*Fishery Technology*
2002, Vol. 39(2) pp : 124 - 128

# A Process for Convenient Production of Hygienic Fish Sauce by Lactic acid Fermentation of Shark Meat Gel

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A process is described to prepare hygienic sauce from shark meat by lactic acid fermentation. Whole shark (*Scoliodon laticaudus*) was eviscerated, beheaded and deboned. The meat pieces were soaked overnight in cold water, drained, homogenized in water and converted into a gel by lowering the pH to 3.5 by acetic acid. The gel was fermented by *Lactobacillus casei* for 3 days at 30°C. Fermentation was monitored by measuring growth of the organism, pH fall and utilization of carbohydrates. The product was characterized by sour taste and negligible fishy odour. Since the shark meat gel has a refrigerated shelf life of 2 months, the process helps in convenient preparation of sauce.

**Key words: Lactic acid fermentation, shark gel, fish sauce, *Lactobacillus casei***

Production of pastes and sauces by fermentation has been one of the popular techniques for utilization of low-cost, by-catch fish species (Venugopal & Shahidi, 1995; Owen & Mendoza, 1985, Lee, 1989) and shellfish by-products (Fagbenro & Bello Olusoji, 1996) as human food and also as aquaculture feeds in many Southeast Asian countries. Lactic acid bacteria (LAB) belonging to the genera, *Streptococcus, Pediococcus* and *Leuconostoc,* are used for the production of fermented fishery, meat and dairy products. During fermentation, apart from lactic acid, the main end product, a variety of low mol. wt. compounds including acids, alcohols, carbon dioxide, diacetyl, hydrogen peroxide etc. are also produced, many of which have antimicrobial activities against growth of undesired microbial species in the product (Helander *et al.,* 1997; Levin, 1994; Shelef, 1994; Han-Ching *et al.,* 1992). Fermentation of fish-carbohydrate mixtures by LAB offers scope for the development of a variety of products (Lee, 1989). Recently, we have developed a process to preserve fish meat making use of gel forming property of fish meat proteins (Venugopal, 1994; Venugopal *et al.,* 1997). The process involves washing fish mince in water to remove soluble components, and reduction of the pH of the washed fish meat homogenate in water to 3.5 to 4.0 by weak organic acids such as acetic or lactic acid. The gel could be used to prepare a variety of novel products such as restructured steaks, spray dried powder etc. The present paper discusses production of fish sauce by fermentation of the gel prepared from shark meat by *Lactobacillus casei.*

## Materials and Methods

Fresh Indian dog shark (*Scoliodon laticaudus*) from the local market was brought to the laboratory in ice. The fish were beheaded, eviscerated, skinned, deboned and the meat was cut into small (4-5g each) pieces. After washing in tap water, the meat pieces were held overnight in three times its weight of cold (< 10°C) water in a cold room maintained at 0-2°C in order to remove soluble and odour bearing compounds and also to induce hydration of structural proteins. The meat was drained, washed once again in cold water, and was homogenized in equal amount of cold water using a 'Sumit' kitchen homogenizer. Gel was

prepared from the homogeneate, by dropwise addition of glacial acetic acid to a final concentration of 0.5% (w/w) of the homogenate, which was gently stirred during acidification. The gel was stored at 0-2°C until used.

Gel strength of unheated gel was measured using STEVEN-LFRA Texture analyzer (Texture Technologies, Scarsdale, NY, USA). Gel (50g) held in a 50ml beaker was penetrated to a depth of 20mm with a spherical probe (0.5 cm diameter) at a penetration speed of 2 mm per sec. Maximum force exerted on the probe was taken as gel strength which was expressed in g. Negative values were taken as tackiness (cohesiveness) of the gel. A minimum of three readings was taken. Results were expressed as mean value ± standard deviation. Measurements were also made after heating the gel for 10 min in a water bath followed by cooling to room temperature.

The shark gel (1-25 g) and glucose, fructose or invert sugar (up to 5 g) were homogenized in 30 ml water. The pH of the homogenate was brought to 7.0 by adding 1N NaOH and the final volume was made up to 50 ml. Pre-sterilized glucose, lactose or invert sugar was added at 1% (w/v) level. The substrate in 100 ml flasks was seeded wth $10^4$ cells of active culture of *L. casei*. The flasks were incubated at 30°C for 1 to 5 days. After every 24 h of incubation, samples were withdrawn and analysed for bacterial counts, pH and utilization of sugar. In some cases, the shark gel homogenate in water (pH adjusted to 2.0 with a few drops of 1 N HCl, protein content, 20 mg per ml) was incubated with pepsin (Sigma Chemical Co., St.Louis, Mo., specific activity, 10,000 units per g) at 37°C for 2h). After the treatment, pH was raised to 7.0 with 1N NaOH and was used for fermentation, as described above.

*Lactobacillus casei* was obtained from Radio-biology Division, Bhabha Atomic Research Centre, Mumbai. The culture was maintained on plate count agar (Hi-Media, Mumbai, India). For enumeration of the organism, the fermented products were plated at appropriate dilutions on Man Rogosa Sharp Agar (MEA) (Hi-Media, Mumbai, India) plates which were incubated at 24-48 h at 30°C before determining viable counts, as described by Adams *et al.*,(1987). For determining total plate counts, the gel was homogenized in sterile saline at 10% level and total plate count was determined using nutrient agar (Difco, Detroit, USA).

Moisture, protein and crude fat of the gel were determined according to A.O.A.C. (1990) procedures. For protein determination by Kjeldahl method in terms of nitrogen, (N x 6.25), a Kjelplus KPS-012 digestion system and Distil M semi-automatic distillation system (Pelican Instruments Co., Chennai, India) were used. pH was determined using a laboratory pH meter (Global Electronics, Hyderabad, India). Pepsin hydrolysis of the gel was carried out by treating 1 to 30 g of the gel in 0.2% HCl in presence of one mg pepsin per g gel, the enzyme having a specific activity of 2500-3500 units per g) (Sigma Chemical Co., St. Louis, Mo.) up to 22 h at 37°C. Tyrosine was determined by Lowry's method. Total reducing sugar was determined by dinitrosalicylate method of Miller (1959).

## Results and Discussion

The fish used for the studies had proximate composition, namely, 74.9±1.5% moisture, 19.3±0.5% protein and 3.2±0.2% crude fat, while the corresponding values for the gel were 87.3±1.4%, 7.4±0.2% and 0.3±0.1%, respectively. As compared with whole shark meat, which was opaque, the gel was translucent and of hard texture. The gel was characterized by high water holding capacity, since centrifugation at 10,000 x g for 30 mn did not result in separation of water. Fig 1 gives the characteristics of the gel as measured by STEVEN-LFRA texture analyzer. The gel had a strength of 508±12 g and tackiness (cohesiveness) of 272±8 g.

After heating and cooling, 40% reduction in gel strength was noticed.

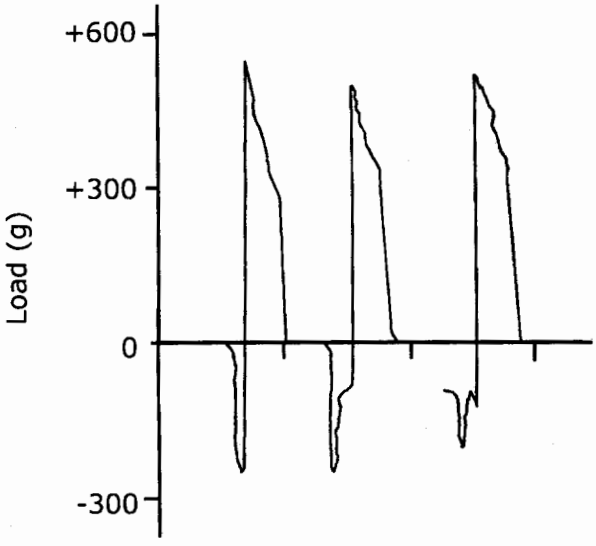


Fig. 1. Gel strength and cohesiveness of acetic acid induced shark gel. Figure shows data from three independent experiments.

The freshly prepared gel had an initial count of 5.0 ± 0.5 x $10^1$/g. After storage of the gel for 2 months at 10°C, the total bacterial count did not increase and remained as low as 7.0 ± 0.4 x $10^1$/g . Storing the gel at ambient temperature for one month gave a bacterial count of 5.0 ± 0.4 x $10^1$/g only, suggesting its remarkable stability against bacterial growth. This was essentially due to the low pH and the preservative effect of acetic acid (Marshall & Kim, 1996). However, prolonged storage could result in slight yellowing of the gel, due to Maillard type reactions.

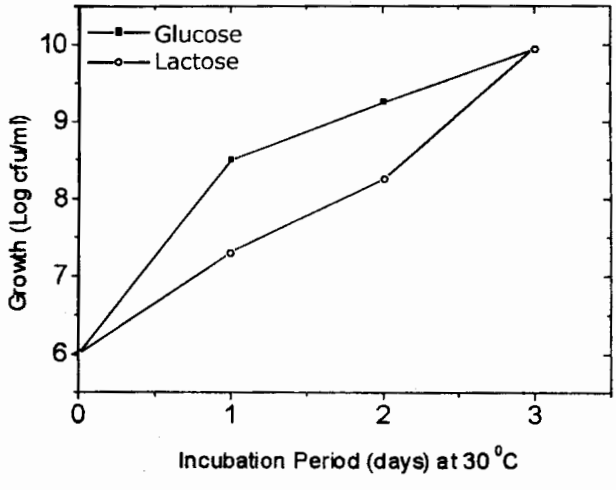


Fig. 2. Growth of *L. casei* in shark gel medium

*L. casei,* grew in the shark gel medium to a maximum of $10^9$ per mL within an incubation period of 3 days at 30°C, as shown in Fig.2. The growth was supported by the gel medium containing any one of the carbohydrate employed, which included glucose, lactose, invert sugar or molasses. Growth of bacterium was associated with fall in pH from 7.0 to 4.5 within an incubation period of 4 days (Fig. 3). Utilization of glucose or lactose during fermentation is depicted in Fig. 4. It can be seen that 40% of glucose or 32% of lactose was utilized during fermentation. Glucose was found to be a better carbohydrate source than lactose. Invert sugar was, however, found to be a better carbohydrate source than lactose. Invert sugar was, however, found to be ideal for fermentation. More than 80% of invert sugar was utilized when fermentation was carried out using shark gel in the range of 1 to 20 g (Fig 5). There was no need of additional nutrients such as yeast extract or vitamin mix for growth of the bacterium.

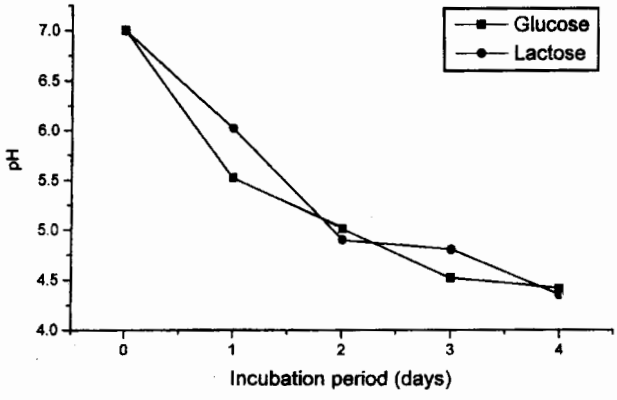


Fig. 3. Change in pH of shark gel medium during fermentation of shark gel by *L. casei*

The use of lactic acid bacteria and also propionic acid producing bacteria for fermentation of dairy products has been quite well known. Both groups of bacteria are capable of fermenting sugars to respective organic acids, resulting in significant reduction in pH. The susceptibility of sugars and the maximum reduction in pH due to fermentation depends upon the species. A

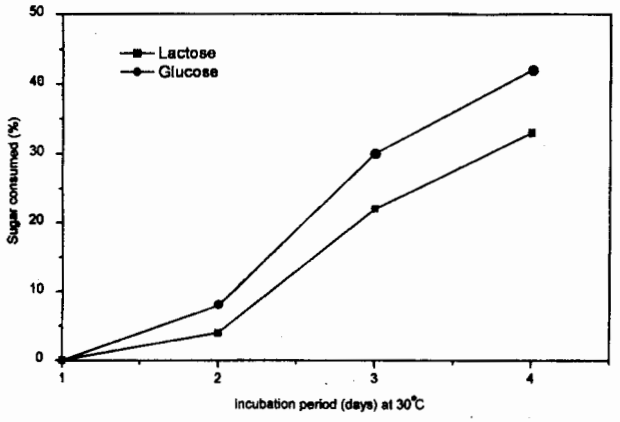


Fig. 4. Consumption of glucose or or lactose during fermentation of shark gel by *L. casei*. The sugars were incorporated at 1% level in the medium

reduction of pH to 4.0 within 72 h has been chosen as the criterion of successful lactic acid fermentation, since under the conditions, growth of contaminating microorganisms in the non-sterile substrates is avoided (Levin, 1994). It was also observed that molasses at 7.5% concentration could be used for fermentation of cod gurry hydrolyzates which was fermented by *L. plantarum* to give a final pH of 3.9 after 72 h incubation (Levin, 1994). Raw heads of river prawn, *Macrobrachium vollenhovenii* fermented with *Lactobacillus plantarum* at 30°C for 7 days gave a product which could be used as an aquafeed ingredient (Fagbenro & Bello-Olusoji, 1977). We observed that 1% of any of the carbohydrates including glucose, lactose, invert sugar or molasses, could be used as source of carbohydrate. Alternatively, rice or cassava could be used for lactic acid fermentation of fish, which can help reduction of pH to 4.5 within 2 h

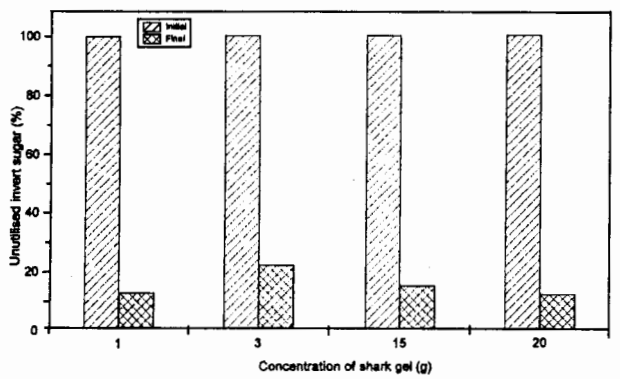


Fig. 5. Consumption of invert sugar during fermentation. The sugar was incorporated at 1% level in the medium

(Owens and Mendoza, 1985). We have recently reported that the shark gel could be used for production of convenient, restructured steaks (Venugopal, 2002).

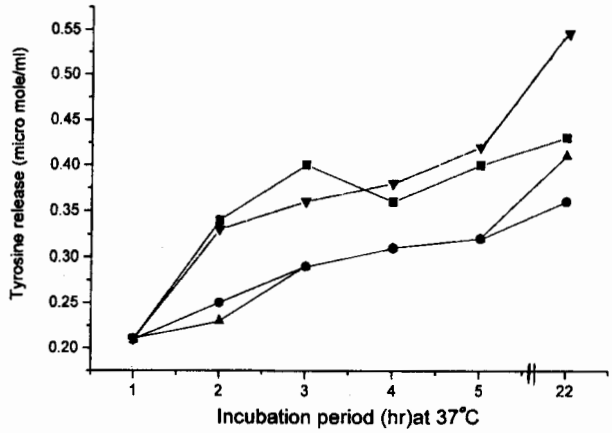


Fig. 6. Release of tyrosine during digestion of shark gel by pepsin. Concentration of shark gel: *, 5%; *, 10%; *, 15%; and *, 30%.

The present study suggests that shark gel could be used for the production of sauce by lactic acid fermentation. The remarkable microbial stability of the gel helps its convenient use for fermentation. The above results suggested that shark gel in presence of glucose, lactose or molasses was a good substrate for *L. casei* fermentation. The product had typical odour of lactic acid fermented foods and was devoid of any significant fish odour. However, presence of sediments, presumably due to undigested proteins during fermentation, affected the appearance of the product. The problem was overcome when the shark gel pre-digested with pepsin was used for fermentation. Fig 6 shows degradation of the gel protein by pepsin. Pre-treatment of the shark gel using pepsin for 5 h followed by fermentation resulted in a product having negligible sediments and uniform appearance. Addition of protease or protease sources such as pineapple is known to accelerate fermentation processes including lactic acid fermentation of fishery products (Beddows & Ardeshir, 1979; Lee, 1989). The present product prepared from shark gel could also be additionally flavoured, using ingredients such as shrimp head extract or other flavouring agents.

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