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# Effect of Storage on Physicochemical Properties of Spiced Fish Sauce

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## Abstract

The study encompasses the influence of storage on physicochemical properties of spiced sauce at ambient temperature on 15 days interval up to 60 days. The results pertaining to physicochemical properties studied on fresh day and 60 days demonstrate that the moisture content of sauce was gradually increased from (65.81 g/100 g) to (66.20 g/100 g), fat was slightly decreased from (6.20 g/100 g) to (6.08 g/100 g), protein was increased from (5.72 g/100 g) to (6.25 g/100 g), carbohydrate steadily decreased during storage time from (16.15 g/100 g) to (15.10 g/100 g), total ash was constantly between 2.88 g/100 g to 2.90 g/100 g during whole study, Salt found 3.20 g/100 g to 3.18 g/ 100 g during storage, the pH was (5.62) to (4.87), the total organic acidity was 1.05 to 1.23, TSS of sauce was slightly increased during storage was increased from fresh (21° Bx) to 60$^{th}$ days (23° Bx) simultaneously. And the viscosity of sauce drastically decreases with storage during study as from fresh (282.0 cP) to 60$^{th}$ day (260.4 cP). The results obtained reveals that change in the physicochemical properties of spiced fish sauce was due to lactic acid fermentation during storage. The dominant flora was counted on same day of chemical analysis simultaneously and the results revealed gradual increase in growth from fresh day $2.9\times10^3$ to $4.1\times10^3$ at 60$^{th}$ day.

**Keywords:** Spiced fish sauce; Storage; LAB's fermentation; Physicochemical properties

## Introduction

India is the second largest producer of marine water fish and the second largest producer of fresh water fish in the world. Fish production has increased from 41.57 lakh tonnes (24.47 lakh tonnes for marine and 17.10 lakh tonnes for inland fisheries) in 1991-92 to 86.66 lakh tonnes (33.71 lakh tonnes for marine and 52.95 lakh tonnes for inland fisheries) in 2011-2012 [1].

Fish is a rich source of animal protein and its culture is an efficient protein food production system from aquatic environment. The main role of fish culture is its contribution in improving the nutritional standards of the people. Fish and fishery products represent a valuable source of nutrients of fundamental importance for diversified and healthy diets. Fish provides not only high-value protein, but also a wide range of essential micronutrients, including various vitamins (D, A and B), minerals (including calcium, iodine, zinc, iron and selenium) and polyunsaturated omega-3 fatty acids (docosahexaenoic acid and eicosapentaenoic acid) [2,3]. The nutritive value of fish proteins is comparatively high because of the favorable essential amino acid pattern. Fish proteins are rich in all the essential amino acids (particularly methionine and lysine), in contrast with most proteins from plant sources, which lack adequate amounts of one or more essential amino acids. As compared with red meat, fish proteins are considered nutritionally equivalent or slightly superior [4].

Fermentation is one of the oldest techniques in food preservation as it not only extends the shelf-life but also enhances the flavour and nutritional quality of the product [5]. In Southeast Asia, fish sauce is made from various types of fish, from both freshwater and marine fish species, by various methods. Each has its unique taste and characteristic. Fish sauce is known as '*nampla*' in Thailand, '*patis*' in Philippines, '*kecapikan*' in Indonesia, '*budu*' in Malaysia, '*nuocnam*' in Vietnam, '*toeuk trey*' in Cambodia and '*nganpyaye*' in Myanmar [6-9].

Fish sauce is a translucent amber liquid with a unique aroma and flavour and is rich in amino acids. Fish sauce is basically a protein hydrolysate that results from a natural fermentation of fish and salt [9]. Protein hydrolysis is induced by endogenous proteinases in fish muscle and digestive tract as well as proteinases produced by halophilic bacteria [10,11]. Degradation of fish protein to free amino acids is primarily responsible for the delicious taste of fish sauce [12]. Various volatile compounds, including acids, carbonyls, nitrogen-containing compounds, and sulfur-containing compounds, are formed during fermentation and believed to be responsible for the distinct aroma of fish sauce [13,14]. Fish sauce has been used as a condiment and an important ingredient in Southeast Asia cooking. In addition, fish sauce is also a rich source of essential amino acids especially lysine. Many vitamins and mineral were also found in fish sauce. Fish sauce is a very good source of vitamin B12 and many minerals such as sodium (Na), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn) and phosphorus (P) [15]. Fish sauces contain about 20 g/l of nitrogen, of which 80% is in the form of amino acids; thus they may be considered an important sources of protein [16].

Spices and herbs have been used for thousands of centuries by many cultures to enhance the flavor and aroma of foods. Early cultures also recognized the value of using spices and herbs in preserving foods and for their medicinal value [17].

In the view of the importance of freshwater fish like Catla, to reduce the post-harvest losses and to increase the production of processed fish

products in country, the sincere effort were taken to carry the mentioned study in laboratory. Hence the results are narrated here.

## Materials and Methods

### Preparation of plain and spiced fish sauce

The plain fish sauce was prepared by using sweet water fish variety i.e., Catla (Indian major carp). Firstly the fish was washed and eviscerated then clean by water to remove blood clots and other unwanted parts. The fish was cut into small strips of required size and shape then mixed with salt in 3:1 proportion and mixed well. The mixture was kept in glass chamber and the lid was closed tightly, to avoid air contamination. The mixture was allowed to ferment for 2 months, after fermentation clear amber liquid i.e., plain fish sauce was extracted from fermented mass and used for further study. The spiced fish sauce was prepared by using plain fish sauce 1 kg, Wheat 1.5 kg, Cumin seeds 500 g, coriander 1 kg, mustard 1 kg, fennel seed 750 g, black pepper 100 g and water 8 litre [18]. All spices were roasted, ground and then mixed thoroughly with plain fish sauce.

The spiced sauces were prepared by using three recipes made i.e., T1, T2 and T3 and the spice mix was prepared according to standard recipe adopted from [18]. The recipe was standardized by using 10 g of plain fish sauce and 80 ml water with 30, 40 and 50 g spice mix in T1, T2 and T3 respectively. The panel of semi-trained judges consisting of 25 members was given the spiced fish sauce samples for evaluation of organoleptic characteristics viz. colour, taste, flavour and overall acceptability. After organoleptic assessment of spiced fish sauce, the spiced sauce containing 40 g spice mix having a best sensory acceptability than remaining other two samples.

### Physicochemical analysis of spiced fish sauce

Chemical constituents like moisture, fat, protein, carbohydrate, total ash, salt, TSS, TS and total organic content and pH were determined by AOAC (for salt only) [19,20] and Ranganna [21].

**Determination of moisture:** Moisture was estimated by accurately weighing the 5 g sample, it was ground and subjected to oven drying at 105°C for 4 hr. It was again weighed after cooling in desiccators until constant weight. The resultant loss in weight was calculated as moisture content [19].

**Determination of fat:** 5 g ground de-moisturised sample was weighed accurately in thimble and defatted with petroleum ether in Soxhlet apparatus for 6-8 hrs. at 60°C. The resultant ether extract was evaporated and lipid content was calculated [19].

**Determination of protein:** Protein was determined by micro-Kjeldahl method using 0.2 g of sample by digesting the same with concentrated sulfuric acid ($H_2SO_4$) containing catalyst mixture for 3-4 hrs at 70°C. Then it was distilled with 40 per cent NaOH and liberated ammonia was trapped in 2 per cent boric acid containing methyl red indicator and then it was titrated with 0.01 N $H_2SO_4$. The per cent nitrogen was calculated and protein percentage was estimated in the sample by multiplying with appropriate factor. To calculate protein content %N was determined by given formula [19].

**Determination of carbohydrate:** Total carbohydrate content of foods has, for many years, been calculated by difference, rather than analysed directly. Under this approach, the other constituents in the food (protein, fat, water, alcohol, ash) are determined individually, summed and subtracted from the total weight of the food. This is referred to as total carbohydrate by difference and is calculated by the following formula:

100-(Weight in grams [protein+fat+water+ash+other constituent] in 100 g of food)

It should be clear that carbohydrate estimated in this fashion includes fibre, as well as some components that are not strictly speaking carbohydrate, e.g. organic acids [22].

**Determination of ash:** 5 g sample was weighed into silica crucible and heated at low flame till all the material was completely charred and cooled. Then it was kept in muffle furnace for about 4 hr at 600°C. It was again cooled in desiccator and weighed and repeated until two consecutive weights were constant. The per cent ash was calculated by knowing the difference between the initial and final weight [19].

**Determination of total organic acidity:** A total acidity was determined as a total organic acidity Take 25-40 ml of the sauce, (previously filtered to remove suspended matter if any) in a 200 ml flask, add 25-50 ml water if desired and titrate against standard Sodium hydroxide solution using phenolphthalein as indicator till a faint pink color persists for 15 seconds. Calculate acidity by using a standard formula [23].

**Determination of salt:** Weigh 10 g sample was taken into 250 ml Erlenmeyer flask. Add 20 ml of the standardized 0.1 N $AgNO_3$ or more than enough to precipitate all Cl as AgCl. Add 20 ml of $HNO_3$. It was boiled gently on a hot plate until all solids except AgCl solids dissolve (usually 15 min). Cooled, then 50 ml $H_2O$ and 50 ml indicator was added and titrated with 0.1 N $NH_4SCN$ solution until solution becomes permanent light brown. Subtracted the ml 0.1 N $NH_4SCN$ used from ml 0.1 N $AgNO_3$ added and calculate difference as NaCl. With 10 g sample each ml 0.1 N $AgNO_3$ = 0.058% NaCl [20].

**Determination of total soluble solids**: Total soluble solids were measured by using hand refractometer (Erma Japan) and reported as °Bx. The TSS of sauce was affected by the concentration of salt, protein, fat and minerals in the sauce.

**Determination of viscosity:** Viscosity was determined to check the flowing nature and thickness or viscosity (resistance to flow) of sauce which is one of the quality criteria of fish sauce, determined by using the Brookfield viscometer DV-E at different speed and at constant temperature 25°C with a spindle number S-62 and it were expressed in terms of centipoises (cP).

## Result and Discution

### Effect of storage (ambient temperature) on physicochemical properties of spiced fish sauce

The storage of spiced sauce at ambient temperature affects the physicochemical properties of fish sauce. The study involves the effect of storage on physicochemical properties of spiced sauce and the results were noted on 15 days period intervals up to 60$^{th}$ day.

The moisture content of spiced fish sauce indicates the prime quality parameter and it drastically affects the shelf life of product and also other components of food. The results revealed the moisture content of spiced was gradually increased and the values narrate to moisture was from fresh day (65.81 g/100 g) to 60$^{th}$ day (66.20 g/100 g). The increase in moisture content of spiced sauce may be the effect of degradation and hydrolysis of protein and carbohydrate during storage by fermentation of lactic acid bacteria's. The fat percentage was slightly

decreased during storage period from fresh (6.20 g/100 g) to 60th day (6.08 g/100 g). The similar results pertaining to fat changes during storage were observed by Magdi. The protein content of spiced sauce in the Table 1 demonstrates that the protein content was drastically increased during storage. The data exists to protein shows that it was increased from fresh (5.72 g/100 g) to 60th day (6.25 g/100 g). The steady increase in protein concentration of spiced fish sauce was the effect of increase in bulk concentration of microbes in the sauce. The similar findings were quoted in Nicole. scientific literature. The data pertaining to carbohydrates in the Table 1 prove that the carbohydrate steadily decreased during storage from fresh (16.15 g/100 g) to 60th day (15.10 g/100 g). The ultimate results states that carbohydrate content was decreased during storage and this may the effect of utilization of carbohydrate by microbes in the sauce. The similar results were noted by Magdi. The total ash content of sauce indicates the concentration of minerals in the sauce. It could be observed from the Table 1 that the ash content of sauce was found between 2.88 g/100 g to 2.90 g/ 100 g while during study. The salt content of spiced sauce was not much affected during storage. And it was constantly between 3.20 g/100 g to 3.18 g/ 100 g.

| No. | Characteristics | Values in g/100 g | | | | | Mean | SE ± |
|---|---|---|---|---|---|---|---|---|
| | | Fresh | 15 days | 30 days | 45 days | 60 days | | |
| 1 | Moisture | 65.81 | 65.85 | 65.89 | 65.95 | 66.2 | 65.94 | 0.0191 |
| 2 | Fat | 6.2 | 6.16 | 6.15 | 6.12 | 6.08 | 6.142 | 0.0103 |
| 3 | Protein | 5.72 | 5.79 | 5.84 | 5.97 | 6.25 | 5.914 | 0.0129 |
| 4 | Carbohydrates | 16.15 | 15.87 | 15.7 | 15.57 | 15.1 | 15.678 | 0.0214 |
| 5 | Salt | 3.2 | 3.2 | 3.19 | 3.18 | 3.18 | 3.19 | 0.0115 |
| 6 | Total Ash | 2.9 | 2.9 | 2.89 | 2.88 | 2.88 | 2.89 | 0.0096 |
| 7 | pH | 5.62 | 5.45 | 5.32 | 5.1 | 4.87 | 5.272 | 0.0187 |
| 8 | Total organic acidity | 1.05 | 1.08 | 1.12 | 1.17 | 1.23 | 1.13 | 0.032 |
| 9 | Total soluble solids | 21 | 22 | 22 | 23 | 23 | 22.2 | 1.0645 |
| 10 | LAB's | $2.9\times10^3$ | $3.1\times10^3$ | $3.4\times10^3$ | $3.6\times10^3$ | $4.1\times10^3$ | - | - |

**Table 1:** Effect of storage (ambient temperature) on physicochemical properties of spiced fish sauce (*Each value represents the mean of three determinations).

The pH of spiced sauce was gradually decreased during storage and it affected the quality of sauce, the pH declined drastically affected the micro flora of sauce. The pH of sauce during storage was noted from fresh (5.62) to 60th day (4.87). The results reveals that fall in pH was due to increase in acidity of sauce and this may be the effect of lactic acid fermentation during storage. Also decline in pH was indications of increase in LAB's and decrease in other pH dependent micro flora. The total organic acidity of fish sauce refers to the lactic acid dominantly and the results were noted on first day 1.05 and 60th day 1.23 of study.

The total soluble solids content shows the amount of soluble fraction of food components in the product. The data related to total soluble solids from Table 1 expresses that TSS of sauce was fairly decreased during storage from fresh (21° Bx) to 60th day (23° Bx). The increase in TSS of sauce may be due to degradation and hydrolysis of carbohydrate and fat during storage by microbes.

The crucial factor for the changes occurred in physicochemical properties of spiced fish sauce during storage was ongoing fermentation. The halophilic LAB's was found dominant at the final stage of fermentation, coinciding with a period when color, aroma and flavor are fully developed. This has led to an assumption that halophilic LAB's could play a significant role in the distinct characteristics of fish sauce [10]. According to Jedah et al. [18] the addition of spices can act as stimulants for the growth of LAB's. Also LAB's are able to improve the shelf life of several food products. Result concerning to the LAB's count during storage has noted in the above Table 1 revealed that the LAB's count was progressively increased during storage right from the fresh day ($2.9\times10^3$ cfu/ml) to 60th day ($4.1\times10^3$ cfu/ml). Similar interpretations were found in the work (Figure 1) [18].

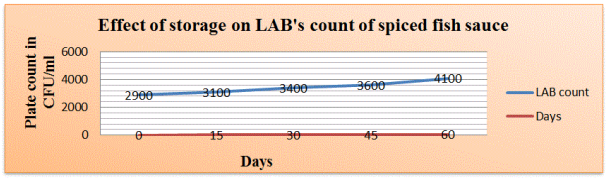


**Figure 1:** Effect of storage on LAB count of spiced fish sauce.

The viscosity of sauce (other viscous products also) is a major physical quality parameter in respect to consumer acceptance. Therefore, reliable and accurate rheological data are necessary for designing and optimization of various foods processing equipment. The viscosity of sauce was initially increased by addition of wheat flour. The various parameters affect the viscosity of food viz. temperature, chemical composition, storage and etc., during the study the effect of storage was carefully considered on fresh to 60th day by 15 days intervals regularly.

It could be observe from Table 2 that the viscosity was constantly decreased during storage. The results obtained from the table shows that the viscosity was gradually decreased from fresh (282.0 cP) to 60$^{th}$ day (260.4 cP). The change in viscosity during storage may be the effect of fermentation and hydrolysis of food components. Similar results were expressed by Beal et al. (Figure 2) [24].

| No. | Days | Viscosity in Centipoise (cP) |
|---|---|---|
| 1 | Fresh | 282.0 |
| 2 | 15 | 278.2 |
| 3 | 30 | 275.3 |
| 4 | 45 | 268.6 |
| 5 | 60 | 260.4 |
| 6 | Mean | 272.9 |
| 7 | SE ± | 3.1166 |

**Table 2:** Effect of storage (ambient temperature) on Viscosity of spiced fish sauce (*Each value represents the mean of three determinations).

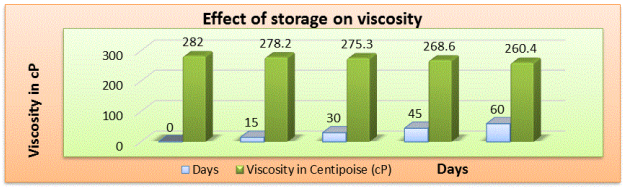


**Figure 2:** Effect of storage (ambient temperature) on viscosity of spiced fish sauce.

## Conclusion

A good quality spiced fish sauce can prepare by using given formulation and mature for valid time which carries various changes during storage or alteration of physicochemical properties of sauce due to the fermentation. The change in properties was effect of fermentation and degradation of chemical components of sauce during storage by fermentation. Also it causes loss of viscosity of product at the end of study.

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