Indian J. Fish., 70 (4): 150-157, 2023 doi:10.21077/ijf.2023.70.4.136119-16

Available online at: epubs.icar.org.in/index.php/IJF

Reduction in salt fermentation time of Indian mackerel (*Rastrelliger kanagurta*) using *Halobacterium salinarum* as a starter culture

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Abstract

Salt fermentation is a preservation technique in which higher salt concentrations are introduced to inhibit the growth of most spoilage microbes. This process takes several months to attain maturity. The present study was designed to accelerate the fermentation rate and shorten the maturation time employing the haloarchaeal strain *Halobacterium salinarum* (HS1). *H. salinarum* was inoculated in the salt fermentation medium and the fermentation time and quality were compared to the control salt fermentation without inoculation of HS1. The lot inoculated with HS1 matured by the 60th day with higher sensory scores, whereas the control (C) took 120 days for maturation, indicating that the salt fermentation process can be accelerated using haloarchaea. The final moisture content at the end of 120 days in the control and HS1 were 56.31 and 56.78% respectively. pH showed decreasing trend initially and was found to be stable in later phases. Protein content reduced from 24.29 to 18.95% and 15.03% in control and HS1 respectively. Fat content fluctuated throughout the fermentation period (6.99 to 8.81%) showing an increasing pattern initially and then a reduction during later stages of fermentation. Trimethylamine (TMA, 3.12 to 14.09 mg%), total volatile base nitrogen (TVBN, 18.23 to 38.35 mg%), alpha-amino nitrogen (AAN, 31.87 to 126.43 mg%) and non-protein nitrogen (NPN, 0.18 to 0.83%) contents increased throughout the salt fermentation process but were found to be within limits suggesting controlled degradation. Present results suggest that *H. salinarum* (HS1) as a starter culture can accelerate salt fermentation and maturation can be attained by 60 days compared to 120 days of maturation time taken by control without HS1.

Introduction

Fermentation is a preservation technique where microbes or inherent enzymes undertake controlled degradation of meat leading to formation of stable products (Zang *et al*., 2020; Belleggia and Osimani, 2023). In salt fermentation, a high concentration of salt is incorporated into the fermentation process and is one of the most commonly used preservation techniques worldwide. In India, *Iona ilish* is one of the most consumed salt fermented products, mainly in the north-eastern parts of India and a few regions of Bangladesh (Majumdar and Basu, 2010). Due to the higher price and lesser availability of hilsa fish used for the preparation of the *Iona ilish,* Indian mackerel, was found to be the best alternative for preparation of salt fermented product, owing to its availability and higher fat content (Uchoi *et al.,* 2021; Sukmawati *et al.*, 2023). One of the significant concerns in salt fermentation is the time taken for maturation. Salt fermentation using autocthonous microbes and enzymes will take around 4-6 months to get matured (Majumdar *et al*., 2006; Dhar *et al.*, 2021). Hence there is a need to reduce the maturation time and the present study focused on this issue.

Das *et al.* (2020) investigated the microflora of several salt fermented fishery products and reported higher archaeal abundance in the salt fermented fish products compared to bacteria. Since all the salted fish products had over 20% salt, they used media incorporated with over 20% salt and isolated 75% of archaea compared to 25% bacteria. They also opined that

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Keywords:
Haloarchaea, *Halobacterium salinarum,* Indian mackerel, Maturation, Salt fermentation

Received : 08.05.2023
Accepted : 14.12.2023

earlier studies that used 10% salt in the isolation media have likely to have missed the archaea. Salt fermented fish products usually contain more than 20% salt, thus needing an isolation procedure using more than 20% salt in the media. Haloarchaea are a group of microorganisms that grow optimally at higher salt concentrations (above 10%). Extremely halophilic, aerobic members of archaea are classified within the family Halobacteriaceae. Halobacteriaceae comprises 150 species in 40 genera (Oren, 2012). Most of them are known to live in environments like salt lakes or salterns with very high salt concentrations. It has recently been reported that halophilic archaea exist in all the salt-fermented fish products from India after analysing over 105 samples (Das *et al.,* 2020). In addition, metagenomic analyses also indicate an increasing presence of halophilic archaea not only in foods but also in many environments (Borrel *et al.,* 2020).

Halophilic archaea are also known to have a role in fermenting food products. It is reported that the addition of *Halobacterium* sp. SP1(1) reduced the fermentation period of fish sauce, in addition to having an improved amino acid profile which leads to better flavour (Akolkar *et al.*, 2010). The fascinating element is that in the case of salted anchovies added with haloarchaea, the production of histamine was inhibited during the initial ripening process (Aponte *et al*., 2010).

The present study aimed to reduce the time taken for the salt fermentation of mackerel and hypothesise that the addition of haloarchaea will reduce the fermentation time without affecting the quality of the product. In normal fermentation, the acclimatisation and growth of fermenting microbes take time as they compete with other microorganisms, leading to prolonged maturation period. In contrast, the addition of starter cultures can address this challenge. In the present study, *Halobacterium salinarum* (HS1) was used as the starter culture and changes were studied.

Materials and methods

Raw material

Indian mackerel (*Rastrelliger kanagurta*) were procured from Four Bungalows Fish Market, Mumbai, India. Care was taken to get fishes of similar sizes. The average length and weight of fish ranged from 22-25 cm and 190- 220 g, respectively.

Microbial strain and growth conditions

Halobacterium salinarum strain (MN000357) was used in this study which was isolated and characterised by Das *et al.* (2020). This culture was grown in a medium composed of casamino acids (7.5 g l^{-1}), yeast extract 10 g l^{-1}), KCl 2 g l^{-1}), trisodium citrate (3 g l^{-1}), $MgSO_4.7H_2O$ (20 g l^{-1}), $FeSO_4.4H_2O$ (0.0023 g l^{-1}), $MnSO_4.4H_2O$ (0.005 g l^{-1}), supplemented with 20% NaCl, pH 7.2 at 37ºC for 15 days in shaking incubator at 150 rpm. Cells were pelletised by centrifugation at 4000 rpm for 10 min, supernatant discarded, and replaced with an equal volume of 20% NaCl solution and total archaeal cell count was enumerated.

Fermentation procedure

The fish were cleaned, dressed and cut into 2-3 steaks. The pieces were then dry salted (fish to salt ratio 4:1, w/w) with crystallised unprocessed sea salt in PVC trays with salt and fish layers, covering the top layer with salt (Fig. 1). The trays were covered with a lid to prevent direct contact with sunlight and kept for 48 h to release moisture. After 48 h, the self-brine was drained and the salted fish pieces were cleared from adhering salt and tightly packed in two PVC containers of 5 l capacity. Each container was packed with

Fig. 1. Salt fermentation flow chart

around 3.5 kg of mackerel steaks. Saturated brine solution was boiled, cooled and poured upto the brim of the PVC container. One of the PVC containers was added with the 5 log CFU of 15 days old *H. salinarium* (HS1) at 1% of the mackerel weight (v/w). Second container without addition of starter culture served as the control. Both containers were closed tightly and kept at room temperature for maturation.

Sampling

Sampling was done at an interval of 15 days. Muscles from at least four specimens belonging to a single lot of each container were pooled in a petri-dish. Fresh pieces sampled prior to salting were considered as fresh fish samples and dry-salted pieces after 48 h were considered as 0^{th} day samples.

Proximate composition

Proximate composition in terms of moisture, crude protein, fat content and ash of the samples was determined as described in AOAC (2005). Moisture content was estimated by drying the fish muscle at 105°C in the oven until constant weight attained. Nitrogen content was estimated by the micro-Kjeldahl distillation method and the nitrogen values were multiplied by 6.25 to estimate the total protein values. Fat content was determined using a soxhlet extractor and petroleum ether was used as extracting solvent. The ash content was estimated by incinerating the samples at 550°C in a muffle furnace.

pH and total titratable acidity (TTA)

A digital pH meter (Pico+, Lab India) was calibrated and used to measure the pH. Ten grams of fish sample was mixed with 90 ml of distilled water using a homogeniser (PT 2100, Kinematica, Polytron system) for 30 s (AOAC, 2005) and pH was checked using the digital pH meter. The TTA was estimated using the prepared homogenate and titrated with 0.1 N NaOH in the presence of 1% phenolphthalein as an indicator to a final pH value of 8 and expressed as % lactic acid equivalent (AOAC, 2005).

Salt (NaCl) content

Ten grams of muscle samples from fermented fish were pooled in a blender and mixed with distilled water (50 ml) and homogenised. The homogenate was transferred into a 250 ml conical flask. After settling of the homogenate, 10 ml of supernatant was taken in a 100 ml conical flask, added with 1 ml of 5% potassium chromate solution and then titrated against 0.1N silver nitrate ($AgNO_3$) solution until a brick red colour was obtained (Belcher *et al.,* 1957).

Protein degradation products

Trimethylamine (TMA) and total volatile base nitrogen (TVBN) was estimated using the official European steam distillation method (EU, 1995). For the TMA estimation, Pooled fermented fish samples weighing 10 g was blended with 7.5% trichloroacetic acid (TCA). The supernatant that carried the TMA was distilled in the presence of 10% NaOH and 35% formaldehyde. The distillate was collected in 4% boric acid in the presence of Tashiro's indicator and was titrated against 0.025N H_2SO_4, whereas for TVBN extraction, the distillation was performed in the absence of 35% formaldehyde. Alpha-amino nitrogen (AAN) and non-protein nitrogen (NPN) contents were estimated by preparing TCA extract of the sample following the methods of Pope and Stevens (1939) and AOAC (2005), respectively.

Fat degradation products

The fat oxidation product, thiobarbituric acid reactive substances (TBARS) was estimated following Tarladgis *et al.* (1960). Pooled muscle (10 g) from fermented fish was homogenised with 97.5 ml of distilled water and 2.5 ml of 4% hydrochloric acid. Distillation was performed and the distillate was mixed with TBA reagent and kept in boiling water at 110°C for 55 min. The TBARS was calculated by measuring the OD at 538 nm in a spectrophotometer. Free fatty acid was estimated following AOAC (2005).

Microbial analysis

Samples were collected aseptically from the fermentation containers. Total halophilic counts were performed according to Ramakrishna *et al.* (2020) with slight modifications. Ten grams of fish samples were added to a conical flask with 90 ml of saline (15%). Subsequent dilutions were prepared by 10-fold serial dilution with 20% saline and 0.1 ml aliquot from each dilution was spread plated on solid agar medium and inoculated plates were kept for incubation at 37ºC for 15 days.

Sensory analysis

Sensory evaluation of the salt-fermented mackerel was conducted by a panel comprising 10 seasoned consumers of fermented fish. The assessment encompassed key attributes such as colour, texture, odour, general appearance, and overall acceptance. Ratings were assigned using a 9-point hedonic scale, as outlined by Jones *et al.* (1955), providing a comprehensive insight into the sensory qualities of the fermented mackerel.

Statistical analysis

In the present study, the statistical analysis was executed by using a standard statistical package of IBM, SPSS Statistics for windows version 26.0. The representation of data was done as mean±standard deviation (SD) and the differences of the means was analysed by performing Duncan's multiple range test to estimate the statistical significance ($p<0.05$) between the treatments.

Results and discussion

Changes in proximate composition and NaCl content during fermentation

Proximate composition of fermented mackerel sampled during salt fermentation are presented in Table 1. The moisture content of fresh mackerel was 72.12% which reduced to 52.08% after dry salting. The reduction in moisture is due to leaching out of the water from tissue in the presence of the salt. There after, an increase in moisture level was observed, which may be due to the reabsorption of moisture from the brine solution in both lots. At the same time,

Table 1. Changes in proximate composition during salt fermentation

Parameter	Fresh	Days of salt fermentation																	
		0		15		30		45		60		75		90		105		120	
		Control	HS1	Control	HS1	Control	HS1	Control	HS1	Control	HS1	Control	HS1	Control	HS1	Control	HS1	Control	HS1
Moisture	72.19± 0.57	53.48^{a} ±0.25	53.48^{A} ±0.25	54.94^{b} ±0.09	54.89^{B} ±0.05	56.02^{c} ±0.05	56.22^{E} ±0.07	56.46^{d} ± 0.03	55.78^{C} ± 0.03	56.82^{ef} ± 0.04	55.96^{D} ± 0.06	56.85^{f} ± 0.05	56.17^{E} ± 0.03	56.65^{e} ± 0.08	56.32^{E} ± 0.02	56.47^{d} ± 0.08	56.55^{F} ± 0.04	56.31^{d} ± 0.03	56.78^{H} ± 0.05
Protein	22.2± 0.12	24.29^{h} ±0.25	24.29^{I} ±0.25	23.05^{g} ±0.12	22.89^{H} ±0.10	22.98^{g} ±0.04	22.08^{G} ±0.08	22.16^{f} ± 0.11	21.64^{F} ± 0.19	21.53^{e} ± 0.11	20.71^{E} ± 0.19	20.91^{d} ± 0.16	19.05^{D} ± 0.27	20.44^{c} ± 0.01	17.15^{C} ± 0.11	19.77^{b} ± 0.31	16.46^{B} ± 0.22	18.95^{a} ± 0.25	15.03^{A} ± 0.04
Fat	5.31± 0.15	6.99^{a} ±0.07	6.99^{A} ±0.07	7.94^{c} ±0.04	7.87^{C} ±0.12	8.41^{e} ±0.11	8.31^{F} ±0.08	8.66^{f} ± 0.14	8.81^{F} ± 0.07	8.18^{f} ±0.09	8.30^{E} ± 0.12	8.22^{d} ± 0.13	8.10^{D} ± 0.05	8.03^{cd} ± 0.06	7.87^{C} ± 0.03	7.92^{c} ± 0.11	7.15^{B} ± 0.05	7.36^{b} ± 0.08	7.03^{AB} ± 0.06
Ash	1.47± 0.08	13.31^{a} ±0.03	13.31^{A} ±0.03	13.37^{b} ±0.05	13.44^{B} ±0.04	14.46^{c} ±0.49	14.23^{C} ±0.34	14.13^{c} ±0.22	14.66^{C} ± 0.26	14.96^{d} ± 0.13	15.68^{D} ± 0.25	15.05^{d} ± 0.27	16.16^{E} ± 0.28	16.16^{e} ± 0.32	17.89^{F} ± 0.37	17.23^{f} ± 0.34	18.14^{F} ± 0.38	18.26^{g} ± 0.31	18.31^{F} ± 0.35

Values presented as mean ± SD with n = 3. $^{a-i}$ Different superscripts in lower case letters in each row indicate significant ($p<0.05$) changes in control during fermentation days. $^{A-I}$ Different superscripts in upper case letters in each row indicates significant ($p<0.05$) changes in HS1inoculated lot during fermentation days.

a slight reduction in the moisture content was found on the 45th and 60th day in the HS1 lot, which may be due to the release of moisture and water soluble components as a part of the maturation process in HS1. On the other hand, in the control lot, moisture content increased initially and slightly reduced during maturation on the 120th day. In both the lots, the reduction in moisture content was reflected by an increase in ash and salt content. Uchoi *et al.* (2021) studied the salt fermentation of mackerel and also found a reduction in moisture content by the time of maturation. Dhar *et al.* (2021) studied salt fermentation of pangas and found a decrease in moisture content during maturation and similar findings were reported by Majumdar *et al.* (2006) in salt fermentation of Indian shad. A slight increase in moisture content was observed during the later stages of fermentation. Wheaton and Lawson (1985) attributed this increase to prolonged immersion in brine solution resulting in absorption of moisture and tissue swell.

The crude protein content of fresh mackerel is 22.2% which increased to 24.29% after dry salting. There was a significant ($p<0.05$) reduction in crude protein content during salt fermentation in both control and HS1 lots. The final protein contents of samples from control and HS1 were 18.95% and 15.03%, respectively. Compared to the control, in HS1, there was a higher reduction in protein contents. The reduction in protein content may be due to the leaching out of water-soluble protein and the action of proteolytic microbes as well as enzymes. The higher protein reduction may be due to the proteolytic activity of Haloarchaea. Similar results were found by Mathew and Ragunath (1996) in salt-cured mackerel, where the leaching of proteins continued throughout the fermentation.

The fat content of fresh mackerel was 5.31% which increased to 6.99% after dry salting. Fat content fluctuated throughout the fermentation process. There was an increase in fat during the initial days of fermentation till 45 days and later, there was a decrease in the fat content till 120 days in both lots. The increase in fat in the initial 45 days was despite the increase in moisture content during the period. Since protein was continuously decreasing, an increase in fat may balance the mass lost through protein. The reduction in the fat content may be due to the lipolytic activity of enzymes and microbes. The HS1 lot was found to have lower fat content compared to the control, which may be due to the higher lipolytic activity of Haloarchaea. El-Shamery (2010) suggested that reduction may also be the result of the degradation of fat due to hydrolysis and oxidation of fat to aldehydes, ketones and other non-fatty substances.

Ash content was found to increase throughout the fermentation period in both control and HS1 (Table 1). An increase in ash content is directly correlated to an increase in salt content. Ash content increased significantly ($p<0.05$) after dry salting, with initial ash content of 1.47%, which increased to 13.31% after dry salting. Similar observations were made by Dhar *et al.* (2021). The salt content significantly ($p<0.05$) increased after dry salting from 0.21% in fresh fish to 8.15% in dry salted fish (Fig. 2), due to the absorption of salt into fish tissue during salting. The salt content continued to increase throughout the fermentation period in both lots. The final salt content in control and HS1 on the 120th day was 15.16 and 15.35%, respectively. Similar observations were made by Kumar *et al.* (2021) and Majumdar *et al.* (2006) for pangas and hilsa, respectively. The increased salt concentration will lead to reduced water activity (a_w) which helps to preserve salt fermented products. Eyo (1993) reported that at 15% NaCl concentration, the water activity (a_w) lies around 0.92 or below.

Changes in pH and TTA

Changes in pH and TTA during salt fermentation are represented in Fig. 3 and Fig. 4, respectively. pH was found to reduce throughout the fermentation period in both lots. There was a significant ($p<0.05$) decrease in pH of HS1 lot on the 60th day, which may be due to the breakdown of proteins into acidic amino acids due to the microbial action as starter culture was added. The final pH of control and HS1 on the 120th day was 5.52 and 5.55, respectively. Minimal changes were observed in pH after 60 days in the HS1 lot, which indicated that the product became stable. TTA has shown an increasing trend in both control and HS1 which was reflected in reduced pH. The Initial TTA levels on the 0th day were 0.97% and final TTA contents in control and HS1 were 4.58 and 4.81%, respectively. The increase in TTA may be attributed to production of acids by the microbes.

Changes in TMA, TVBN and AAN content

An increase in TMA content was recorded throughout the fermentation period (Fig. 5). The initial TMA content was 2.96 mg% which increased to 3.12 mg% after dry salting and reached 13.84 mg and 14.09 mg% on the 120th day of fermentation in control and HS1, respectively. The TMA content varied similarly to that of TVBN content. There was a sharp rise in TMA content from the 45th day to the 60th day in HS1, which generated the typical flavour which could be due to degradation products of protein by microbial action.

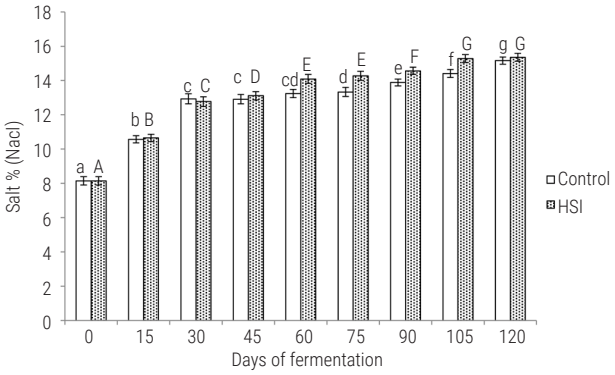

Fig. 2. Changes in salt concentration during fermentation

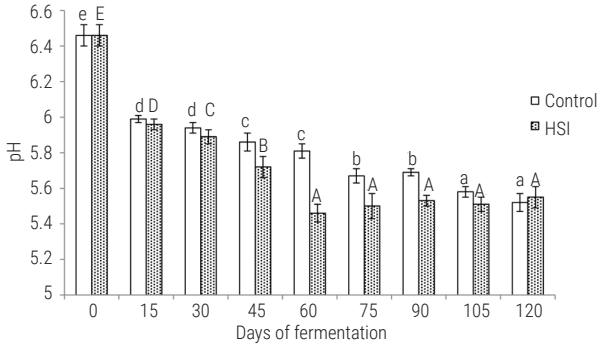

Fig. 3. Changes in pH during salt fermentation

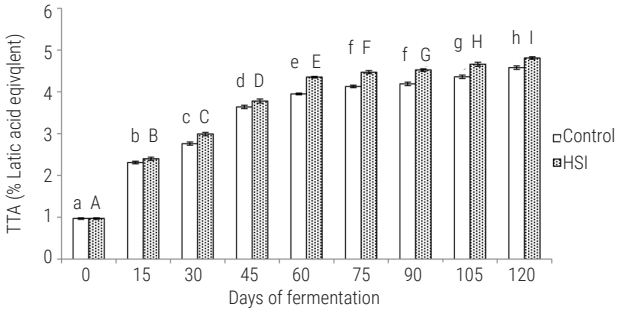

Fig. 4. Changes in TTA during salt fermentation

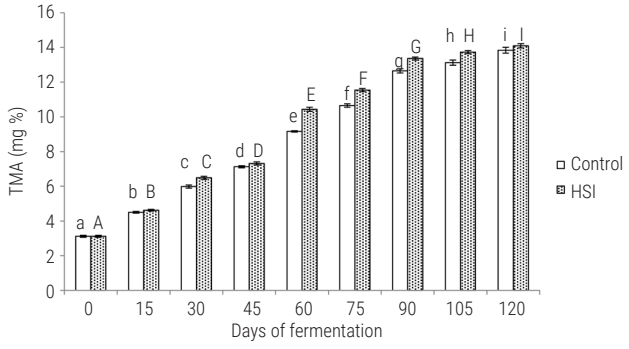

Fig. 5. Changes in TMA content during salt fermentation

TVBN content in the fresh fish was 16.46 mg% which increased to 18.23 mg% after dry salting and then increased throughout the fermentation period in control and HS1 to 38.15 mg and 38.35 mg% respectively (Fig. 6). Filsinger *et al.* (1984) reported a positive correlation between TVBN and time. The rapid increase in TVBN content till 60 days in HS1 compared to control could be attributed to the action of haloarchaea, which was inoculated as a starter culture and this resulted in typical maturation flavour in the HS1 lot. However, in control also, there was an increase in TVBN in later stages coinciding with the increase in microbial count. As such, there is no regulatory standard prescribed for TVBN content in salt fermented products. The acceptability range of 100-200 mg per 100 g for salt fermented sardines was suggested by Connell (1978).

A significant ($p<0.05$) increase in AAN content was observed throughout the fermentation period (Fig. 7). The initial AAN content in the fresh mackerel was 27.23 mg% which increased to 31.87 mg% after dry salting. A continuous rise in AAN throughout the fermentation indicated gradual degradation of protein by the enzymes and bacteria originating from the muscle and gut (Dhar *et al*., 2021). There was a sharp rise on the 60th day in HS1, which suggests that the product has undergone maturation. Whereas in control, it took 120 days to attain maturation. Majumdar *et al.* (2006) observed an increase in the AAN content from 59.59 to 140 mg% during Indian shad salt fermentation.

Comparison of changes in PN and NPN

There was an increase in protein nitrogen after dry salting, which may be due to a reduction in moisture content after dry salting.

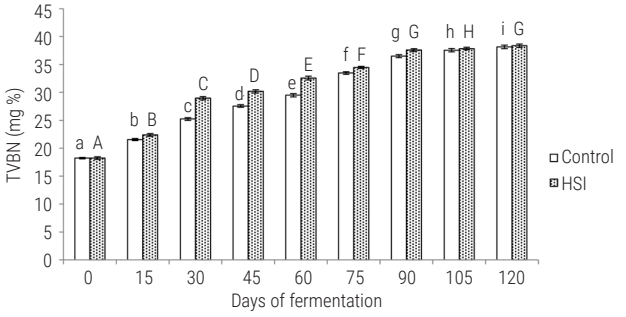

Fig. 6. Changes in TVBN content during salt fermentation

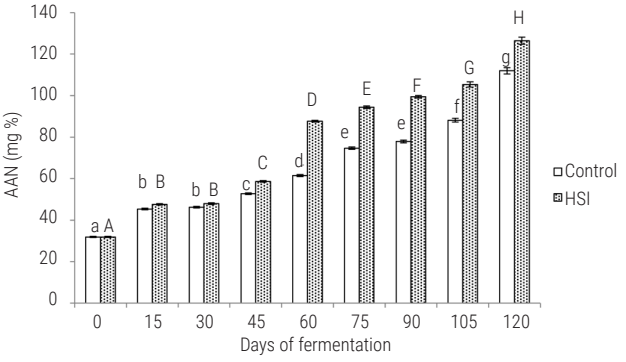

Fig. 7. Changes in AAN content during salt fermentation

Thereafter, a decrease in the protein nitrogen from 3.71 to 2.24 and 3.71 to 1.57% in control and HS1, respectively by 120th day was observed (Table 3). Protein nitrogen decreased significantly ($p<0.05$) in HS1 compared to control. The proteolytic activity of *H. salinarium* might have contributed to the faster reduction than control. The reduction in PN content during salt fermentation could be attributed to leaching out of salt soluble nitrogen during the prolonged storage in the brine solution. On the other hand, there was a significant ($p<0.05$) increase in the NPN content from 0.18 to 0.79% and 0.18 to 0.83% in control and HS1, respectively. There was a negative correlation between PN and NPN throughout the fermentation. An increase in NPN content may be due to the breakdown of protein molecules into low molecular peptides and amino acids (Mahanta and Muzaddadi, 2012). The HS1 showed a significant ($p<0.05$) increase during the 45th and 60th day in NPN content which represents the breakdown of the protein macromolecules. Fermentation flavour had also started appearing during this period and the product got matured by the 60th day. There was also a significant ($p<0.05$) increase in AAN during this period in the HS1 inoculated lot, which indicates the maturation of the product. In control. These changes were observed much later and maturation was attained only by the 120th day. Similar observations of an increase in NPN and a decrease in PN were recorded by Majumdhar *et al.* (2006) in salt fermentation of Indian shad. Dhar *et al.* (2021) also recorded a decrease in PN and an increase in NPN content during 150 days of salt fermentation of pangas.

Changes in FFA and TBARS

FFA content in the fresh fish was 0.1% oleic acid which increased to 0.11% oleic acid after dry salting and then increased initially and decreased in the later stage of fermentation (Fig. 8). Final FFA content by 120th day in control and HS1 reduced to 0.53% oleic acid and 0.45% oleic acid respectively. Similar observations were made by Uchoi *et al*. (2021) in salt fermented mackerel, where FFA content ranged from 0.22 to 0.67% during the 120 days of fermentation.

TBARS content showed fluctuations throughout the fermentation period (Fig. 9). Initial TBARS content in fresh mackerel wass 0.37 mg malonaldehyde kg^{-1} after salt drying, which increased to 0.41 mg malonaldehyde kg^{-1}. There was a significant ($p<0.05$) increase in the initial days of fermentation and a decreasing trend in the later phase of fermentation. The final TBARS content in control and HS1 was

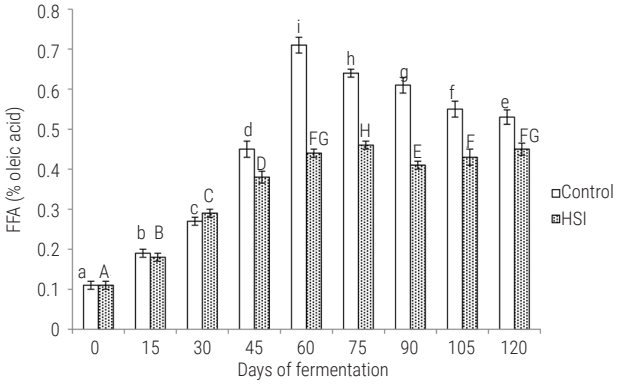

Fig. 8. Changes in FFA content during salt fermentation

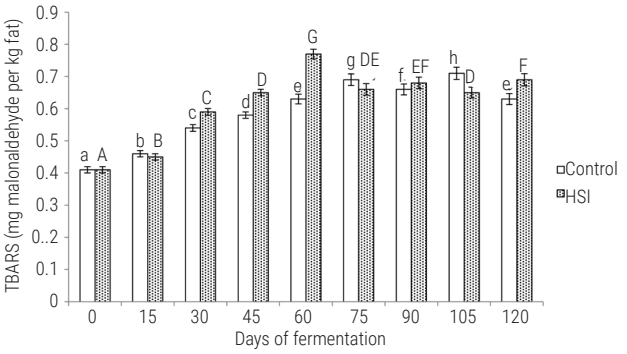

Fig. 9. Changes in TBARS content during salt fermentation

0.8 and 0.69 mg malonaldehyde kg^{-1}. The decrease in later phases may be due to interactions of malonaldehyde with protein degraded products leading to the formation of some tertiary products (Reddy and Shetty, 1996). Similar observations were made by Kumar *et al.* (2021) in Pangas where TBARS values ranged from 0.12 to 0.49 mg malonaldehyde kg^{-1} and Uchoi *et al*. (2021) reported average TBARS content 0.81 mg malonaldehyde kg^{-1} on 120th day of salt fermented mackerel.

Microbial counts

Changes in microbial counts during salt fermentation of mackerel are represented in Fig. 10. The initial TPC on the 0th day was 3.11 log cfu g^{-1}, then increased to 4.31 and 5.03 log cfu g^{-1} in control and HS1, respectively, by the 120th day. Comparatively higher microbial counts were observed in the HS1 throughout the fermentation period. The increase in the microbial count has been reflected in the increase of TVBN, TMA, NPN and AAN, indicating bacterial breakdown of macromolecules and leading to flavor and taste. Similar observations were made by Dhar *et al.* (2021) in Pangas salt fermentation and by Uchoi *et al.* (2021) in salt fermentation of mackerel.

Sensory analysis

Sensory scores are represented in Table 2. Scores were based on colour, texture, odour, general appearance and overall acceptance. The final overall acceptance score on the 120th day of control and HS1 were 8.52 and 8.65.

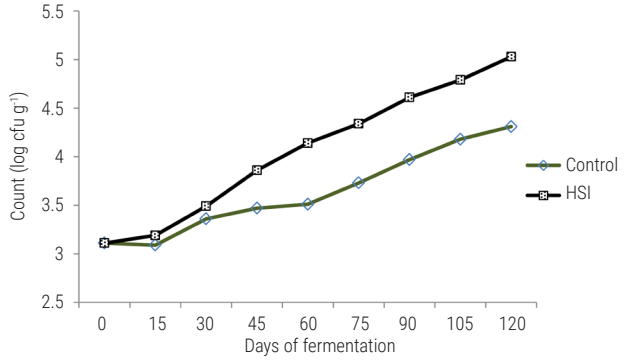

Fig. 10. Changes in microbial count during salt fermentation

Table 2. Changes in sensory parameters during salt fermentation

Sensory parameters	Days of salt fermentation																	
	0		15		30		45		60		75		90		105		120	
	Control	HS1	Control	HS1	Control	HS1	Control	HS1	Control	HS1	Control	HS1	Control	HS1	Control	HS1	Control	HS1
Colour	7.08^{a}± 0.20	7.08^{A} ±0.20	7.02^{a} ±0.13	7.19^{A} ±0.18	7.04^{a} ± 0.11	7.88^{B} ±0.15	7.03^{a} ± 0.13	8.03^{B} ±0.07	7.06^{a} ±0.13	8.20^{C} ±0.04	7.27^{a} ±0.14	8.34^{CD} ±0.2	8.13^{b}± 0.09	8.50^{D} ±0.13	8.26^{c} ±0.04	8.51^{D} ±0.17	8.46^{d} ±0.08	8.55^{D} ±0.15
Texture	7.33^{a} ±0.12	7.33^{A} ±0.12	7.29^{a} ±0.15	7.34^{A} ±0.20	7.5^{ab} ± 0.13	7.63^{AB} ±0.09	7.43^{a} ± 0.15	7.66^{B} ±0.21	7.40^{a} ±0.12	7.70^{B} ±0.12	7.56^{ab} ±0.19	7.95^{C} ±0.09	7.70^{b} ± 0.18	8.30^{D} ±0.14	8.15^{c} ±0.07	8.33^{D} ±0.14	8.38^{d} ±0.12	8.40^{D} ±0.15
Odour	7.67^{b} ±0.20	7.67^{B} ±0.20	7.07^{a} ±0.16	7.06^{A} ±0.19	7.01^{a} ± 0.17	7.13^{A} ± 0.15	6.99^{a} ±0.25	8.01^{C} ±0.16	7.00^{a} ±0.13	8.53^{D} ±0.16	7.09^{a} ±0.24	8.61^{D} ±0.13	7.20^{a} ±0.15	8.80^{D} ±0.12	7.83^{b} ±0.15	8.83^{D} ±0.13	8.83^{c} ±0.19	8.85^{D} ±0.14
General appearance	7.50^{b} ±0.12	7.50^{A} ±0.12	7.28^{a} ±0.14	7.37^{A} ±0.11	7.31^{a} ± 0.16	7.56^{A} ±0.19	7.23^{a} ±0.17	7.94^{B} ±0.09	7.20^{a} ±0.22	8.50^{C} ±0.13	7.38^{a} ±0.18	8.63^{C} ±0.24	7.50^{b} ±0.13	8.70^{C} ±0.18	7.79^{c} ±0.06	8.69^{C} ±0.13	8.60^{d} ±0.17	8.72^{C} ±0.15
Overall acceptance	7.33^{a} ±0.18	7.33^{A} ±0.18	7.21^{a} ±0.20	7.42^{A} ±0.08	7.26^{a} ±0.18	7.56^{B} ±0.20	7.19^{a} ±0.18	7.96^{C} ±0.08	7.22^{a} ±0.18	8.50^{D} ± 0.13	7.42^{b} ±0.08	8.57^{D} ±0.20	7.61^{c} ±0.06	8.60^{D} ±0.12	7.94^{d} ±0.19	8.62^{D} ± 0.14	8.62^{e} ±0.19	8.65^{D} ±0.17

Values presented as mean ± SD with n = 3. $^{a-i}$ Different superscripts in lower case letters in each row indicate significant (p<0.05) changes in control during fermentation days. $^{A-I}$ Different superscripts in upper case letters in each row indicate significant (p<0.05) changes in HS1 inoculated lot during fermentation days.

Table 3. Changes in TN, PN and NPN contents during salt fermentation

Control	Days of salt fermentation								
	0	15	30	45	60	75	90	105	120
TN	3.89^{I} ± 0.04	3.69^{h} ± 0.01	3.68^{g} ± 0.01	3.55^{f} ± 0.01	3.44^{e} ± 0.01	3.35^{d} ± 0.04	3.27^{c} ± 0.01	3.16^{b} ± 0.05	3.03^{a} ± 0.01
PN	3.71^{i} ± 0.03	3.48^{h} ± 0.01	3.30^{g} ± 0.01	3.12^{f} ± 0.01	2.93^{e} ± 0.01	2.77^{d} ± 0.04	2.63^{c} ± 0.01	2.49^{b} ± 0.05	2.24^{a} ± 0.01
NPN	0.18^{a} ± 0.01	0.21^{b} ± 0.01	0.38^{c} ± 0.02	0.43^{d} ± 0.01	0.51^{e} ± 0.00	0.58^{f} ± 0.01	0.64^{g} ± 0.00	0.67^{h} ± 0.00	0.79^{I} ± 0.01
HS1									
TN	3.89^{I} ± 0.04	3.66^{H} ± 0.01	3.53^{G} ± 0.01	3.46^{F} ± 0.03	3.31^{E} ± 0.03	3.05^{D} ± 0.04	2.74^{C} ± 0.01	2.63^{B} ± 0.03	2.40^{A} ± 0.00
PN	3.71^{I} ± 0.03	3.31^{H} ± 0.01	3.02^{G} ± 0.02	2.87^{F} ± 0.03	2.66^{E} ± 0.03	2.36^{D} ± 0.04	2.02^{C} ± 0.01	1.86^{B} ± 0.03	1.57^{A} ± 0.00
NPN	0.18^{A} ± 0.01	0.35^{B} ± 0.01	0.51^{C} ± 0.02	0.59^{D} ± 0.01	0.65^{E} ± 0.01	0.69^{F} ± 0.01	0.72^{G} ± 0.01	0.77^{H} ± 0.01	0.83^{I} ± 0.00

Values presented as mean ± SD with n = 3. $^{a-i}$ Different superscripts in lower case letters in each row indicate significant (p<0.05) changes in control during fermentation days. $^{A-I}$ Different superscripts in upper case letters in each row indicate significant (p<0.05) changes in HS1inoculated lot during fermentation days

The overall acceptance showed an initial decrease in trend and increased in the later phase of fermentation in control, whereas in HS1, there was a significant (p<0.05) increase on the 60th day, suggesting ripening of tissue. The same has been reflected in biochemical composition with an increase in AAN, NPN and TBARS.

From the present study, it is evident that exogenous addition of *H. salinarium* (HS1) as starter culture accelerated the salt fermentation process. Maturation in salt fermented mackerel inoculated with HS1 was attained on the 60th day, whereas control took 120 days. Sensory scores were found to be highly accepted in the treatment group which used HS1 as starter culture. To the best of our knowledge, this is the first report on the possibility of using Haloarchaea as starter culture in salt fermentation of fish.

Acknowledgements

The authors are grateful to the Director, ICAR-CIFE, Mumbai for providing facilities to conduct the experiment.

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