Producing fish sauce from Caspian kilka

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Received: January 2006 Accepted: February 2009

Abstract

Fish sauce is a fermented product which is used in south Asian countries. In the present study, Caspian Kilka was used to produce the sauce, using either cooked or raw fish subjected to four different treatments: 1) traditional method, where fish and salt were used; 2) an enzymatic method, where fish, salt and proteolytic enzymes, including Protamex and Flavourzyme (Novo Nordisc Co., Bagsvaerd, Denmark), were used; 3) a microbial method, where fish, salt, and *Bacillus* and *Pediococcus* species were used; and 4) a combination of the enzyme and microbial methods. Fermentation of the ingredients was carried out in 400cc bottles for a period of 6 months with microbiological and chemical tests at intervals of one week and then one month. The results of molds, yeast, and aflatoxin detection tests were negative. The total bacterial count ranged between log 2.1 and 6.18. Chemical tests included TVN and pH. pH of the final products ranged from 6.5 to 7.0. The speed of fermentation as determined by examining the bottles every two or three days was as follows: Traditional< Microbial<Enzymatic = Enzymatic+Microbial Method. However different treatments could be used to speed up the fermentation, but the traditional method is considered to be better as the quality is concerned.

Keywords: Fish sauce, Fermentation**,** Lab bacteria, Enzyme, Kilka, Caspian Sea

Introduction

Lactic acid bacteria (LAB) are widely used for processing of food in fields as varied as dairy products, meat and vegetables. LAB has been used by seafood processors for a long time in traditional processes, such as salt curing of fish. Knowledge of the temperature, pH and salinity conditions enables the satisfactory use of LAB. Scandinavian countries, among Europeans, are still producing and consuming fermented fish. Southeast Asian countries are the principal consumers of fish sauces. It is estimated that hundreds of millions of liters of fish sauce are produced annually for daily consumption by at least 300 million people (Hull, 1992). Fish sauces are produced under different names,

such as “NAM-PLA” in Thailand, “BAKASANG” in Indonesia, (France *et al*., 1995) “NOUC–MAM” in Vietnam, “TEUK-TREY” in Cambodia, “PATIS” in the Philippines and “BUDU” in Malaysia. Almost every species of fish and even shellfish, such as crab and shrimp, can be used as a raw material. In Iran, there are two traditional, domestically produced fish sauces called "Mahyaveh and Solakh", which are occasionally consumed in the southern part of Iran, *i.e.*, northern coast of the Persian Gulf. Herrings are washed and cleaned and kept in barrels, with added salt and a special red clay soil (which is only available in Hormoz Island). After fermentation a red color fish sauce is being obtained called "Solakh". "Mahyaveh" is in fact spicy form of Solakh.

The whole fish is mixed with 15-30% salt, and after two days most of the constituent water of the fish is released by salts as a result of osmotic pressure.

To prepare fish sauce from the Caspian Sea kilka the whole fish was mixed with 20% salt and some samples had LAB and/or commercial proteolytic enzymes added. The aim of the research was to optimize the techniques for the production of a high quality nutritional fish sauce.

Materials and methods

Kilka fish, including *Clupeonella engrauliformis* and *Clupeonella grimmi*, were procured from Bandar Anzali fishing port with a post-mortem age of about 7 hour. The fish at the time of purchase were in a chilled seawater tank at 0^{o}C. Ice was used when transferring the fish to the laboratory. Samples for TVN were taken immediately (Lakshimi Naira *et al*., 1986). The fish were caught at the end of November and had 15.3% protein, 4.7% fat and 16.8mg/100g of TVN and had an average total length of 10cm and an average total weight of 8g. The fish were chosen in a month when they would have less fat content because the fat in these fish can reach up to 13% of the body weight during its yearly cycle. With less fat content there is more water in the fish so a juicier meat will yield more sauce with less lipid and low oxidation activity (Andrews & Hammack, 2003). The fish on board were kept in a cold sea water tank and delivered with the tank to laboratory.

The fish were prepared and used in three forms: 1) whole fish, 2) whole cooked fish, prepared by dipping in boiling water for one min and 3) headless gutted raw fish. The fish were washed with clean tap water and after draining each batch weighing 400g was placed in a bottle. In total, 120 bottles were prepared for each of the 3 forms of fish in 4 different treatments each sample containing 10 replicates. One sample was mixed with 20% salt, the second was mixed with 20% salt and LAB, the third was mixed with 20% salt and enzymes, and the fourth was mixed with 20% salt, LAB and enzymes. The enzymes were Protamex, with optimum pH range of 5.5-7.5 and optimum temperature of 30-60^{o}C, and Flavourzyme, with optimum pH of 5.2-6.2 and optimum temperature of 45-90^{o}C (Novo Nordisc Co., Bagsvaerd,

Denmark). 1 and 2g, Protamex and Flavourzyme, respectively, were used per 1kg of fish and mixed well before adding salt. Fish were packed in layers with salt above and below each fish layer, in 400cc bottles, then covered with cotton and paper on the top and capped. Bottles were stored at a constant temperature of 37^{o}C for a period of 6 month under light (Raksakuhtine, 1992).

Samples were withdrawn periodically while fermenting by decanting about 40ml of the supernatant liquid for physicochemical and microbiological analysis. The pH was measured using pH paper (short range 6-8.5, USA). Crude protein was determined by Kjeldahl method (AOAC, ISO 5983.1979) using a 6.25 Kjeldahl conversion factor. Total lipid was determined by Soxhlet extraction (AOAC, An301), and salt content was measured using a salinometer (densimeter g/ml, tp 20^{o}C, moyenne, n, 0906, France) density determined from a table of density and salt percentage obtained from the manufacturer. Total volatile nitrogen (TVN), total solids, and specific gravity were determined using the standard kjeldahl methods of Iran (Vida Parvaneh, 1994).

Microbiological analyses (Lalita, 1995) of the fish sauces were carried out weekly during the first month and at monthly intervals thereafter. 25g of all samples was taken aseptically from the bottles and homogenized in 225ml of sterilized 0.9% NaCl solution using a rotating homogenizer. Serial dilutions of the homogenates were made with physiological saline and the total plate counts (TPC) for both aerobic and anaerobic microorganisms were determined using the pour plate method using nutrient agar. Five percent salt was included in nutrient agar (Chayovan, 1983).

The LAB counts were determined using Rogosa agar total coli form were determined using deoxycholate agar (Merck). The spore forming bacteria were determined on nutrient agar by plate count method, serial dilutions of homogenates were boiled for 10 min before plating, and the total count of *Staphylococcu*s were determined using mannitol salt agar incubated at 37^{o}C for 48h (Bennet & Lancette, 2001). Microorganisms were diagnosed using media cultures observations and biochemical tests (Lakshimi Naira *et al*., 1995).

The yield was evaluated by filtering the bottle content by cloth and the supernatant liquid or sauce poured in to a calibrated glass cylinder to obtain the yield. Also the percentage of hydrolysis in 4 samples in each form was estimated by observation and measuring every week. The amount of liquid or sauce extracted through fermentation in each bottle was an average of 15% of the total volume of material at the end of the first week. It was 40-50% at the end of one month and it was 80-100% after 6 month. The organoleptic tests were ran to compare the laboratory kilka fish sauce product with a foreign commercial Thai fish sauce (Golden Boy brand, Thailand). The organoleptic tests were conducted after 4 and 6 months of preparation by the method of panel test **(**Ahmed *et al*., 2006**).**

Results

Here the color of both sauces was brown yellow, the taste was almost the same but the kilka sauce was a little saltier. All

samples were fairly similar in pH, protein and salt content. The TVN varied significantly, ranging from 170-238meqv/g samples. The TVN varied significantly ranging from 170-238meqv/g samples. The TVN in all of the sauces was initially low, then increased sharply and towards end remained constant (Table 1). The total count of the micro flora for Fish + Salt + Enzyme + LAB was highest at 4.2 log cfu/ml. TPC was highest at the 8th week of fermentation. The total anaerobic counts were similar for all the samples. There were no coli form and SFB bacteria detected in any samples. The total *Staphylococcus* count was high in all samples. Fish sauce prepared from the headless gutted Kilka showed the lowest TPC count. *Staphylococcus* sp. and *Lactobacillus* sp. were the predominant microorganisms isolated (Figs. 1 – 3).

All the samples of fish sauce except the cooked fish gave maximum yield of product around 60% and an average yield of about 45%. Organoleptic evaluations for all samples were similar. The percentage of hydrolysis in raw fish was faster than the cooked fish and the speed of fermentation as determined by examining the bottles every two or three days as follows (Table 2):

Traditional<Microbial<Enzymatic=enzymatic + microbial method

The PH in the final products ranged from 6.5-7.

Table 1: Chemical composition of different fish sauce samples

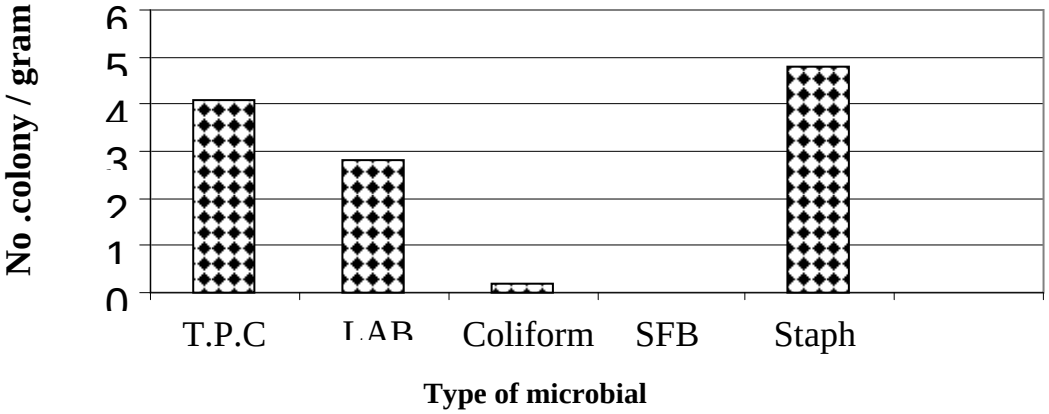

Figure 1: The average of total microbial count from the whole Kilka fish sauce

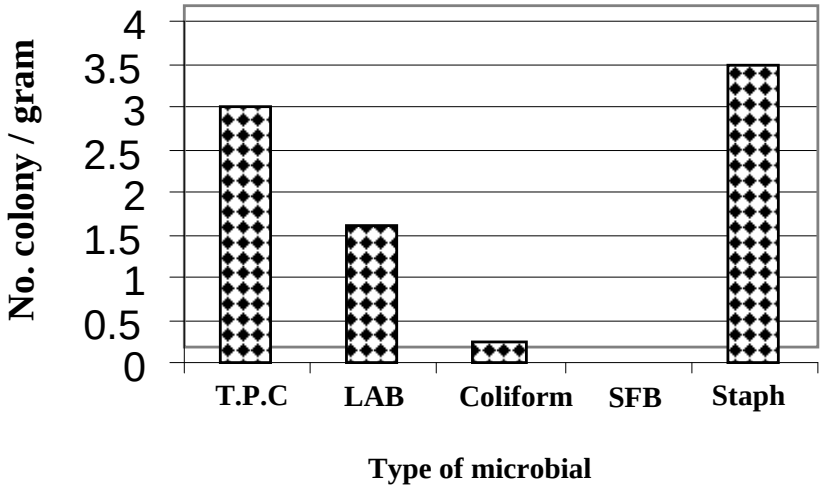

Figure 2: The average of total microbial count from the whole cooked Kilka fish sauce

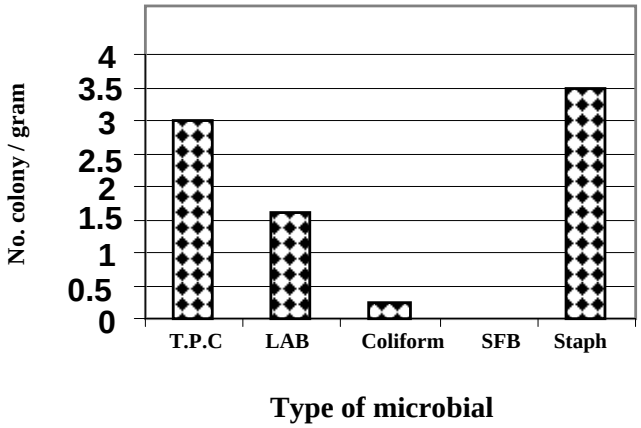

Figure 3: The average of total microbial count from the whole dressed Kilka fish Sauce

Table 2: Percent hydrolysis of fish sauce samples after 4 week

Samples	Traditional	Microbial	Enzymatic	Enzymatic + microbial
Percent hydrolysis Whole Kilka Sauce	80 %	90 %	100 %	100%
Percent hydrolysis Cooked Kilka Sauce	40%	50%	90%	80%
Percent hydrolysis Dressed Kilka Sauce	50%	70%	100%	100%

Discussion

Compare to a foreign commercial Thai fish sauce (Golden Boy brand, Thailand), the laboratory kilka fish sauce in the present study was slightly saltier, however both had similar color of brown yellow, and the taste was almost the same. Compare with Iranian traditional fish sauce, it is similar to Solakh but the color is different. Compare with an anchovy sauce made in Korea and Indonesia with the name Bakasang chemical analysis is almost the same as kilka fish sauce. As per table 3, the shelf life of fish sauce is three years when caped (Fujii, 1992). The production date and expiry date is usually written on the cap on bottles (Coles *et al*., 2003) the sign of expiry is usually noticed by the decalcification and change of color of the fish sauce, in this experiment the maintenancy of kilka fish sauce was not studied due to limitation of time.

Table 3: Chemical comparison between Bakasang and Kilka fish sauce

Composition	Bakasang fish sauce	Kilka fish sauce
Water	66.8%	73%
pH	7%	7
Salt	20%	20%
Fat	1%	1.5%
Crude protein	13%	13%

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