ORIGINAL ARTICLE

Antioxidant Activities and Sensory Evaluation of Ngari, A Non-Salted Traditionally Fermented Fish, During Different Fermentation Period

Somishon Keishing[1*] and Thahira Banu A[2]

[1]Ph.D., Department of Home Science, G.P Women's College, Dhanamanjuri University, Imphal, Manipur, India.
[2]Ph.D., Department of Home Science, Gandhigram Rural Institute-Deemed to be University, Tamil Nadu, India.

ABSTRACT **Background:** Ngari is a non-salted fermented fish product of Manipur, situated in the northeastern part of India. It is known for its unique taste and aroma in Manipuri cuisine. It has occupied a major role in enhancing the taste of Manipuri foods and is considered an essential ingredient in every Manipuri kitchen. Sundried fish, Puntius sophore (Ham) is typically used in the fermentation of ngari. Though extensively used, data on antioxidant activity and sensory quality of the product are not available in a systematic way for a prolonged period of fermentations. **Aim:** The main aim of the present study is to analyse the Antioxidant activity and sensory quality of dry fish used as raw material for processing ngari and matured at different periods (0, 3, 6, 9, and 12 months). **Methods and Material:** The antioxidant of the products was determined based on the scavenging activity by DPPH, H_2O_2 and FRAP. Sensory characteristics of the ngari samples were evaluated using 9 points hedonic scale. **Results and Conclusion:** The antioxidant activity increased markedly after the fermentation process, and on prolonged fermentation, its activity increased. DPPH radical scavenging activity, Hydrogen peroxide H_2O_2 scavenging activity, and Ferric Reducing Antioxidant Power (FRAP) ranged from 40% to 98%, 70% to 87%, and 34 to 90 mg/g of AAE respectively. The sensory qualities improved as the fermentation period increased. The results suggest that prolonging fermentation periods could bring about improved antioxidant activity and sensory characteristics of the ngari samples.

Keywords: Ngari, *Puntius sophore*, Fermented fish, Antioxidants, Sensory quality

Address for correspondence: Somishon Keishing, Ph.D, Department of Home Science, G.P Women's College, Dhanamanjuri University, Imphal, Manipur, India. E-mail: soshonkeishing@gmail.com

Submited: 09-Feb-2022 **Accepted:** 04-Jul-2022 **Published:** 29-Aug-2022

INTRODUCTION

Fermentation is a natural process that unavoidably affects the human food supply worldwide, and fermented foods have worldwide acceptance that arises in the human relationship to the microbial environment.[1] Fermented foods and beverages are consumed as regular food items in Manipur, one of the states in Northeast India. The people of Manipur consumed different types of fermented food prepared traditionally at home. They have contributed significantly to the food supply chain and dietary patterns. Bamboo shoot (soibum, soidon), soya (Hawaijar), fish (Ngari, hentak), and mustard leave (inziangsang dui) are some of the commonly consumed fermented foods, particularly in Manipur. Among the fermented foods, fish-based products are extensively consumed from time immemorial. Ngari is a non-salted fermented fish product known for its characteristics flavor and taste prepared with sundried *Puntius sophore (Ham)*. It forms an intrinsic part of the diet and an essential commodity amongst Manipur's people.[2, 3] No starter culture was added during its preparation; it is a naturally fermented product.

Studies have evidenced that fermented food products possess antioxidant properties, a good source of peptides[4] and peptides of fermented fish products act as an excellent antioxidant.[5] The antioxidant activity of Philippine salt-fermented shrimp paste increased with prolonged fermentation time.[6] Antioxidant activity in several fermented

Access this article online
Website: www.ijfans.org
DOI: 10.4103/ijfans_218_22

How to cite this article: Somishon Keishing and Thahira Banu A. Antioxidant Activities and Sensory Evaluation of Ngari, A Non-Salted Traditionally Fermented Fish, During Different Fermentation Period. Int J Food Nutr Sci 2022; 11:182-187.

fishery products such as fermented blue mussels, fish sauces, fermented mussel sauce, fermented shrimp paste, and Thai fermented shrimp and krill products were recorded to be potential sources by several researchers. [7, 8, 9, 10] Fermented fish products will have a higher content of small peptides and amino acids because of hydrolysis, which possesses antioxidant properties.[11]

Studies on fermented fish have established substantial evidence of promising health benefits. Many investigators have reported that the antioxidants activity of ngari increased considerably during fermentation when compared to its unfermented counterparts. However, findings of the same on different intervals of fermentation periods during prolonged maturation time are scanty. Although ngari is very popular, studies on sensory evaluation and consumer acceptability are not carried out so far during the periods of fermentation. Therefore, the present study aimed to investigate the changes in ngari regarding its antioxidant activity and sensory characteristics during the fermentation period.

MATERIALS AND METHODS

Sample Collection

Sundried fish (*Puntius sophore*) locally known as phabou nga was purchased from the local market and was segregated by cleaning debris. Segregated sundried fish was fermented traditionally by skilled artisans. The sundried fish and fermented fish samples collected at 3, 6, 9, and 12 months were analyzed for antioxidant activity and evaluated for sensory characteristics.

Methods

Determination of Antioxidant Activity

Preparation of Aqueous Extracts: One gram of the ngari samples (dry fish, 3, 6, 9, and 12 months fermented fish) was grounded and blended with 20 ml of aqueous water (4:1) and placed in an incubator at 45 °C for 6 hours at 150 rpm and filtered. The filtrate was kept in freezing condition and the aqueous extract was used for the determination of antioxidant activity. Butylated Hydroxy Toluene (BHT) was used as the standard.

DPPH Radical Scavenging Activity

The antioxidant of the extracts, based on the scavenging activity of the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radicals, was determined by the method described by Braca *et al.* (2001).[12] The aqueous extract (0.1 ml) was added to 3 ml of a 0.001 M DPPH in methanol. After 30 minutes the absorbance at 517 nm was determined, using the formula and the percent inhibition of activity was calculated as:

(Ao – Ae)/Ao)/100

Ao = absorbance without extract; Ae = absorbance with extract)

Hydrogen Peroxide Method (H_2O_2) Radical Scavenging Assay

Hydrogen peroxide radical scavenging assay was determined as described by Ruch *et al.* (1989).[13] A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extract (500 ml) were added to hydrogen peroxide solution (0.6 ml). The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide and compared with ascorbic acid, the reference compound.

% Scavenged (H_2O_2) = [(AC – AS)/AC] × 100

A.C. is the absorbance of the control, and AS is the absorbance in the extracts or standards.

Ferric Reducing Antioxidant Property Assay (FRAP)

FRAP was assayed as per the methods of Benzie and Strain (1999).[14] Antioxidant activity of the standard was estimated by measuring increased absorbance caused by ferrous ions. The working FRAP reagent was made of 0.3 M acetate buffer (pH 3.6), 10 mM TPTZ (2, 4, 6-tri (2-pyridyl)-S-triazine), 40mM Hcl and 20 mM $Fecl_3$ $6H_2O$ in the ratio of 10:1:1 (freshly prepared and warmed to 37 °C). 2.7 ml of this working solution was mixed with 100 µl of sample extract to initiate the reaction. Absorbance was recorded after 10 min at 593 nm and the antioxidant capacity was expressed as ascorbic acid equivalent.

Sensory Evaluation and Consumer Acceptability of the Ngari Samples

The fermented fish collected at 3, 6, 9, and 12 months were evaluated for physical and sensory characteristics. Semi-trained panel members from Manipur who were familiar with ngari evaluated the physical characteristics by using quality scores for assessing the fermented fishes as described by Mahanta and Muzaddadi.[15] The appearance, color, flavor, texture, and overall acceptability were evaluated using the nine points hedonic scale. Fermented fish (ngari) purchased from the market was used as a standard. The color of the products was recorded based on visual observation and texture by applying pressure with fingertips.[16]

Consumer Acceptability of the Ngari Samples

The 9-point hedonic scale is used to evaluate the Consumer acceptability of the products. Fifty women of Imphal,

Manipur, who regularly prepared food for the family, were engaged in assessing the overall acceptability of the food product.

Statistical Analysis

All the analysis was performed in triplicate. Statistical Packages for Social Science (SPSS 20th version) was used for the statistical analysis. The data were presented in mean±SD and the groups were compared by using ANOVA for the expression of the results.

RESULTS AND DISCUSSION

In vitro Antioxidant Activity of the Dry Fish and Ngari Samples

Water-soluble fractions of the ngari samples possessed good antioxidant activity, and the changes obtained at various fermentation times as determined by DPPH, H_2O_2 radical scavenging activities, and FRAP are depicted in Table 1.

DPPH Radical Scavenging Activity

The present investigation has shown that the extracts of all the ngari samples exhibited DPPH scavenging activity. The most effective being the 12 months fermented ngari, which exhibited significantly higher DPPH scavenging activity of 98.7±1.05% followed by nine months fermented ngari (98.2±0.5%) and 6-month fermented ngari (84.8±1.68%). The radical scavenging activity of dry fish (40.74±0.06%) increased during the fermentation process showing the role of fermentation in enhancing antioxidant activity. The percentage of scavenging activity of the 6, 9, and 12 months fermented ngari extracts was higher than that of the standard BHT (70.22±0.40%). The radical scavenging activity increases as the period of fermentation increases and is in line with the findings of other investigators studying kapi, miso, and anchovy sauce which may have been influenced by fermentative products as a result of the prolonged fermentation process.[17, 18, 19] The current study indicates that the DPPH scavenging activity significantly (P<0.001) varied within dry fish, and the ngari samples were fermented for a different period.

Hydrogen Peroxide (H_2O_2) Scavenging Activity

The result indicates the hydrogen peroxide scavenging activity as 70±1.2%, 84.6±2.1, 81.0±1.0 86.0±2.3%, and 87.6±2.6 for dry fish, 3, 6, 9, and 12 months ngari respectively. The most robust H_2O_2 activity was observed in the F12 sample collected 12 months after the fermentation period. In general, all the fermented samples showed prominent H_2O_2 inhibition activity above 80 percent. In the six-month fermented sample, there was a sudden decrease in its scavenging power which increases prominently at 9 and 12-month fermentation periods. The results show that the hydrogen peroxide scavenging activity was not significant at (P<0.01).

The inhibition of hydroxyl radicals by fish *miso* extracts indicated that the substrate responsible for hydroxyl radical scavenging rapidly developed during the early stages of fermentation and in the advanced stages of fermentation (over 135 days) for fish miso stored at 25 °C, the scavenging activity mostly remained stable.[18] The antioxidant activity could have been due to the fermentative products', like peptides, amino acids, and other low molecular compounds that possessed antioxidant properties.[20]

Ferric Reducing Antioxidant Power (FRAP)

The results showed that the ferric reducing ability was prominent in F12 months fermented sample (90.5±1.9

Table 1: *In vitro* Antioxidant Activity of the Dry Fish and Ngari Samples

Sample	DPPH (%)	H_2O_2	FRAP (mg/g of AAE)
PF	40.7 ± 0.06	70.0 ± 1.2	34.0 ± 2.3
F3	64.9 ± 2.88	84.6 ± 2.1	46.6 ± 1.4
F6	84.8 ± 1.68	81.0 ± 1.0	54.9 ± 1.4
F9	98.2 ± 0.50	86.0 ± 2.3	88.0 ± 1.7
F12	98.7 ± 1.05	87.6 ± 2.6	90.5 ± 1.9
F	36.917	4.378	8.35
P	0.000***	0.027*	0.003**
BHT	70.22 ± 0.40	66.60 ± 0.90	2.30 ± 1.60

Note: PF: Pre-fermented dry fish, F3: Fermented for 3 months, F6: Fermented for 6 months, F9: Fermented for 9 months, F12: Fermented for 12 months. Results are means ± standard deviation (n = 3). *** Significant at 0.001% level.

mg/g of AAE) followed by F9 (88.0±1.7 mg/g of AAE), F6 (54.9±1.4 mg/g of AAE), F3 (6.6±1.4 mg/g of AAE) and dry fish (34.0±2.3 mg/g of AAE). It is evident from the table that the reducing ability increased with the duration of fermentation. The results indicate that the ferric reducing ability was not significant at ($P<0.01$). Another investigator has reported that FRAP significantly increased with the increase in the protein concentration and fermentation period in ngari and suggested their capability of providing the electron.[11]

The study reported that all the selected ngari samples showed good antioxidant activity. The F12 sample fermented for 12 months showed the maximum DPPH, H_2O_2, and FRAPS activity. The antioxidant assay results imply that fermentation could have contributed to a marked increase in its initial antioxidant activity.[20] It was observed that there were differences in their antioxidant activities when determined with different assays suggesting that the peptides responsible for its activities have a different mode of action.

Physical Characteristics of the Dry Fish and the Ngari Samples

The physical characteristics and sensory qualities are essential aspects considered for the quality of a product. Semi-trained panelists studied the physical characteristics of the pre-fermented dry fish and fermented fish (ngari) collected at different maturation periods. The findings are depicted in the following Table 2. It is clear from the table that the physical characteristics of dry fish (*Puntius sophore*) changed drastically from dry hard surface to moist, soft, and sticky yet intact in its shape during the fermentation. The putrid smell of the dry fish transformed into a good flavor and aroma at the final stage of fermentation. Further, there was a gradual change in the color of the dry fish from silvery color to pale brown, brownish, and finally golden brown.

Similar improvement was reported in fermented shidol fish characteristics at different fermentation periods.[21]

Sensory Characteristics of the Ngari Samples

The fermented fish samples F3, F6, F9, and F12 collected at 3,

Table 2: Physical Characteristics of Dry Fish and Ngari in a Different Stage of Fermentation

Fermentation Periods	Characteristics of the Product	Remark
Dry fish (P.F.)	Dry hard surface and texture, silvery fish color	---
Three months (F3)	Dry fish like no characteristic smell of ngari, hard texture and no color change	The initial stage of fermentation
Six months (F6)	Moderate characteristic ngari smell, moist with a slightly soft and sticky surface, softened texture and color change to pale brown	Under-fermented, pre-mature stage, low quality but acceptable
Nine months (F9)	Characteristic ngari smell, moist and sticky surface, soft texture with the shape of the fish intact and brownish.	Matured, good quality
12 months (F12)	Strong characteristics ngari smell, moist and sticky surface, soft texture, and golden brown color.	Matured, high quality

Table 3: Sensory Quality of the Selected Ngari Samples

Products	Appearance	Colour	Flavor	Texture	Overall Acceptability
Standard	8.40±0.55	8.40±0.55	8.40±0.55	7.80±0.84	7.80±0.84
F3	1.60±0.55	1.60±0.55	1.80±0.45	2.00±0.71	2.20±0.84
F6	5.00±0.71	6.00±1.00	6.40±0.89	5.20±1.92	6.00±1.58
F9	7.00±1.22	7.40±0.55	7.80±0.45	7.60±0.55	7.80±0.45
F12	8.40±0.55	8.20±0.45	8.60±.55	8.80±0.45	8.00±1.00
F	70.793	93.476	110.278	34.593	29.745
P	0.000***	0.000***	0.000***	0.000***	0.000***

Note: All traits measured on the 9-point scale, with one being least and nine being the most. F3: Fermented for 3 months, F6: Fermented for 6 months, F9: Fermented for 9 months, F12: Fermented for 12 months; Results are means ± standard deviation (n = 5). ***Significant at 0.001% level.

Table 4: Consumer Acceptability of the Ngari Samples

Products	Appearance	Colour	Flavor	Texture	Overall Acceptability
Standard	7.52±1.01	7.46±0.97	7.54±0.89	7.55±0.99	7.62±0.75
F3	1.64±0.53	1.44±0.58	1.60±0.70	1.68±1.22	1.56±0.64
F6	4.34±1.56	4.30±1.50	4.29±1.24	4.62±1.21	4.74±1.34
F9	6.32±0.94	6.36±1.14	6.40±1.09	6.32±1.02	6.74±0.96
F12	7.62±0.81	7.66±0.82	7.76±0.87	7.40±0.83	7.66±0.99
F	302.731	308.028	281.751	263.326	357.278
P	0.000***	0.000***	0.000***	0.000***	0.000***

Note: UF: Unfermented dry fish, F3: Fermented for 3 months, F6: Fermented for 6 months, F9: Fermented for 9 months, F12: Fermented for 12 months; Results are means ± standard deviation (n = 50). ***Significant at 0.001% level.

6, 9, and 12 months of fermentation respectively were evaluated for their sensory quality using 9 points hedonic scale. The mean sensory scores of ngari samples are shown in Table 3. The results showed that attributes such as appearance, color, flavor, and texture of the standard and ngari samples (F3, F6, F9 and F12) had significant variation except for variations F12 and standard. The highest mean score for appearance was 8.40±0.55 for the variation F12 and standard, followed by the variation F9 with 7.00±1.22. This F12 sample had the maximum score for all the attributes followed on F9, F6, and the least score registered for the F3 specimen. The result indicates that the time of fermentation has a positive effect on the appearance of the ngari product, and it imparts desirable sensory characteristics. It is statistically proven that there exists a significant difference in appearance, color, texture, and overall acceptability of the ngari samples fermented for 3, 6, 9, and 12 months. Better sensory characteristics were exhibited in samples fermented for more extended and optimum periods and statistically proven that there exists a significant difference ($P<0.001$) in the sensory attributes of the standard ngari and samples of 3, 6, 9, and 12 months maturation time. Similar results were reported in the production of shidol (fermented fish of Assam), where the sensory attributes improved gradually from 30 days to 180 days of fermentation.[21]

Consumer Acceptability of the Ngari Samples

Fifty untrained panel members were selected for the evaluation of consumer acceptability of the ngari samples. The results are given in the following Table 4. One of the essential attributes in selecting ngari among the selected consumers was the flavor, and the highest score reported for F12 (7.66±0.99) and hence was the most preferred. A significant difference was observed in the overall acceptability score of F12 (7.66±0.99) and the F3 sample (1.56±0.64). The score for all the attributes, namely color, texture, flavor, taste, appearance, and the overall acceptability of ngari samples, increased with increasing fermentation.

CONCLUSION

The fermented fish (Ngari) are fermented by the people from *Puntius sophore* and eaten by Manipuris' people since time immemorial for its taste and aroma. The present study revealed that prolonged fermentation of ngari could enhance the antioxidant activity and sensory qualities throughout the 12 months. Among the ngari samples, 9 and 12 months fermented ones were found to have a high antioxidant capacity and good sensory quality. The present results may help to shed light on further studies in exploring its therapeutic and functional properties, which is the need of the hour.

REFERENCES

1. Tanasupawat S. and Visessanguan W. (2014). Fish Fermentation. In: Boziaris IS Seafood processing: technology, quality, and safety, Wiley Blackwell, Oxford, pp. 182-185.
2. Das A. J. and Deka S. C. (2012). Fermented foods and beverages of North-East India. Intl Food Res Journal; 19(2): pp. 377-392.
3. Sarojnalini C. H. and Suchitra T. (2009). Microbial profile of starter culture fermented fish product 'Ngari' of Manipur. Indian J Fish; 56(2): pp. 123-127.
4. Shimada K., Fujikawa K., Yahara K. and Nakamura T. (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agri Food Chem; 40(6): pp. 945-948.
5. Majumdar R. K., Roy D., Bejjanki S. K. and Narayan B. (2016). Chemical and microbial properties of shidal, a

traditional fermented fish of Northeast India. J Food Sci Tech; 53(1): pp. 401-410.

6. Peralta E. M., Hatate H., Kawabe D., Kuwahara R., Wakamatsu S., Yuki T. and Murata H. (2016). Improving antioxidant activity and nutritional components of Philippine salt-fermented shrimp paste through prolonged fermentation. Food Chem; 111(1): pp. 72-77.
7. Harada K., Okano C., Kadoguchi H., Okubo Y., Ando M., Kitao S. and Tamura Y. (2003). Peroxyl radical scavenging capability of fish sauces measured by the chemiluminescence method. Interl J Mol Med; 12(4): pp. 621-625.
8. Rajapakse N., Mendis E., Jung W. K., Je J. Y. and Kim S. K. (2005). Purification of a radical-scavenging peptide from fermented mussel sauce and its antioxidant properties. Food Res Interl; 38(2): pp. 175-182.
9. Peralta E. M., Hatate H., Watanabe D., Kawabe D., Murata H., Hama Y. and Tanaka R. (2005). Antioxidative activity of Philippine salt-fermented shrimp and variation of its constituents during fermentation. J Oleo Sci; 54(10): pp. 553-558.
10. Faithong N., Benjakul S., Phatcharat S. and Binsan W. (2010). Chemical composition and antioxidative activity of Thai traditional fermented shrimp and krill products. Food Chem; 119(1): pp. 133-140.
11. Phadke G., Elavarasan K. and Shamasunder B. A. (2014). Angiotensin-I converting enzyme (ACE) inhibitory activity and antioxidant activity of fermented fish product Ngari influenced by the fermentation period. Int J Pharm Bio Sci; 5(2): pp. 134-142.
12. Braca A., De Tommasi N., Di Bari L., Pizza C., Politi M. and Morelli I. (2001). Antioxidant principles from Bauhinia tarapotensis. S J Nat Prod; 64: pp. 892-895.
13. Ruch R. J., Cheng S. J. and Klaunig J. E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis; 10(6): pp. 1003-1008.
14. Benzie I. F. and Strain J. J. (1999). Ferric reducing/ antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. In: Methods in enzymology; 299, Academic Press, pp. 15-27.
15. Mahanta P. and Muzaddadi A. U. (2012). Post-fermentation preservation of Shidal-A fermented fish product of North-East India. Fishery technol; 49(2), p. 177.
16. Lilabati H. and Viswanath W. (1999). Changes in Bacterial and fungal quality during storage of smoked esomus danricus of Manipur. Fishery technol; 36(1).
17. Majumdar R. K., Bejjanki S. K., Roy D., Shitole S., Saha A. and Narayan B. (2015). Biochemical and microbial characterization of Ngari and Hentak-traditional fermented fish products of India. J Food Sci Tech 52(12): pp. 8284-8291.
18. Singh S. S., De Mandal S., Lalnunmawii E. and Kumar N. S. (2018). Antimicrobial, antioxidant and probiotics characterization of dominant bacterial isolates from traditional fermented fish of Manipur, North-East India. J Food Sci-Tech.; 1: 55(5): pp. 1870-9.
19. Faithong N. and Benjakul S. (2014). Changes in antioxidant activities and physicochemical properties of Kapi, a fermented shrimp paste, during fermentation. J Food Sci Tech; 51(10): pp. 2463-2471.
20. Giri A., Nasu M. and Ohshima T. (2012). Bioactive properties of Japanese fermented fish paste, fish miso, using koji inoculated with Aspergillus oryzae. Int. J. Nutr. Food Sci; 1: pp. 13-22.
21. Muzaddadi A. U. (2015). Minimisation of fermentation period of shidal from barbs (*Puntius spp*.). Fishery Technol; 52: pp. 34-41.