. and V. Diez. 2002. The effect of nitrite and starter culture on microbiological quality of "Chorizo" – a Spanish dry cured sausage. **Meat Science** 60(3): 295-298.

Greco, M., R. Mazzette, E. P. L. de Santis, A. Corona and A. M. Cosseddu. 2005. Evolution and identification of lactic acid bacteria isolated during the ripening of Sardinian sausages. **Meat Science** 69: 733-739.

Hernández-Jover, T., M. Izquierdo-Pulido, M. T. Vecina-Nogués, A. Mariné-Font and M. C. Vidal-Carou. 1997a. Biogenic amine and polyamine contents in meat and meat products. **Journal of Agricultural and Food Chemistry** 45: 2098-2102.

Hernández-Jover, T., M. Izquierdo-Pulido, M. T. Vecina-Nogués, A. Mariné-Font and M. C. Vidal-Carou. 1997b. Effect of starter cultures on biogenic amine formation during fermented sausage production. **Journal of Food Protection** 60: 825-830.

Holzapfel, W. 1997. Use of starter cultures in fermentation on household scale. **Food control** 8: 241-258.

Honikel, K. -O. 2008. The use and control of nitrate and nitrite for the processing of meat products. **Meat Science** 78: 68-76.

Hugas, M. and Monfort, J. M. 1997. Bacterial starter cultures for meat fermentation. **Food chemistry** 59(4): 547-554.

Hughes, M. C., J. P. Kerryb, E. K. Arendtb, P. M. Kenneallyc, P. L. H. McSweeneya and E. E. O'Neilla. 2002. Characterisation of proteolysis during ripening of semi-dry fermented sausages. **Meat Science** 62: 205-216.

Ingolf, F. N. and R. Skjelkvale. 1982. Effect of natural spices and oleoresins on *Lactobacillus plantarum* in fermentation of dried sausage. **Journal of Food Science** 47: 1618-1621.

Kaban, G. 2009. Changes in the composition of volatile compounds and in microbiological and physicochemical parameters during Pastrima processing. **Meat Science** 82:17-23.

Khieokhachee, T., W. Praphailong, C. Chowvalitnitithum, S. Kunawasen, S. Kumphati, V. Chavasith, S. Bhumiratana, and R. Valyasevi. 1997. **Microbial interaction in the fermentation of Thai pork sausage.** pp. 312-318. Proceedings of the Sixth ASEAN Food Conference. Singapore.

Kongtong, K. 2008. **Comparison of microbial diversity during natural and starter culture Nham fermentation.** Master degree thesis. Kasetsart University. 161 p.

Khuanburi, P. 2005. **The role of ingredients on flavor profiles of Nham.** [Online]. Available http://www.stkc.go.th/stportalDocument/stportal_1116920587.doc. (18 June 2008).

Kröckel, L. 1996. Bacterial fermentation of meats. In G. Campbell-Platt and P.E. Cook, (eds.). **Fermented Meats.** London: Blackie Academic Professional. 242 p.

Kumar, M. and J. S. Berwal. 1998. Sensitivity of food pathogens to garlic (*Allium sativum*). **Appiled and Environmental Microbiology** 84: 213-215.

Kunawasen, S. 2000. **Molecular typing of lactic acid bacteria isolated during Nham fermentation.** Master degree thesis. Mahidol University. 162 p.

Kurt, S. and Ö. Zorba. 2009. Effect of ripening period, nitrite level and heat treatment on biogenic amine formation of "sucuk" –A Turkish dry fermented sausage. **Meat Science** 82: 179-184.

Kwasiborski, A., T. Sayd, C. Chambon, V. Santé-Lhoutellier, D. Rocha and C. Terlouw. 2008. *Pig Longissimus lumborum* proteome: Part II: Relationships between protein content and meat quality. **Meat Science** 80: 982-996.

Latorre-Moratalla, M. L., T. Veciana-Nogués, S. Bover-Cid, M. Garriga, T. Aymerich, E. Zanardi, A. Ianieri, M. J. Fraqueza, L. Patarata, E. H. Drosinos, A. Lauková, R. Talon and M. C. Vidal-Carou. 2008. Biogenic amines in dry fermented sausages produced in selected European countries. **Food Chemistry** 107: 912-921.

Laxananil, P., R. Promchai, S. Wanasen, S. Kamdee, P. Thepkasikul, V. Plengvidhya, W. Visessanguan and R. Valyasevi. 2009. Monitoring *Lactobacillus plantarum*

BCC9546 starter culture during fermentation of Nham, a traditional Thai pork sausage. **International Journal of Food Microbiology** 129: 312-315.

Lebert, I., S. Leroy, P. Giammarinaro, A. Lebert, J. P. Chacornac, S. Bover-Cid, M. C. Vidal-Carou and R. Talon. 2007. Diversity of micro-organisms in environments and dry fermented sausages of French traditional small units. **Meat Science** 76: 112-122.

Lee, C. H. 1997. Lactic acid fermented foods and their benefits in Asia. **Food Control** 8: 259-269.

Lee, S. H., R. G. Cassens and O. R. Fennema. 1976. Effect of muscle type on residual nitrite in cured meat. **Journal of Food Science** 41: 100-101.

Leroy, F. and L. D. Vuyst. 2004. Lactic acid bacteria as functional starter cultures for the food fermentation industry. **Trends in Food Science and Technology** 15: 67-78.

Limsuwan, S. 2004. **Effect of starter cultures and temperatures on evolution of biogenic amines in Nham during fermentation and storage.** Master degree thesis. King's Mongkut University of Technology Thonburi. 97 p.

Lin, K. -W. and S. -N. Lin. 2002. Effect of sodium lactate and trisodium phosphate on the physicochemical properties and shelf-life of low-fat Chinese-style sausage. **Meat Science** 60: 147-154.

Losso, J. N. and S. Nakai. 1997. Molecular size of garlic fructooligosaccharides and fructopolysaccharides by Matrix-Assisted Laser Desorption Ionization Mass Spectrometry. **Journal of Agricultural and Food Chemistry** 45(11): 4342-4346.

Mah, J. -H., Y. J. Kim and H. -J. Hwang. 2009. Inhibitory effect of garlic and other spices on biogenic amine production in *Myeolchi-joet*, Korean salted and fermented anchovy product. **Food control** 20: 449-454.

Maijala, R. L. and S. Eerola. 1993. Contaminant lactic acid bacteria of dry sausages produce histamine and tyramine. **Meat Science** 35: 387-395.

Maijala, R., S. Eerola, M. Aho, J. Hirn. 1993. The effect of GDL induced pH decrease on the formation of biogenic amines in meat. **Journal of Food Protection** 56: 125-129.

Malinowski, E. R (ed.). 1991. **Factor analysis in chemistry** 2nd ed. New York: John Wiley. 368 p.

Mancini, R. A., M. C. Hunt, M. Seyfert, D. H. Kropf, K. A. Hachmeister, T. J. Herald and

D. E. Johnson. 2007. Effect of ascorbic and citric acid on beef lumbar vertebrae marrow color. **Meat Science** 76: 568-573.

Masniyom, P., S. Benjakul and W. Visessanguan. 2005. Combination effect of phosphate and modified atmosphere on quality and shelf-life extension of refrigerated seabass slices. **LWT** 38: 745-756.

Masson, F., R. Talon and M.C. Montel. 1996. Histamine and tyramnie production by bacteria from meat products. **International Journal of Food Microbiology** 32: 199-207.

Mataragus, M., P. Skandamis, G. J. E. Nychas and E. H. Drosinos. 2007. Modeling and predicting spoilage of cooked, cured meat products by multivariate analysis. **Meat Science** 77: 348-356.

McCabe-Sellers, B., C. G. Staggs and M. L. Bogle. 2006. Tyramine in Foods and monoamine oxidase inhibitor drugs: a crossroad where medicine, nutrition, pharmacy and food industry converge. **Journal of Food Composition and Analysis** 19: S58-S65.

Molenaar, D., J. S. Bosscher, B. ten Brink, A.J.M. Driessen and W.N. Konings. 1993. Generation of a proton motive force by histidine decarboxylation and electrogenic histidine/histamine antiport in *Lactobacillus buchneri*. **Journal of Bacteriology** 175(10): 2864-2870.

Molly, K., D. Demeyer, G. Johansson, M. Raemaeker, M. Ghistelinck and I. Geenen. 1997. The importance of meat enzymes in ripening and flavor generation in dry fermented sausages. **Food chemistry** 59: 539-545.

Molly, K., D. Demeyer, T. Civera and A. Verplaetse. 1996. Lipolysis in a Belgian sausage: relative importance of endogenous and bacterial enzymes. **Meat Science** 43: 235-244.

Montel, M.C., F. Masson and R. Talon. 1999. Comparison of biogenic amine content in traditional and industrial French dry sausages. **Sciences des Aliments** 19: 247-254.

Mukerjea, R., G. Slocum and J. F. Robyt. 2007. Determination of the maximum water solubility of eight native starches and solubility of their acidic-methanol or -ethanol modified analogues. **Carbohydrate Research** 342: 103-110.

Nakao, Y., Konno, A., Taguchi, T., Tawada, T., Kasai, H., Toda, J. and Terasaki, M. 1991. Curdlan: properties and application to food. **Journal of Food Science** 33: 42-46.

Neves, A. R., W. A. Pool, J. Kok, O. P. Kuipers and H. Santos. 2005. Overview on sugar

metabolism and its control in *Lactococcus lactis*-The input from in vivo NMR. **FEMS Microbiology Review** 29: 531-554.

Olesen, P. T., A. S. Meyer and L. H. Stahnke. 2004. Generation of flavor compounds in fermenred sausages-the influence of curing ingredients, *Staphylococcus* starter culture and ripening time. **Meat Science** 66: 675-687.

Ongol, M. P., Y. Sawatari, Y. Ebina, T. Sone, M. Tanaka, F. Tomita, A. Yokota and K. Asano. 2007. Yoghurt fermented by *Lactobacillus delbrueckii* subsp. *Bulgaricus* H^+ ATPase-defective mutants exhibits enhanced viability of *Bifidobacterium breve* during storage. **International Journal of Food Microboilogy** 116: 358-366.

Olympia, M., H. Fukuda, H. Ono, Y. Kaneko, and M. Takano. 1995. Characterization of starch-hydrolysing lactic acid bacteria isolated from fermented fish and rice food "Burong isda", and its amylolytic enzyme. **Journal of Fermentation and Bioengineering** 80(2): 124-130.

Opaswatcharanon, U. 2004. **Development of simulated Nham broth to study growth and lactic acid production of *Lactobacillus sakei* in culture Nham fermentation.** Master degree thesis. Mahidol University. 112 p.

Owens, J. D. and L. S. Mendoza. 1985. Enzymatically hydrolysed and bacterially fermented fish products. **Journal of Food Technology** 20: 273-293.

Østergaard, A., P. K. Ben Embarek, J. Yamprayoon, C. Wedel-Neer gaard, H. H. Huss and L. Gram. 1998. Fermentation and spoilage of Som-fak, a Thai low-salt fish product. **Tropical Science** 38: 105–112.

Özogul, F. and Y. Özogul. 2006. Biogenic amine content and biogenic amine indices of sardines (*Sardina pilchardus*) stored in modified atmosphere packaging and vacuum packaging. **Food Chemistry** 99: 574-578.

Paludan-Müller, C., H. H. Huss, and L. Gram. 1999. Characterization of Thai low-salt fermented fish product and the role of garlic as substrate for fermentation. **International Journal of Food Microboilogy** 46: 219-229.

Paludan-Müller, C., M. Madsen, P. Sophanodora, L. Gram and P. L. Møller. 2002a. Fermentation and microflora of Plaa-som, a Thai fermented fish product prepared with different salt concentrations. **International Journal of Food Microbiology** 73: 61-70.

Paludan-Müller, C., R. Valyasevi, H. H. Huss and L. Gram. 2002b. Genotypic and phenotypic characterization of garlic-fermenting lactic acid bacteria isolated from Som-fak, a Thai low-salt fermented fish product. **Journal of Applied Microbiology** 92: 307-314.

Papamanoli, E., N. Tzanetakis, E. Litopoulou-Tzanetaki and P. Kotzekidou. 2003. Characterization of lactic acid bacteria isolated from a Greek dry fermented sausage in respect of their technological and probiotic properties. **Meat Science** 65: 859-867.

Pastan, I. and S. Adhya. 1976. Cyclic adenosine 5'-monophosphate in *Escherichia coli*. **Bacteriology Reviews** 40: 527-551.

Passos, F. V., H. P. Fleming, D. F. Ollis, R. M. Felder and R. F. McFeeters. 1994. Kinetics and modeling of lactic acid production by *Lactobacillus plantarum*. **Applied Environmental Microbiology** 60(7): 2627-2636.

Patarata, L., I. Judas, J. A. Silva, A. Esteves and C. Martin. 2008. A comparison of the physicochemical and sensory characteristics of *alheira* samples from different-sized producers. **Meat Science** 79: 131-138.

Paukatong, K.V. and S. Kunawasen. 2001. The Hazard Analysis and Critical Control Points (HACCP) Generic Model for the Production of Thai Pork Sausage (Nham). **Berl. Münch. Tierärzlt. Wschr** 114: 1-4.

Pearson, A. M. and T. A. Gillett. 1996. Casings, extenders, and additives. In A. M. Pearson and T. A. Gillett (eds.). **Processed meats.** New York: Chapman & Hall. 448 p.

Pereira, C. I., M. T. Barreto Crespo and M. V. San Romão. 2001. Evidence for proteolytic activity and biogenic amines production in *Lactobacillus curvatus* and *Lactobacillus homohiochii*. **International Journal of Food Microbiology** 68: 211-216.

Perez-Juan, M., M. Flores and F. Toldrá. 2007. Binding of aroma compounds by isolated myofibrillar proteins: Effect of protein concentration and conformation. **Food Chemistry** 105: 932-939.

Perez-Juan, M., M. Flores and F. Toldrá. 2008. Effect of pork meat proteins on the binding of volatile compounds. **Food Chemistry** 108: 1226-1233.

Petchsing, U., M. J. Woodburn. 1990. *Staphylococcus aureus* and *Escherichia coli* in Nham (Thai-style fermented pork sausage). **International Journal of Food Microbiology** 10: 183-192.

Phithakpol, B., W. Varanyanond, S. Reungmaneepaitoon and H. Wood. 1995. **The traditional fermented food of Thailand.** Institute of Food Research and Product Development, Kasetsart University, Thailand.

Pighin, D. G., A. M. Sancho and C. B. Gonzalez. 2008. Effect of salt addition on the thermal behavior of proteins of bovine meat from Agentina. **Meat Science** 79: 549-556.

Plumed-Ferrer, C., K. M. Koistinen, T. L. Tolonen, S. J. Lehesranta, S. O. Kälenlampi, E. Mäkimattila, V. Joutsjoki, V. Vertanen and A. von Wright. 2008. Comparative study of sugar fermentation and protein expression patterns of two *Lactobacillus plantarum* strains grown in three different media. **Applied and Environmental Microbiology** 74(17): 5349-5358

Pohlman, F. W., M. R. Stivarius, K. S. McElyea, Z. B. Johnson and A. L. Waldroup. 2002. Reduction of *E. coli*, *Salmonella typhimirium*, coliforms, aerobic bacteria and improvement of ground beef color using trisodium phosphate or cetylpyridinium chloride before grinding. **Meat Science** 60: 349-356.

Pösö, A. R. and E. Puolanne. 2005. Carbohydrate metabolism in meat animals. **Meat Science.** 70: 423-434.

Postma P. W and W. Lengeler. 1985. Phosphoenolpyruvate:carbohydrate phosphotransferase system of bacteria. **Microbiology and Molecular Biology Reviews** 49: 232-269.

Prescott, S. C. and C. G. Dunn. 1959. **Industrial Microbiology.** 3rd ed. New York: Mc. Graw Hill Book Co. 541 p.

Price, J. F. and B. S. Schweigert. 1973. **The Science of Meat and Meat products.** 2nd ed. San Francisco: W. H. Freeman & Co. 639 p.

Qiao, J., M. O. Ngadi, N. Wang, C. Gariépy and S. O. Prasher. 2007. Pork quality and marbling level assessment using a hyperspectral imaging system. **Journal of Food Engineering** 83: 10-16.

Rantsiou, K., E. H. Drosinos, M. Gialitaki, R. Urso, J. Krommer, J. Gasparik-Reichardt, S. Tóth, I. Metaxopoulos, G. Comi and L. Cocolin. 2005. Molecular characterization of *Lactobacillus* species isolated from naturally fermented sausages produced in Greece, Hungary and Italy. **Food Microbiology** 22: 19-28.

Rashchi, F. and J. A. Finch. 2000. Polyphosphate: a review their chemistry and application with

particular reference to mineral processing. **Mineral Engineering** 13(10): 1019-1035.

Reddy, G., Altaf, Md., Naveena B. J., Venkateshwar, M. and Kumar, E. V. 2008. Amylolytic bacterial lactic acid fermentation - A review. **Biotechnology Advances** 26: 22-34.

Riebroy, S., S. Benjakul, W. Visessanguan, M. Tanaka, U. Erikson and T. Rustad. 2007. Effect of irradiation on properties and storage stability of Som-fak produced from bigeye snapper. **Food Chemistry** 103(2): 274-286.

Romano, C.H., J.D. Trifone and M. Brustolon. 1979. Distribution of the phosphoenolpyruvate: glucose phosphotransferase system in fermentative bacteria. **Journal of Bacteriology** 139: 93-97.

Roseiro, L. C., C. Santos, M. Sol, M. J. Borges, M. Anjos, H. Gonçalve and A. S. Carvalho. 2008. Proteolysis in Painho de Portalegre dry fermented sausage in relation to ripening time and salt content. **Meat Science** 79: 784-794.

Ruiz Pérez-Chacho, M. P., H. Galán-Sodevilla, F. Léon-Crespo and G. Molina recio. 2005. Determination of the sensory attributes of a Spanish dry-cured sausage. **Meat Science** 71: 620-633.

Sahoo, J. and A. S. R. Anjaneyulu. 1997. Quality improvement of ground buffalo meat by preblending with sodium ascorbate. **Meat Science** 46(3): 237-247.

Saisithi, P., P. Yongmanitchai, P. Chimanage, C. Wongkhaluang, Boonyaratanakornkit, Maleehuan, S. 1986. **Improvement of a Thai traditional fermented fish product: Som-fug.** Institute of Food Research and Product Development, Kasertsart University, Bangkok, FAO report.

Sallam, Kh. I., M. Ishioroshi and K. Samejima. 2004. Antioxidant and antimicrobial effects of garlic in chicken sausage. **Lebensm.-Wiss. u. Technol** 37: 849-855.

Sánchez-Escalante, A., D. Djenane, G. Torrescano, J. A. Beltrán and P. Roncaléz. 2001. The effects of ascorbic acid, taurine, carnisine and rosemary powder on color and lipid stability of beef patties packaged in modified atmosphere. **Meat Science** 58: 421-429.

Schaefer, D. M., Q. Liu, C. Faustman and M. C. Yin. 1995. Supranutritional administration of vitamins E and C improves oxidative stability of beef. **Journal of Nutrition** 125: 1792S-1798S.

Scheffler, T. L. and D. E. Gerrard. 2007. Mechanisms controlling pork quality development: The

biochemistry controlling postmortem energy metabolism. **Meat Science** 77: 7-16.

Shalaby, A. R. 1996. Significance of biogenic amines to food safety and human health. **Food Research International** 29(7): 675-690.

Sharaf, M. A., D. L. Illman, and B. R. Kowalski. 1986. **Chemometrics.** New York: John Wiley. 352 p.

Sharma, A. D., S. Kainth and P. K. Gill. 2006. Inulinase production using garlic (*Allium sativum*) powder as a potential substrate in *Streptomyces* spp. **Journal of Food engineering** 77: 486-491.

Sheard, P. R., G. R. Nute, R. I. Richardson, A. Perry and A. A. Taylor. 1999. Injection of water and polyphosphate into pork improve juiciness and tenderness after cooking. **Meat Science** 51: 371-376.

Silla Santos, M. H. 1996. Biogenic amines: their importance in food. **International Journal of Food Microbiology** 29: 213-231.

Sofos, J. N. 1986. Use of phosphate in low-sodium meat products. **Food Technology** 40(3): 52-69.

Somboonpanyakul, P., S. Barbut, P. Jantawat and N. Chinprahast. 2007. Textural and sensory quality of poultry meat batter containing malva nut gum, salt and phosphate. **LWT** 80: 498-505.

Spaziani, M., M. D. Torre and M. L. Stecchini. 2009. Changes in physicochemical, microbiological and textural properties during ripening of Italian low-acid sausages. Proteolysis, sensory and volatile profiles. **Meat Science** 81: 77-85.

Suman, S. P., C. Faustman, S. Lee, J. Tang, H. A. Sepe, P. Vasudevan, T. Annamalai, M. Manojkumar, P. Marek and K.S. Venkitanarayanan. 2005. Effect of erythorbate, storage and high-oxygen packaging on premature browning in ground beef. **Meat Science** 69: 363-369.

Suzzi, G. and F. Gardini. 2003. Biogenic amines in dry fermented sausages: a review. **International Journal of Food Microbiology.** 88: 41-54.

Swetwiwathana, A., U. Leutz and A. Fischer. 1999. Role of garlic on growth and lactic acid production of starter cultures. **Fleischwirtschaft International** 1(99): 124-128.

Takahashi, M. and S. Soutome. 1975. On the properties of β-fructofuranosidase from

Lactobacillus plantarum. **Bulletin College Agricultural Utsunomiya University** 9: 89-95.

Tanasupawat, S. and K. Komagata. 1995. Lactic acid bacteria in fermented foods in Thailand. **World Journal of Microbiology and Biotechnology** 11(3): 253-256.

Thompson J. 1987. Sugar transport in the lactic acid bacteria. In Reizer J. and A. Peterkofsky. (eds.). **Sugar transport and metabolism in gram-positive bacteria.** New York: John Wiley & Sons. 796 p.

Thompson, J. and T. D. Thomas. 1977. Phosphoenolpyruvate and 2-phosphoglycerate: endogenous energy source(s) for sugar accumulation by starved cells of *Streptococcus lactis*. **Journal of Bacteriology** 130: 583-595.

Toldrá, F., and M. Flores. 1998. The role of muscle proteases and lipases in flavour development during the processing of dry-cured ham. **Critical Reviews in Food Science and Nutrition** 38: 331-352.

Valyasevi, R., P. Jungsiriwat, T. Smitinont, W. Praphailong and C. Chowalitnitithum. 2001. Improvement of starter culture for Nham fermentation. In **Final report submitted to National Center for the Genetic Engineering and Biotechnology.** National Science and Technology Development Agency.

Valyasevi, R. and R. S. Rolle, 2002. An overview of small-scale food fermentation technologies in developing countries with special reference to Thailand: scope for their improvement. **International Journal of Food Microbiology** 75: 231-239.

Valyasevi, R., T. Smitinont, W. Praphailog, C. Chowalitnitithum, S. Kunawasen, V. Chavasith. 1999. **The microbiology and development of starter culture for Nham, the traditional Thai pork sausage.** pp. 709-711. Proceedings of the Seventeenth International Conference of the International Committee on Food Microbiology and Hygience (ICFMH). Veldhoven, Netherlands.

Valyasevi, R., T. Smitinont, W. Visessanguan, S. Lertsiri, A. Tongta, M. Nopharatana, P. Thepkasikul, C. Kittikun, M. Rattanaprasert. 2003. The fermentation rate and production of metabolite compounds during the fermentation of cultured Nham. In **Final report submitted to National Center for Genetic Engineering and Biotechnology.** National Science and Technology Development Agency.

Vidal-Carou, M. C., M. Izquierdo-Pulido, M. C. Martín-Morro and Marine-Font, A. 1990. Histamine and tyramine in meat products: Relationship with meat spoilage. **Food Chemistry** 37: 239-249.

Vinci, G. and M. L. Antonelli. 2002. Biogenic amines: quality index of freshness in red and white meat. **Food Control** 13: 519-524.

Virgili, R., G. Saccani, L. Gabba, E. Tanzi and C. S. Bordini. 2007. Changes of free amino acids on biogenic amines during extented aging of Italian dry cured ham. **LWT** 40: 871-878.

Visessanguan, W., S. Benjakul, A. Panya, C. Kittikun and A. Assavanig. 2005. Influence pork and rind ratios on physico-chemical and sensory quality of Nham –A Thai fermented pork sausage. **Meat Science** 69: 355-362.

Visessanguan, W., S. Benjakul, S. Riebroy and P. Thepkasikul. 2004. Changes in composition and functional properties of proteins and their contributions to Nham characteristics. **Meat Science** 66: 579-588.

Visessanguan, W., S. Benjakul, T. Smitinont, C. Kittikun, P. Thepkasikul and A. Panya. 2006. Changes in microbiology, biochemical and physico-chemical properties of Nham inoculated with different inoculum levels of *Lactobacillus cuvatus*. **LWT** 39: 814-826.

Wager, M. K. and F. F. Busta. 1985. Inhibition of *Clostridium botulimun* 52A toxicity and protease activity by sodium acid pyrophosphate in meat systems. **Applied and Environment Microbiology** 50: 16-20.

Warner, R. D., R. G. Kauffman and M. L. Greaser. 1997. Muscle protein changes post-mortem in relation to pork quality traits. **Meat Science** 45: 339-352.

Watts, B. M. and B. T. Lehmann. 1952. Ascorbic acid and meat color. **Food Technology** 6: 194-198.

Wiriyacharee, P., L. Rujanakraikarn, P. Vibulseth, S. Sitthipojna, A. Hunpongkittikul, S. Chusangjan. 1990. The development of Nham formulation and controlled process made with mixed bacterial starter cultures. In **Final report submitted to National Center for the Genetic Engineering and Biotechnology.** National Science and Technology Development Agency.

Wood, B. J. B. (ed.). 1985. **Microbiology of Fermented Foods.** England: Elsevier Applied Science Publisher Ltd. 292 p.

Yan, P. -M., W. -T. Xue, S. -S. Tan, H. Zhang and X. -H. Chang. 2008. Effect of inoculating lactic acid bacteria starter cultures on the nitrite concentration of fermenting Chinese paocai. **Food Control** 19: 50-55.

Yang, G. C., P. M. Yasaei and S. W. Page. 1993. Garlic as anti-oxidants and free radical scavenges. **Journal of Food and Drug Analysis** 1(4): 337-364.

ไพโรจน์ วิริยจารี, ลักขณา รุจนะไกรกานต์, ปานจิตต์ ชะวลี, อิศรพงษ์ สิริกุล, วิวรรธน์ วรรธนัจฉริยา และ สุธยา บุญถนอม. 2537. น้ำตาลที่เหมาะสมต่อการผลิตแหนมโดยใช้เทคโนโลยีเชื้อบริสุทธิ์เริ่มต้นผสม. **วารสารเกษตร** 10(1): 92-102.

ไพโรจน์ วิริยจารี, ลักขณา รุจนะไกรกานต์ และ ปานจิตต์ ชะวลี. 2536. การพัฒนาผลิตภัณฑ์แหนมโดยการใช้เทคโนโลยีเชื้อบริสุทธิ์เริ่มต้นผสม 2. ผลของข้าวเจ้าและข้าวเหนียวต่อการผลิตกรดแลคติกในผลิตภัณฑ์. **วารสารเกษตร** 9(1): 61-74.

สมบุญ เตชะภิญญาวัตน์. 2518. **การศึกษาจุลินทรีย์ที่เป็นตัวการในระหว่างการทำแหนม.** วิทยานิพนธ์ปริญญาโท. มหาวิทยาลัยเกษตรศาสตร์. 126 น.

สำนักงานมาตรฐานผลิตภัณฑ์อุตสาหกรรม กระทรวงอุตสาหกรรม. 2546. **มาตรฐานผลิตภัณฑ์ชุมชน แหนม มผช 145/2546.** มาตรฐานอุตสาหกรรม มอก. 1219-2546. 5 น.

FUTURE WORKS

In this study, a controlled strategy relies mainly on the estimation of the actual amounts of glucose and fructose in both total and free forms. Therefore, for industrial application, further study is still needed to enable the processors and Nham formulators to limit sugar substrates in Nham based on the actual amount of raw materials added. Due to a variation of sugar composition in raw materials, specially for garlic and cooked rice, more data on total glucose and fructose of these raw materials as affected by variety, season, storage time, and processing methods is needed. Lastly, to ensure the safety of products, the effect of limiting carbohydrate substrates on the survival of some important food-borne pathogens should also be studied.

APPENDICES

APPENDIX A

MEDIUM PREPARATION

Medium preparation

MRS agar + 0.5% calcium carbonate (1 L)

Proteose peptone No. 3	10	g
Meat extract	10	g
Yeast extract	5	g
Glucose	10	g
Tween 80	1	g
Di-potassium hydrogen phosphate	2	g
Sodium acetate	2	g
Di-ammonium citrate	0.2	g
Magnesium sulfate heptahydrate	0.5	g
Manganese sulfate monohydrate	0.05	g
Agar	15	g
Calcium carbonate	5	g
Distilled water	1000	ml

Note: Mix the ingredients and sterilize at 121 °C for 15 min.

APPENDIX B

BALLOT FOR SENSORY EVALUATION

Ballot for sensory evaluation

แบบสอบถามการประเมินผลทางประสาทสัมผัสผลิตภัณฑ์แหนม

ชื่อ.....นามสกุล.....

วันที่.....รหัสผลิตภัณฑ์.....

กรุณาทดสอบตัวอย่างผลิตภัณฑ์อาหารต่อไปนี้ โดยทำเครื่องหมาย √ หน้าข้อความเพื่อแสดงระดับความชอบและไม่ชอบต่อลักษณะต่างๆของผลิตภัณฑ์ และโปรดให้เหตุผลในการอธิบายความรู้สึกชอบและไม่ชอบของท่านที่มีต่อผลิตภัณฑ์

หมายเหตุ: กรุณาบ้วนปากหลังการชิมทุกครั้งที่เปลี่ยนตัวอย่างผลิตภัณฑ์

1. สีของผลิตภัณฑ์
   - ☐ ชอบมากที่สุด
   - ☐ ชอบมาก
   - ☐ ชอบปานกลาง
   - ☐ ชอบเล็กน้อย
   - ☐ เฉยๆ
   - ☐ ไม่ชอบเล็กน้อย
   - ☐ ไม่ชอบปานกลาง
   - ☐ ไม่ชอบมาก
   - ☐ ไม่ชอบมากที่สุด

2. กลิ่นแหนมของผลิตภัณฑ์
   - ☐ ชอบมากที่สุด
   - ☐ ชอบมาก
   - ☐ ชอบปานกลาง
   - ☐ ชอบเล็กน้อย
   - ☐ เฉยๆ
   - ☐ ไม่ชอบเล็กน้อย
   - ☐ ไม่ชอบปานกลาง
   - ☐ ไม่ชอบมาก
   - ☐ ไม่ชอบมากที่สุด

3. รสเปรี้ยวของผลิตภัณฑ์

☐ ชอบมากที่สุด
☐ ชอบมาก
☐ ชอบปานกลาง
☐ ชอบเล็กน้อย
☐ เฉยๆ
☐ ไม่ชอบเล็กน้อย
☐ ไม่ชอบปานกลาง
☐ ไม่ชอบมาก
☐ ไม่ชอบมากที่สุด

4. เนื้อสัมผัสของผลิตภัณฑ์

☐ ชอบมากที่สุด
☐ ชอบมาก
☐ ชอบปานกลาง
☐ ชอบเล็กน้อย
☐ เฉยๆ
☐ ไม่ชอบเล็กน้อย
☐ ไม่ชอบปานกลาง
☐ ไม่ชอบมาก
☐ ไม่ชอบมากที่สุด

5. ความชอบโดยรวมที่มีต่อผลิตภัณฑ์

☐ ชอบมากที่สุด
☐ ชอบมาก
☐ ชอบปานกลาง
☐ ชอบเล็กน้อย
☐ เฉยๆ
☐ ไม่ชอบเล็กน้อย
☐ ไม่ชอบปานกลาง
☐ ไม่ชอบมาก
☐ ไม่ชอบมากที่สุด

เหตุผลของความชอบหรือไม่ชอบผลิตภัณฑ์ : .....

.....

.....

APPENDIX C

VITAE

VITAE

NAME	Ms. Sujitra Piluk
DATE OF BIRTH	March 30, 1985
EDUCATION	1997-2002: Bunyawatwittayalai School, Lampamg 2003-2006: Maejo University, Chaingmai Bechelor of Science (Food Science and Technology)
SCHOLARSHIP	2007-2008: Thailand Graduate Institute of Science and Technology (TGIST) scholarship
ORAL PRESENTATION	Effect of rice and garlic content on physico-chemical properties of Nham during post-acidification, The 20th Annual Meeting and International Conference of Thai Society for Biotechnology, October 14-17, 2008, Thailand
HOME ADDRESS	91/5 Charoen Muang Rd., Suandok, Muang, Lampang, Thailand, 52000
E-MAIL ADDRESS	sujitra_jaa@hotmail.com