CHEMICAL AND MICROBIOLOGICAL CHANGES DURING FERMENTATION OF *NONSOM*, AN INDIGENOUS FERMENTED FISH IN SABAH.

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DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE DEGREE BACHELOR OF FOOD SCIENCE WITH HONOURS IN THE FIELD OF FOOD TECHNOLOGY AND BIOPROCESS

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### ABSTRACT

The physicochemical, proximate properties and microbiological changes of *nonsom*, which is traditionally produced fermented fish with rice in Sabah, were studied during fermentation. Value of pH dropped significantly at first 5 days. Reversely, the value of titratable acidity rapidly increased at that period. The correlation between pH, titratable and salt content was studied throughout fermentation. The value of crude fat and crude fiber were gradually increased, while crude protein content of sample decreased in this spontaneous fish fermentation. There are no significant changes in ash content, moisture content, carbohydrate and salt content. Total aerobic mesophile, proteolytic bacteria, halotolerant bacteria, lactic acid bacteria, yeast and mould were included in microbiological counting. Total aerobic mesophiles was varied in 10<sup>7</sup> and 10<sup>8</sup>. Lactic acid bacteria comprised the dominant microbial group in *nonsom* compared to other microbial groups except total aerobic mesophiles. The LAB strains isolated namely *Lactobacillus brevis, Lactobacillus sakei, Lactobacillus plantarum, Lactobacillus hilgardii, Leuconostoc* spp., *Pediococcus* spp., which exhibiting starch-fermenting characteristic.



# ABSTRAK

#### PERUBAHAN KIMIA DAN MIKROBIOLOGI SEMASA FERMENTASI NONSOM, SEJENIS IKAN FERMENTASI DI SABAH.

Perubahan fizikokimia, proksimat dan mikrobiologi bagi nonsom, sejenis ikan fermentasi di Sabah, dikaji sepanjang masa fermenatasi. Nilai pH menurun dengan nyata dalam 5 hari pertama. Sebaliknya, keasidan titrat meningkat dengan cepat pada masa itu. Korelasi antara pH, keasidan titrat dna kandungan garam juga dikaji sepanjang masa fermentasi. Nilai bagi lemak kasar dan serabut kasar meningkat beransur-ansur, manakala protin kasar menurun semasa fermentasi semulajadi ini berlaku. Perubahan bagi nilai kandungan abu, kandungan air, karbohidrat dan garam tiada perubahan yang nyata. Jumlah mesofilik aerobik, bakteria proteolitik (LAB), bakteria halotolerant, bakteria asid laktik, yis dan mould termasuk dalam perhitungan mikrobiologi untuk menentukan kuantiti setiap kumpulan mikroorganisma. Jumlah mesofilik aerobik berbeza antara 10<sup>7</sup> and 10<sup>8</sup>. Bakteria asid laktik didapati merupakan kumpulan dominan dalam nonsom berbandingan dengan kumpulan lain selain total aerobic mesophiles. Strains LAB yang dipencilkan merupakan Lactobacillus brevis, Lactobacillus sakei, Lactobacillus plantarum, Lactobacillus hilgardii, Leuconostoc spp., Pediococcus spp., yang berupaya mefermentasikan karbohodrat.



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# **CHAPTER 1**

## INTRODUCTION

Fermentation has been used for several thousand years as an effective and low cost means to preserve the quality and safety of foods. Animals and plant tissues subjected to the action of microorganisms and/or enzymes to give desirable biochemical changes and significant modification of food quality are referred to as fermented food (Campbell-Platt, 1994). Food fermentation is the oldest known form of biotechnology. The traditional fermented foods contain high nutritive value and developed a diversity of flavors, aromas, and texture in food substrates (Steinkraus, 1995).

As food expand and diversify, there is a growing interest in traditional fermented products manufactured at the farm level due to the uniqueness of such foods and the difficulties in mimicking them on an industrial scale(Souza *et al.*, 2003). Besides, it may also help to alleviate the nutritional problems that face the developing world. Food fermentation is especially important for developing countries where the lack of resources limits the use of techniques such as vitamin enrichment of foods, and the use of energy and capital intensive processes for food preservation. Examination of the indigenous fermented foods may, therefore, provide clues as to how food production and preservation can be expanded and thereby contribute to improved nutrition in the developing world in the future.



The so-called 'fermented' products can be divided into two main categories: the first is a high salt (20-30%) product in which the microbial count decreases during curing, and the second has a lower salt content to which fermentable carbohydrates are added (Cooke *et al.*, 1993). The latter category is characterized by an increase in microbial count during fermentation with a dominance of LAB (Tanasupawat & Komagata, 1995; Steinkraus, 1996). During fermentation, there are physical, microbiological and biochemical changes, involving tissue enzymes as well as microbial enzyme take place in food. The fermentation process depends on different parameters, such as the proportions and the quality of raw material, the use of starter cultures, temperatures and relative humidity and microbial flora (Rosello *et al.*, 1995).

Some important indigenous foods include Indonesian kocap (fermented vegetables proteins), Malaysian tempe kedela (fermented cereal), Korean kimchi (fermented cabbage), fish sauce in South-east Asia and so on. In South-East Asia, the fermented fish normally produced for condiments. However there are some indigenous fermented fish that are lightly salted fermented fish products (Ishige, 1993; Adams *et al.*, 1985). They are typically composed of freshwater fish species, salt, a carbohydrate source (boiled/ roasted rice, millet, sugar or fruit) and spices (garlic, ginger, chilli, pepper) (Paludan-Müller *et al.*, 1998). There is closely product called burong dalog in Philippine which is the raw fish are mixed with boiled rice and solar salt and allowed to ferment (Steinkraus, 1994). Besides, Som-fak is another Thai product composed of minced fish fillet, salt, ground boiled rice and minced garlic (Paludan-Müller *et al.*, 1998). In Som-fak, rapid growth of lactic acid bacteria (LAB) causing pH to decrease below 4.5



in 2 days is essential to prevent spoilage (Østergaard *et al.*, 1986) and to ensure safety of the product.

In Sabah, Malaysia, one type of fermented fish known as *Nonsom* among the Kadazan society was found. Traditionally, almost every Kadazan household in Sabah makes their own *nonsom* for domestic consumption. It is manufactured with fresh fish, rice, salt, seed of *Pangium edule* and no commercial starters are used in its production, fermentation occurring, therefore, by a natural process. Ripening depends entirely on natural microflora. These home-made techniques, acidification by the indigenous microbial group in raw material and the absence of control of temperature and relative humidity during ripening period, result in a lack of uniformity and quality of the final product. However there is a lack of knowledge is particularly critical, specially the microbiological quality. Such lack of knowledge is particularly critical, specially the microbiological quality as the spontaneous fermentation of fish.

The knowledge of microbiological and biochemical reactions in *nonsom* can help to introduce process modifications necessary to improve the quality of products, without any loss in its characteristics. The aims of the present study were investigate the changes in levels of the main microbial groups and in the physicochemical characteristics as well as proximate composition throughout the ripening process of *nonsom*. The result from this study is expected to study the beneficial microbial groups involved in fermentation of *nonsom*, as well as to provide further scientific basis for the understanding of ripening process, which is necessary to improve home-made processing.



# **Specific objectives**

- 1. To determine physico-chemical and proximate composition changes during the fermentation of nonsom.
- 2. To enumerate the count of microbial groups at various stages of fermentation.
- To characterize and identify the microorganisms isolated based on biochemical tests.



#### CHAPTER 2

#### LITERATURE REVIEWS

#### 2.1 Food Fermentation

Fermentation is one of the oldest forms of food preservation as well as being a precursor of modern biotechnology (Cook, 1994). It is a process dependent on the biological activity of microorganisms for production of a range of metabolites which can suppress the growth and survival of undesirable microflora in foodstuffs (Ross *et al.*, 2002) in which depend on the principle of oxidation of carbohydrates and related derivatives to generate end-products such as generally acids, alcohol and carbon dioxide. Besides, chemical definition of the term of fermentation is applied to describe a strictly anaerobic process; however, the general understanding of the term now encompasses both aerobic and anaerobic carbohydrate breakdown processes (Caplise & Fitzgerald, 1999).

The raw materials traditionally used for fermentation are as diverse as: fruits, cereals, honey, vegetables, milk, meat and fish. It is possible to obtain a large variety of different food products by selecting different raw materials, starter cultures and fermentation conditions (Cook, 1994). Fermented foods are food substrates that are invaded or overgrown by edible microorganisms whose enzymes, particularly amylases, proteases and lipase, hydrolyze the polysaccharides, proteins and lipids to nontoxic



products with flavors, aromas and textures pleasant and attractive to the human consumer (Steinkraus, 2002). A variety of fermented foods can be found throughout the world, such as beer, bread, sauerkraut, pickles, cheese, yoghurt and sausages in which their principles offer a wealth of possibilities for new product development (Giraffa, 2004). The fermentation process may be conducted either naturally or by adding starter culture. In a spontaneous fermentation, the conditions are set so that the desirable microorganisms grow preferentially and produce metabolic by products, which give the unique characteristic of the product (Hansen, 2002). The process is unpredictable and uncontrollable as it depends on the natural microflora on the raw material is inefficient. Thus the indigenous microflora is very important in determining quality of the fermented food.

### 2.2 Fermentation as a method of food processing

Generally, food fermentation plays important role in enrichment of the human dietary through development of a wide diversity of flavors, aromas, colors and textures in foods. It also contributes in enrichment of food substrates biologically with protein, essential amino acids, essential fatty acids and vitamins. The nutritional impact of fermented foods on nutritional diseases can be direct or indirect. Food fermentation which raise the protein content or improve the balance of essential amino acids or their availability will have a direct curative effect and also for that increase the content or the availability of vitamins such as thiamine, riboflavin, niacin or folic acid can have profound direct positive effects on the health of the consumers (Steinkraus, 1994).



Besides, microorganisms from fermented foods may be are potential sources of useful components including uncommon lipids, antimicrobial agents and biopolymers help in preservation of substantial amounts of foods. Through the soaking or hydration that raw substrates undergo in various fermentation processes ot enzymatic hydrolysis, some natural toxic like trypsin inhibitor, phytate and hemagglutinin exist in food may be detoxified. For example, aflatoxin that frequently found in peanut and cereal grain substrates is reduced in the Indonesian *ontjom* fermentation (Steinkraus, 1983). Fermented foods are also a potential source of microorganisms for biotechnology although relatively few have been exploited by industry. The production of fermented foods is also important in adding value to agriculture raw materials, thus providing income and generating employment (Hansen, 2002).

#### 2.3 Indigenous fermented food

Indigenous food fermentations are one of the oldest forms of biotechnology, although the potential of associated microorganisms has probably not yet been fully realized. The indigenous fermented foods constitute a group of foods that are produced in homes, villages, and small cottage industries at prices within the means of the majority of consumers (Steinkraus, 1985). In most cases, the methodologies and knowledge associated with the production of these products were handed down from generation to generation within local communities, monasteries and feudal estates (Caplise & Fitzgerald, 1999). They can be produced and distributed at a relatively low cost but generally high nutritious, providing calories, protein, vitamins and minerals (Steinkraus, 1994). With the increasing population and the benefits aspects of fermented foods, there is starting of the concentration of studies related to indigenous fermented foods so



that the indigenous fermented foods will be contribute in ensuring the extensive food supplies needed to feed mankind when world population rises in the future.

#### 2.3.1 Classification of indigenous fermented food

There are a number of ways to classify the indigenous fermented foods according to different researchers. Major food preservations by fermentation include lactic acid bacteria fermentation, alcoholic fermentation, acetic acid fermentation and alkaline fermentation. Besides, It is also classified according to the following categories (Steinkraus, 1983): (1) Fermentation producing textures vegetable protein meat substitutes in legume or cereal mixtures. Examples are Indonesian *tempe* and *ontjom*; (2) High salt or savory meat-flavored or amino acid or peptide sauce and paste fermentation. For instance, Chinese soy sauce, Japanese *shoyu* and others in which are predominantly Oriental fermentation; (3) Lactic acid fermentation. Examples are vegetable lactic acid fermentation, fermented milk and leavened bread; (4) Alcoholic fermentation; an example is grape wine; (5) acetic acid or vinegar fermentation, examples are Japanese *natto* and Thai *thua-nao*; (7) Leavened bread, example is sourdough breads; and (8) Flat unleavened bread.

### 2.3.2 Indigenous fermented food in Malaysia

There are a number of indigenous fermented foods have been industrialized in Malaysia, such as product of high salt/savory meat-flavored/ amino acid/ peptide sauce and pasta fermentation: *belachan*; lactic acid fermentation: pickled vegetables, *tempoyak* and *tairu* (soy-bean milk); as well as alcoholic fermentation: *tapai* (Steinkraus, 1983). However,



there are many of the indigenous fermented foods have not been studied and developed especially those produced at household level in rural areas. It is also considered as traditional food from different ethic groups in Malaysia. It may represent a cultural and lifestyle of certain groups of people from different regions in Malaysia besides preserved over many years in order to maintain the uniqueness and identities of these foods.

For instance, *montoku* is a traditional alcoholic beverage in Sabah among Kadazandusun. They drink *montoku* in conjunction with harvest celebrations and social or communal gatherings. Besides, *sasad* is a traditional starter culture produced at home scale production by local Sabah community as well as fermented *bambangan*. It have been consumed by people in Sabah and Sarawak and due to its high yield of production, it have been preserved through fermentation by ancestors and handed through generations. Some of them are similar to the fermented foods from other Orient countries and they are recognized in different name. For example, *sasad* is also can be known as *ragi* and *montoku* is one type of rice wine. Malaysian rice wine or tapai is lighter in colour, ranging from red to pink. It is made from cooked gelatinized rice and red pulverized ragi (yeast cake, or jui-piang) (Aidoo *et al*, 2005).

#### 2.4 Fermented fish

Many fermented fish products are prepared in different parts of the world, and the method of processing depends upon various factors, such as availability of raw materials, consumer preference and the climatic conditions of the region. Microbial fermentation of fish has precedents in the production of various fish sauces and pastes (Mackie *et al.*, 1971; Beddows, 1985; Adams *et al.*, 1985; Saono *et al.*, 1986) and in the production of



fish silages (Raa & Gildberg, 1982). There are some popular fermented fish products in Southeast Asia and is known by various names according to the country of origin (for example, *Nam-pla* in Thailand and Laos; *Budu* in Malaysia, *Patis* in the Philippines; *Ngampi-pya-ye* in Burma; *Ketjap-ikan* or *Bakasang* in Indonesia; or *Nuoc-mam* in Vietnam) (Beddows, 1985). Besides this region, fermented fish sauce also produced in other Asia and Europe countries. It is called *Shottsuru* or *Ika-shoyu* in Japan; *yu lu* in China; *Jeot-kal* in Korea; *Colombo-cure* in India and Pakistan; *Mehiawah* in Middle East and anchovy in France. Table 2.1 shows the acid-fermented fish of different countries (Lee, 1994).

Product name	Country	Major ingredients	Microorganisms	Usage
Sikhae	Korea	Sea-water fish, cooked millet, salt	L.mesenteroides, L. plantarum	Side-dish
Narezushi	Japan	Sea-water fish, cooked millet, salt	L.mesenterioides. L. plantarum	Side-dish
Burong-isda	Philippines	Fresh-water fish, rice, salt	L.brevis, Streptoccus sp.	Side-dish
Pla-ra	Thailand	Fresh-water fish, salt, roasted rice	Pediococcus	Condiment
Balao-balao	Philippines	Shrimp, rice, salt	L.mesenteroides, P.cerevisiae	Side-dish
Kungchao	Thailand	Shrimp, salt, sweetened rice	P.cerevisiae	Side-dish

Table 2.1 Examples of acid-fermented seafood and cereal mixtures (Lee, 1994).

There are another types of low salt fermented fish in which other sources of carbohydrate like rice, millet and garlic would be added and mixed with fish. Preservation of fermented fish products obviously depends on lactic acid and possibly bacteriocin production; however, other factors may also contribute to the overall



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keeping quality of low-salt fermented fish products. These products are often produced using freshwater fish, where addition of salt may have a significance inhibitory effect on mesophilic spoilage flora (Gram, 1991). Similarly, the addition of spices (e.g. garlic) may have an important role in ensuring product safety (Souance *et al.*, 1987; Beuchat, 1994). In addition, salt and spices (such as garlic, pepper or ginger) may add to the safety of products. Also, in some products garlic may serve as a carbohydrate source for the fermentation (Paludan-Müller *et al.*, 1999). In addition, this fermented fish also depend on proteolytic enzymes to hydrolyze the proteins in the substrate to the constituent amino acids and peptides.

### 2.4.1 Low salt fermented fish in South East Asia

There are a few examples of low salt fermented fish products. For instance, *plaa som* in Thailand in which the whole fish is mixed with salt (ratio 8:1 by weight) and left overnight. Cooked rice and minced garlic are added (ratio 20 fish/salt: 4 rice: 1 garlic by weight), then the mixture is packed in jars and fermented at ambient temperature for 5-7 days. The shelf life is reportedly 3 weeks (Phithakpol *et al.*, 1995).

In addition there is another type of low salt fermented fish in Thailand called *som-fug* which is a highly nutritious and served as an excellent source of protein. It varies in color from white to brownish or reddish, depending on the type of fish and the methodology used in its production. The ripened product is slightly sour and salty in flavor, and is of a relatively firm and springy texture, and consumed either in the raw or cooked state (Valyasevi & Rolle, 2000). Besides, there is another closely related product which categorized in boiled rice/ raw shrimp/ raw fish mixtures: Philippine *balao- balao,* 



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