UNIVERSITI PUTRA MALAYSIA

MICROBIAL AND PHYSICOCHEMICAL CHANGES DURING FERMENTATION OF THAI CHICKEN SAUSAGE

APINYA VANICHPUN

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By

APINYA VANICHPUN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
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Dedicated to

My family for their loves and supports and special dedication to Lee Yock Ann who warmed my heart while I was away from home

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science

MICROBIAL AND PHYSICOCHEMICAL CHANGES DURING FERMENTATION OF THAI FERMENTED CHICKEN SAUSAGE

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APINYA VANICHPUN

August 2003

Chairman : Dr. Zaiton Hassan, Ph.D.

Faculty : Food Science and Biotechnology

A newly formulated fermented Thai chicken sausage was developed by mixing chicken meat with glutinous rice, sugar, garlic, salt, and black pepper. Glutinous rice rendered good binding of the mixed ingredients when compared to other carbohydrate sources (rice, glass noodle, rice + glass noodle, and glutinous rice + glass noodle). Moreover, glutinous rice also significantly ($p<0.05$) reduced the pH value from 6.06 to 4.42 when compared to the formulation with added rice (from 5.80 to 4.50), rice + glass noodle (from 5.77 to 4.43), and glutinous rice + glass noodle (from 5.82 to 4.34) within 24h of fermentation. The addition of water (5% w/w) with and without sodium tripolyphosphate (0.2% w/w) significantly ($p<0.05$) improved moisture retention in the sausages. Sodium tripolyphosphate (STPP) and water significantly ($p<0.05$) affect the pH reduction and the growth of lactic acid bacteria. The formation of mainly lactic acid from 4.96 to 23 mmol of acid/ 100 g of samples and a small amount of acetic acid from 1.57 to 3.27 mmol of acid/ 100 g of samples as a result of

carbohydrate fermentation by LAB during 0h-48h fermentation contributed to the acidic aroma and typical tangy taste typically found in Thai fermented sausages. The fermentation process was successfully carried out within 48h as shown by the rapid increase in the growth of LAB from 7.80 log_{10}cfu/g to12.46 log_{10}cfu/g, rapid increase in titratable acidity from 0.90 to 2.25 g of lactic acid/ 100 g of sausages, and sharp decrease in pH values from 5.64 to 4.27. Prolonged fermentation (after 48h) caused an over acidity in the sausages with a final pH between 4.20-4.30. This newly formulated fermented chicken sausage proved to be highly selective for the growth of LAB. Four species of LAB namely, *Pediococcus pentosaceus, Pd. acidilactici, Lactobacillus pentosus,* and *Lb. brevis* were isolated. *Pd. pentosaceus* and *Pd. acidilactici* were found throughout the fermentation process, whereas *Lb. pentosus* and *Lb. brevis* were found after 24h of fermentation, indicating that the latter two species are tolerant to the acidic conditions. *Pd. pentosaceus* was found throughout the fermentation process in higher number than other species.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
Sebagai memenuhi keperluan untuk ijazah Master Sains

PERUBAHAN MIKROBIOLOGI DAN FISIKO-KIMIA SEMASA FERMENTASI SOSEJ AYAM THAI

Oleh

APINYA VANICHPUN

Ogos 2003

Pengerusi: Dr. Zaiton Hassan, Ph.D.

Fakulti: Sains Makanan dan Bioteknologi

Formulasi baru sosej ayam Thai telah dihasilkan mengandungi daging ayam cincang yang dicampur dengan pulut masak, bawang putih, gula, garam dan lada hitam. Pulut memberikan kapasiti keupayaan pengabungan bahan-bahan campuran yang baik berbanding karbonhidrat lain (nasi, tanghun, nasi + tanghun, dan pulut + tanghun). Tambahan pula, pulut memberikan signifikasi penurunan nilai pH dari 6.06 kepada 4.42 ($p<0.05$) berbanding formulasi yang ditambahkan nasi (dari 5.80 kepada 4.50), nasi + tanghun (dari 5.77 kepada 4.43), atau nasi pulut + tanghun (dari 5.82 kepada 4.34) dalam fermentasi 24 jam. Penambahan air (5% w/w) dengan atau tanpa natrium trifosfat (0.2% w/w) memberikan signifikasi ($p<0.05$) pengekalan kelembapan di dalam sosej. STPP dan air memberi kesan signifikan pH penurunan ($p<0.05$). Pembentukan asid utama melibatkan asid laktik dari 4.96 kepada 14.23 mmol asid/100g sampel) dan jumlah asid asetik yang kecil dari 1.57 kepada 3.27 mmol asid /100g sampel sebagai hasil fermentasi karbohidrat oleh LAB semasa fermentasi

0-48 jam turut menyumbangkan aroma asidik dan rasa masam yang biasa ditemui dalam sosej fermentasi Thai. Proses fermentasi telah dijalankan dengan dalam masa 48 jam menunjukkan pertumbuhan pantas di dalam LAB dengan peningatan bilangan LAB dari $\log_{10}$ 7.80 cfu/g kepada $\log_{10}$12.46 cfu/g, titrasi asiditi dari 0.90 kepada 2.25 asid laktik /100 g sosej, dan penurunan nilai pH dari 5.64 kepada 4.27. Fermentasi berpanjangan (selepas 48 jam) menyebabkan asiditi berlebihan di dalam sosej dengan pH akhir 4.20-4.30. Formulasi sosej ayam yang baru ini telah memberikan pemilihan yang tinggi untuk pertumbuhan LAB. Empat jenis LAB yang telah dipencilkan adalah *Pediococcus pentosaceus, Pd. acidlactici, Lactobacillus pentosus,* dan *Lb. brevis. Pd. pentosaceus* dan *Pd. acidilactici* telah ditemui pada setiap peringkat fermentasi, manakala, *Lb. pentosus* dan *Lb. brevis* ditemui selepas fermentasi 24 jam. Ini menunjukkan dua spesis terakhir adalah toleran kepada keadaan asidik. *Pd. pentosaceus* ditemui di sepanjang proses fermentasi dan mempunyai bilangan jumlah yang jauh lebih tinggi berbanding spesis lain.

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LIST OF ABBREVIATIONS

ADP	= Adenosine-5'-diphosphate
ANOVA	= Analysis of variance
AOAC	= Association of Official Analytical Chemists
ATP	= Adenosine-5'-triphosphate
B.C	= Before Christ
ºC	= Degree celcius
CO_2	= Carbondioxide
DNA	= Deoxyribonucleic acid
EMP	= Embden Meyerhof Parnas
FDP	= Fructose diphosphate aldolase
G+C	= Guanine + Cytosine
g	= Gram
HPLC	= High Performance Liquid Chromatography
H_2O	= Water
H_2O_2	= Hydrogen Peroxide
h	= Hour
kg	= Kilogram
LAB	= Lactic Acid Bacteria
LDH	= Lactate dehydrogenes
Min.	= Minute
mmol	= Millimolar
MRS	= de Man Ragosa Shape

CHAPTER I

GENERAL INTRODUCTION

Sausages are popular meat products enjoyed by millions of consumer worldwide (Mendoza *et al.*, 2001). In Thailand, the total consumption of sausage in 1988 was 5,000 tons and this include 1,000 ton of fermented sausage, however the total production of fermented sausage by Sor Khonkean, a Thai company that produces meat products reached up to 9,600 tons/year in 1993 (Sartsittisuk, 1995). In the United States of America, over 2.18 billion kilograms of sausages are produced annually (Matulis *et al.*, 1995). In Germany, a large variety of fermented sausages have been developed with over 350 varieties being produced (Vernam and Sutherland, 1995). The annual per capita consumption of fermented sausages in Germany is in the order of 5 kg (Lücke, 1985). About 50 varieties of fermented sausages have been catalogued in Spain (Fernández *et al.,* 2000) and the production of *Chorizo*, the main fermented sausage produced in Spain was 61,700 tons annually (Pagan-Moreno *et al.,* 1998).

Lactic acid bacteria (LAB) are important in the ripening process of fermented sausages (Hammes *et al.*, 1994), and they are usually the predominant microbial population in this type of sausage (Mäkelä, 1992; Bruna *et al.*, 2001a; Coppola *et al.*, 2000). This is because the production of

organic acids, mainly lactic acid and low concentrations of acetic acid by LAB result in a decrease in pH, which is responsible for the preservative effect. The combined effect between lactic and acetic acid imparts an "acid flavour" which is predominant in semi-dry sausages (Lücke, 2000; Vignolo *et al.*, 1989). In addition, the accumulation of lactic and acetic acid can inhibit meat-borne pathogenic bacteria, coagulate soluble meat proteins, and facilitating drying of the products (Lücke, 2000).

Throughout the industrialized world, a growing concern for health has increased the interest in protein sources that are low in fat and cholesterol (Pedersen, 1995). The use of chicken meat as a raw material for different chicken meat based products is becoming popular (Silva and Glória, 2002). Currently, health-conscious consumers try to reduce dietary fat intake by consuming low fat, reduced fat or fat free foods. This has in turn increased consumer demand for low fat content meat products (Mugumura *et al.*, 2003) and resulted in the development of new formulations or modification of traditional food products to contain less fat (Mendoza *et al.*, 2001).

The traditional Thai fermented sausage is mainly made up of ground lean pork mixed with lard. This resulted in a high fat content of the final product. Thungtakul (1988) reported that the content of fat in Thai fermented sausage was 38.98% (dry basis) after 144h of fermentation.