

# The influence of storage conditions of tuna viscera before fermentation on the chemical, physical and microbiological changes in fish sauce during fermentation

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## Abstract

Effect of storage condition of tuna viscera on chemical, physical and microbiological changes of its sauce were monitored. Results based on microbial counts, protein degradation products, total volatile base (TVB), and trimethylamine (TMA) contents, showed that tuna viscera stored at room temperature underwent more deterioration than that kept in ice, especially with increasing storage time. As a result, fish sauce obtained from tuna viscera stored at room temperature for a longer time rendered the greater amino nitrogen, TVB, TMA contents as well as browning intensity. However, storage conditions had no marked effect on overall changes in chemical, physical and microbiological characteristics of sauce generated during fermentation. Additionally, fish sauce produced from tuna viscera kept at room temperature comprised lower histamine content than that prepared from fresh or ice-stored viscera. Therefore, tuna viscera stored at room temperature for up to 8 h could be used for the production of fish sauce with no detrimental effect on the quality.  
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## 1. Introduction

Fish sauce is the liquid product developed during fermentation of heavily salted fish material in closed tanks at tropical temperatures. Thailand is the largest producer (Saisithi, 1994). The annual production of fish sauce is more than 400 million liters. There are approximately 100 fish sauce producers, however only 20 producers have already held 80% of the market share. Fish sauce is mainly produced from anchovies (*Stolephorus* spp.), mackerel (*Rastrelliger* spp.) and herring (*Clupea* spp.) (Lopetcharat et al., 2001). Traditionally, fish sauce is produced by mixing

one part salt with two or three parts fish and fermenting at ambient temperature (30–40 °C) for 6–12 months or longer (Lopetcharat et al., 2001). However, salt/fish ratio can be different in different regions. During fermentation, protein hydrolysis is induced by endogenous proteinases in fish muscle and digestive tract as well as proteinases produced by halophilic bacteria (Gildberg and Thongthai, 2001). The volatile compounds contributing to flavour of fish sauce are produced by non-enzymatic reactions of various components and enzymatic reactions by endogenous enzymes of fish origin and those of microorganisms surviving during fermentation (Fukami et al., 2004). Fermentation process normally takes a long time to ensure the solubilisation as well as the flavour and colour development of fish sauce. The autolysis of fish proteins during fermentation was accelerated by the addition of fish viscera

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or proteinases (trypsin and chymotrypsin) (Kim et al., 1997; Morioka et al., 1999) or the reduction of salt concentration (<20%) (Morioka et al., 1999). Gildberg and Thongthai (2001) reported that fish sauce from minced capelin was obtained after 6 months of fermentation with the addition of 5–10% enzyme-rich (trypsin and chymotrypsin) cod intestines.

Due to the types of fish and uncontrolled fermentation process used to produce fish sauce, often fish sauces are known to contain high levels of histamine. The overall histamine formation in fish and subsequent fishery products is related to fish species, free histidine content of fish muscle, the presence of bacterial histidine decarboxylase and environmental conditions to promote growth of histamine-forming bacteria (Lehane and Olley, 2000). Generally, histamine formation by bacteria is enhanced at elevated storage temperatures (Kim et al., 2000). The level is related to the combination of time and temperature that fish are exposed to. Therefore, histamine can be accumulated in fish as well as products made from abused or improperly handled fish. The Canadian Fish Inspection Agency set up the maximum limit for histamine in fish sauce at 200 mg/l while the US Food and Drug Administration set it at 500 mg/l (FDA, 2001a,b). Brillantes et al. (2002) reported that fish sauce produced from fish stored without ice contained higher levels of histamine than that produced from iced fish.

Due to the tremendous amounts of solid wastes, especially viscera in tuna industry, more attention has been given to those wastes as the raw material for value-added products. Those wastes have been reported to contain a high amount of protein (Guérard et al., 2001). Thus, tuna viscera can be a potential starting material for fish sauce production owing to the abundant protein as well as high proteolytic activity (Klomklao et al., 2004). They might be hydrolysed to form the liquid having the similar composition and characteristics to commercial fish sauce. After evisceration, viscera may undergo the deterioration, particularly without the appropriate handling. Therefore, the properties and characteristics of fish sauce obtained might be determined by the quality of those wastes. The purpose of this study was to monitor the chemical, physical and microbiological changes of tuna viscera during storage and to investigate the effect of raw material quality on the fermentation and the characteristics of fish sauce.

## 2. Methods

### 2.1. Chemicals

Potassium sulfate, copper sulfate, sodium hydroxide, trichloroacetic acid, hydrochloric acid, sulfuric acid, magnesium oxide, and formaldehyde were obtained from Merck (Darmany, German). Histamine dihydrochloride and *O*-phthaldialdehyde (OPT) were purchased from Sigma (St. Louis, MO, USA).

### 2.2. Raw material

Skipjack tuna (*Katsuwonus pelamis*) viscera, including spleen, stomach, intestine, bile sac, liver, and pancreas were obtained from Chotiwat Industrial Co. Ltd., Songkhla, Thailand. Tuna viscera were transported in ice with the viscera/ice ratio of 1:2 (w/w) to the Department of Food Technology, Prince of Songkla University, Hat Yai, within 30 min and divided into two lots. One lot was immediately iced with the viscera/ice ratio of 1:2 (w/w) and placed in an insulated container with a drain valve. Another lot was allowed to stand at room temperature (28–30 °C). Samples of each lot were taken at the different storage time (0, 4, and 8 h) for analyses and fish sauce fermentation.

### 2.3. Fish sauce fermentation

Tuna viscera kept in ice or at room temperature for different times were subjected to fermentation. Tuna viscera (27 kg) were mixed with solar salt (9 kg) and transferred to earthen jars (50 l). The earthen jars were covered with black plastic bag and aluminum lid to prevent the contamination of foreign materials as well as insects. The earthen jars containing samples were placed outdoor with temperature ranging from 27 to 35 °C. The liquid formed was taken at month 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 10 and 12. Liquid formed at time designated was filtered using Whatman filter paper No. 1. The filtrate was subjected to chemical and physical analysis.

### 2.4. Chemical analysis

#### 2.4.1. Determination of TCA-soluble peptides

TCA-soluble peptides were determined according to the method described by Morrissey et al. (1993). Sample (3 g) was homogenised with 27 ml of 5% TCA (w/v) for 1 min at room temperature using an IKA Labortechnik homogeniser (Selangor, Malaysia). The homogenate was kept in ice for 1 h and centrifuged at 7500g for 5 min. Soluble peptides in the supernatant were measured and expressed as  $\mu\text{mol/g}$ .

#### 2.4.2. Determination of pH

The pH of tuna viscera was measured as described by Benjakul et al. (1997). Sample was homogenised using an IKA Labortechnik homogeniser (Selangor, Malaysia) with 10 volumes of deionised water (w/v) and the pH was measured using a pH meter (Cyberscan 500, Singapore). For the liquid samples, pH was determined directly using a pH meter.

#### 2.4.3. Determination of total volatile bases (TVB) and trimethylamine (TMA) contents

TVB and TMA contents were determined using the Conway microdiffusion assay according to the method of Conway and Byrne (1936). Sample (4 ml) was mixed with

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