Fishery Technology 53 (2016) : 220 - 231

Biochemical and Bacteriological Quality of Rohu (*Labeo rohita*) Head Sauce Produced by Enzymatic and Fermentation Method

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Abstract

A sauce was produced by enzymatic and fermentative methods using the heads of freshwater fish rohu (*Labeo rohita*) with solar salt concentration of 25% (w/w). Commercial papain (3%, w/w) was used for enzymatic hydrolysis and stored at room temperature for 120 d. *Pediococcus pentosaceus* FSBP 4-40 (10%, v/w) with the cell concentration of aproximately 8 log cfu ml^{-1} and dextrose (7.5 %, w/w) was used for fermentative production at 37°C for 120 d. Changes in yield, water activity (a_w), total valatile base nitrogen (TVB-N), total soluble nitrogen (TSN), non protein nitrogen (NPN), titratable acidity (TA), degree of hydrolysis (DH) and fatty acid composition of both enzymatically and fermentativley produced rohu head sauce was observed. Bacteriological parameters such as total plate count, *Escherichia coli*, lactic acid bacteria, staphylococci, total halophile count and yeast and mold counts were determined at different time intervels . The result suggested that TSN, NPN, TA and DH significantly increased ($p<0.05$) in treated samples compared to control and *P. pentosaceus* FSBP4-40. However, a_w, pH, moisture, fat, TVB-N, fatty acid concentration and *L**, *a**, *b** values showed changes. The antioxidant properties like total antioxidant activity, 2,2'-diphenyl-1-picrylhydrazyl (DPPH), super oxide anion scavanging activity and reducing potential of the lyophilized rohu head sauce samples were found to be sigificantly higher ($p<0.05$) in enzyme/bacteria treated samples than control. Bacterial counts after 120 days were significantly lower ($p<0.05$) in the fermentatively produced rohu head sauce than enzymatically prepared sauce.

Keywords: Rohu head sauce, papain, *Pediococcus pentosaceus,* antioxidant, DPPH

Introduction

Fish sauce is considered as an important source of dietary proteins and amino acids, and is a traditional delicacy among Southeast Asian countries (Sanceda et al., 1996). Depending on the degree of hydrolysis or time of fermentation and the separation method, two types of 3 sauce namely, clear and turbid are produced. The turbid type fish sauces are obtained from the exude liquid of cured fish, for example, paties from bagoong production in the Philippines and jeot-kuk from jeot-kal production in Korea. The annual production is estimated to be >400 million liters with 20 out of 100 fish sauce producers contributing more than 80% of the global production (Vidanarachchi et al., 2014). There is high divergence in manufacturing among fish sauce producing countries throughout the world, though the fish and salt are the major raw materials in general production. The leading fish sauce producer in the world is Thailand. Nampla, a traditional Thai fish sauce has become popular among Western consumers, especially in the United States (Lopetcharat et al., 2001).

Fish heads are the major edible by-products generated from fish processing industries, which are rich in lipids and proteins. Hydrolysis processes have been developed to convert underutilized fish and fish waste into marketable and acceptable form, which can be widely used in food rather than animal

Received 01 April 2016; Revised 09 July 2016; Accepted 29 July 2016

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feed or fertiliser (Ruthu et al., 2014). Enzymatic and fermentative hydrolysis of fish and fish by-products have been used in the production of salted fish products *viz.,* fish mince, fish paste and fish sauce. Fish sauce is a clear brown salty liquid with mild fishy flavour that results from the physical, chemical and microbiological changes that occur at high salt concentration and low oxygen levels. Fish sauce is traditionally produced by using whole fish with salt in the ratio of 1:1 and 3:1 and fermented anywhere between 6 and 12 months or even longer. The fermented liquid is rich in fish soluble proteins, peptides and amino acids, and has been characterized by umami(pleasant savory) tastes (Curtis, 2009). These protein fractions are produced during proteolytic degradation by endogenous proteases in the muscles or digestive tracts of fish and various microorganisms exist in the fermentation broth (Yongsawatidigul et al., 2007). The proteolytic enzymes from animal and microbial sources have been employed in the fish sauce production to accelerate the fermentation process and also to reduce the formation of biogenic amines. Various novel halotolerant bacterial cultures have been used to accelerate fermentation process, increase the á-amino content, enhance the sensory characteristics and improve the microbiological quality of fish sauce. Researchers have also focused on effective inhibition of biogenic amines in fish sauces using novel bacterial starter cultures, apart from effectively deciphering the formation of different biogenic amines and their toxicity in fish sauces (Siddegowda et al., 2016).

The rohu head was considered as the minimally utilized by-product because of its less meat content. The extent of the conversion of insoluble fish protein to soluble nitrogen was examined by adding papain (Beddows & Ardeshir, 1979). Papain is the most common commercial protease from plant sources used for the hydrolysis of fish protein (Hoyle & Merritt, 1994). The acceleration of protein hydrolysis occurs during ûsh sauce fermentation with the addition of proteinase-producing halophilic bacteria as a starter culture. The bacteria not only increase the rate of protein solubilization, but also contribute to ûavor development. Two strains of lactic acid bacteria isolated from natural plaa-som fermentation were used as starter culture: *Lactobacillus plantarum* IFRPD P15 and *Lactobacillus reuteri* IFRPD P17. These strains have great potential for use as a mixed starter culture in plaa-som fermentation and may possibly help to reduce fermentation time (Saithong et al., 2010). Use of native proteolytic lactic acid bacteria from salted and fermented fish and fish products effectively hydrolyse fish proteins during fermentation of fish sauce (Siddegowda et al., 2016). Against this background, the objective of the present work to compare the enzymatically and fermentatively produced rohu head sauce with reference to the biochemical, bacteriological and antioxidant properties.

Materials and Methods

Freshwater fish rohu (*Labeo rohita*) head collected from local fish market (Mysore, India) was the raw material of the study. The material was brought to the laboratory in iced condition. *Pediococcus pentosaceus* FSBP4-40, a native proteolytic lactic acid bacteria (LAB) starter isolated from salt fermented fish hydrolysate was used for fermentation. The protease used for the enzymatic hydrolysis papain (Loba chemie), 2,2'-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-3-ethyl-benzothizoline-6 sulphonate (ABTS), peroxidase were purchased from sigma-Aldrich Chemie (Steinheim, Germany). Plate count agar (PCA), eosin methylene blue (EMB) agar, de-Man Rogosa and Sharpe (MRS) agar, Baird Parker agar (BPA), salmonella-shigella agar (SSA) and potato dextrose agar (PDA) were purchased from M/s. Hi-media Laboratories (Mumbai, India). All the other chemicals used in different analysis were of analytical grade unless otherwise mentioned.

The preparation of rohu head sauce is schematically represented in Fig. 1. Briefly, rohu head was sliced into small pieces of 2x1cm size and washed in potable water 3 times. The sliced rohu head was weighed and bottled into clean sterile food grade plastic containers and mixed with commercial enzyme papain (3%, w/w), and was kept at room temperature for 4 h before adding 25% (w/w) salt. A control for the enzymatic production of sauce was maintained by adding only salt without papain. The containers were closed with plastic lids and stored at room temperature. *P. pentosaceus* FSBP 4-40, a proteolytic halotolerant native LAB which was previously isolated from salt fermented fish hydrolysates by our group (Gen Bank accession no: KU933533) was added (10%, v/w) along with 7.5%, w/w sugar (dextrose), 2%, w/w solar salt for fermentative production of sauce from rohu head. This mixture was incubated for 24 h at 37°C and remaining salt of 23%, w/w was added to make up

the total salt concentration of 25%, w/w. Fish head with salt and sugar without added LAB was the control for the fermentation method. After storage period of 120 d, the liquid was filtered through cheese-cloth and further filtered using Whatman no. 1 filter paper. The resulted liquid was considered as fish sauce and the yield of the same was measured as the ratio of original fish-salt and papain mass in the container to the weight of liquid after filtration. The fish sauce was lyophilized and used for chemical, biochemical, microbiological and sensory studies along with *in vitro* antioxidant properties.

Proximate composition (protein, fat and moisture) of the fermented fish sauce samples were determined as per AOAC method (AOAC, 2002). pH measurements were accomplished by directly immersing the combined glass calomel electrode into the sample using pH meter (Cyberscan 1000, Eutech, Singapore). Titratable acidity (TA) was determined as per the method described in Sachindra et al. (2007) by determining the ml of 0.1M NaOH required for increasing the pH of one ml of fermented sauce to 8.0. Total soluble nitrogen (TSN) content of samples was measured using Kjedahl method (AOAC, 1999) and non protein nitrogen (NPN) by precipitation of the proteins with trichloroaceticacid (TCA), followed by analysis by the Kjeldhal method. Water activity (a_w) of the samples was measured by using water activity meter (Aqua Lab Model CX-3T, Decagon Devices Inc., Pullman Wasshington, USA). TVB-N content of the fermented rohu head sauce samples was measured using the Conway microdiffusion assay according to the method of Conway & Byrne (1936). The degree of hydrolysis of the fermented fish sauce was estimated as per the methodology described by Hoyle & Merritt (1994). Briefly, degree of hydrolysis was computed as %DH = (10% TCA soluble N_2 in the sample) / (Total N_2 in the sample) x 100. Fermented rohu head sauce samples were analysed for non-enzymatic browning by measuring melanoidin pigment formation using the method of Hendel et al. (1950).

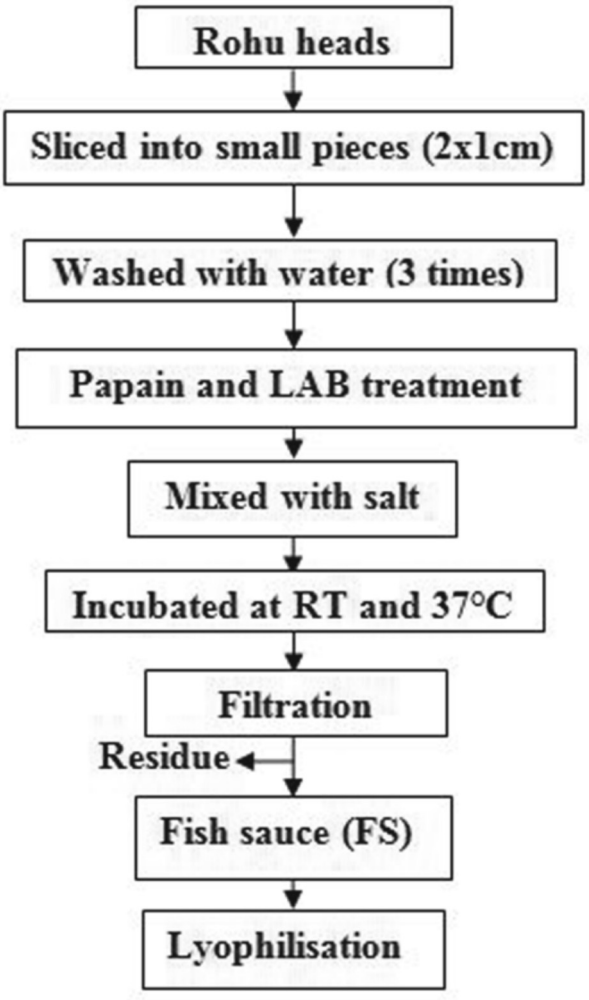

Fig. 1. Schematic flow of sauce production from (*Labeo rohita*) head

Fish sauce samples were dissolved (50 mg ml^{-1}) in double distilled water and homogenized at 10000 rpm for 2 min using homogenizer (Polytron PT 3100) followed by centrifugation at 7000 x g for 15 min. The supernatant was collected and filter through Whatman No. 1 filter paper and protein content in the filtrate was estimated by the method of Lowry et al. (1951). This filtrate was used for assaying various antioxidant activities.

The Total antioxidant activity (TAO) of fermented fish sauce sample was determined according to the method of Prieto et al. (1999). Absorbance of all the sample mixtures was measured at 695 nm and TAO was expressed as of ascorbic acid equivalents in micrograms per gram of sample.

The DPPH radical scavenging capacity of fish sauce samples was determined by the method described in Bijinu et al. (2011). The scavenging activity (%) was determined by measuring the absorbance of samples at 517 nm and calculated using the formula:

Scavenging activity (%) = $[1-\{(A_{sample} - A_{sample\ balnk}) / A_{control}\}] \times 100$

Super oxide anion scavenging activity of the samples was determined by the method as described in Heo et al. (2005). The absorbance was measured at 325 nm from 0 min and 10 min.

ABTS radical scavenging activity ABTS radical scavenging activity of the samples was carried out as explained in Sachindra & Bhaskar (2008). Scavenging activity was calculated as follows: Scavenging activity (%) = $[1-\{(A_{sample} - A_{sample\ balnk}) / A_{control}\}] x100$

Reducing potential of the fermented fish sauce samples was assayed by the method as followed in Bijinu et al. (2011) and absorbance was measured at different concentration (50, 100, 150 and 200 μL). Absorbance of all the samples was measured at 700 nm by using distilled water as blank.

The colour of the fermented fish sauce samples was measured in the *L**, *a** and *b** mode using a Hunter Lab instrument (Minotia CM-5, Konica Minolta Optics inc., Japan) according to CIE Lab scale. The instrument provides the values for three components: lightness (*L**), redness/greenness (*a**) and yellowness/blueness (*b**). The 10-15 ml of samples in quartz cuvettes were illuminated with D65-artificial daylight (10° standard angle) according to the manufacturer's protocol.

Total lipids of the fermented rohu head sauce were extracted by Folch method and the fatty acid composition of the extracted lipids was determined by preparing the fatty acid methyl ester (FAME) as outlined in Majumdar et al. (2015). Briefly, lipid samples were transmethylated using 2 M methanolic NaOH followed by 2 M methanolic HCl to obtain FAME. FAME were analysed by using a gas chromatography-mass spectrometer (GCMS; Shimadzu QP2010 quadruple MS, M/s Shimadzu, Kyoto, Japan) equipped with a Carbowax (30 m x 0.25 mm ID; 0.25 μm film thickness) capillary column (M/s Cromlab, USA). Helium was used as the carrier gas. Injector and detector temperature were set at 250°C. Sample injection was performed in split mode (1: 15). The column temperature was programmed initially at 50°C for 2 min and then ramped at a rate of 10°C per min to a final temperature of 230°C. FAME was separated at a constant pressure (23.1 kpa) and peaks were identified by comparing standard mass spectra with the relative abundances of m/z ranging from 40.00 to 550. The values of fatty acids are presented in area percentage of total identified fatty acids.

In order to identify dominant microflora associated with fermentation, microbial load of fermented *rohu* head sauce was determined at day-0 and 120 d using standard methods (APHA 2001). Ten grams of sample was diluted in 90 ml of sterile physiological saline and then mixed by stomacher blender (Stomacher 400) for 2 min, the stock was then 10-fold serially diluted in sterile saline and 0.1ml of each proper diluted sample was spread with glass spreader on media plates for obtaining total plate count, *Escherichia coli* count, stapylococci count, *Salmonella-Shigella* count. The inoculated plates were incubated at 37°C for 24-48 hours. For lactic acid bacteria count, Deman Rogosa Sharp (MRS) agar supplemented with 0.3% (w/v) calcium carbonate (Maragkoudakis et al., 2006) was used and inoculated plates were incubated in Hi anearo jar (Hi-Media, Mumbai, India) with the vacuum pressure of 10psi at 37°C for 24-48 h (Jini et al., 2011). Yeast and mold count was monitored by inoculating on Potato Dextrose Agar medium (PDA) and incubated at 25±2°C for 5-6 days. Plate count agar with 10% (w/w) NaCl was used for determining total halophiles count and the plates were incubated at 37°C for 10 days. Microbial count was expressed in log CFU g^{-1}.

Results and Discussion

Proximate composition of the rohu head sauce produced by enzymatic and fermentative methods on day-0 and day-120 is given in the Table 1. There was a significant change in the moisture, fat and protein content of papain treated and LAB treated sauce samples. The moisture content was decreased after 120 d of storage in both treated and control (without added papain and *P. pentosaceus* FSBP 4-40). Our findings were in agreement with the results of Hjalmarsson et al. (2007) who found 72-75 and 81-83% moisture content in fish sauce produced from summer capelin and winter capelin respectively at different storage period between 5 to 270 d. Fat content of the papain treated sauce samples was almost unchanged throughout the storage period but, decreased content of fat was found in the LAB treated sample after 120 d of fermentation (Table 1). According to Kilinc et al. (2006) decrease in total lipid content was noticed in all the groups of sauce samples produced from sardine (*Sardina pilchardus*) using different ingredients. The papain treated rohu head sauce samples showed 4 fold increased protein content than control, whereas the LAB treated samples comparatively showed lower

Table 1. Physicochemical properties of fermented rohu head sauce

Parameter		Yield (%)		pH		a_w		% Moisture		% Fat		% Protein	
Treatment	Day	C	T	C	T	C	T	C	T	C	T	C	T
Papain treated	0	28.28	30.27	5.7	5.4	0.789	0.794	69.29 ±0.5	65.64 ±1.1	0.06 ±0.0	0.105 ±0.0	2.25 ±0.1	8.07 ±0.0
	120	39.69	41.45	5.2	5.4	0.756	0.750	67.98 ±0.1	61.52 ±1.8	0.06 ±0.0	0.106 ±0.0	2.37 ±0.0	9.63 ±0.0
LAB treated	0	34.58	36.07	5.9	5.8	0.748	0.761	64.62 ±0.2	62.68 ±0.7	0.20 ±0.0	0.180 ±0.0	4.01 ±0.1	5.31 ±0.0
	120	49.76	51.76	4.9	4.3	0.737	0.749	62.24 ±0.6	65.82 ±0.1	0.15 ±0.0	0.118 ±0.0	4.09 ±0.1	6.76 ±0.0

C-control, T-treated

protein content (Table 1). This difference was due to high degree of hydrolysis of fish protein by papain and insufficient production of proteolytic enzymes produced by the LAB. The protein content ranged from 2.25-9.63 %, which is low when compared to Kilinc et al. (2006) who documented 12.37% crude protein content in fish sauce made from whole Gambusia 12.37%. This difference could be attributed to differences in the protein content of the raw material and condition of fermentation such as pH, temperature and salt concentration.

The LAB treated samples gave higher sauce yield during processing than the papain treated samples after 120 d of fermentation. There was almost 10% difference in the yield of LAB treated samples than the papain treated one. This might be due to the processing method followed, fermentation condition such as temperature employed and acidifying ability of the LAB culture used for the production. Hjalmarsson et al. (2007) reported 42±1 and 59±5% liquid yield in ground winter capelin and summer capelin respectively and filter through cheese cloth with approximately 850 psi after 270 d. Changes in pH values of the fermented rohu head sauce were shown in Table 1. pH value of the sauce treated with LAB decreased during fermentation, from the original 5.9 to 4.3 whereas, in the control, the pH value was decreased to 4.9 from the initial 5.8. There was slight reduction in the pH values of enzyme treated and control samples than LAB treated samples. The decrease of pH may be due to the production of acids by LAB and other autochthonous organisms. The pH values were well suitable for the sourness of the fish sauce. The fish sauce made of squid processing by-products using low salt also showed the pH range 5.26±0.14 to 5.48±0.12 (Xu et al., 2008). Udomsil et al. (2015) reported that the pH values for the fish sauce prepared by added *Staphylococcus* sp. CMC5-3-1 and CMS5-7-5 were 5.38 and 5.36 respectively. The pH of mahyaveh, a traditional Iranian fish sauce samples from different locations was in the range of 4.89-7.55 (Zarei et al., 2012). There was slight decrease in the water activity (a_w) of all the fish sauce samples treated with and without papain and LAB after 120 d of fermentation (Table 1).Water activity values of fermented fish sauce samples made from sardine with different ingredients was in the range of 0.93 to 0.84 (Kilinc et al., 2006). Degree of hydrolysis analysed during the fermentation is shown in Fig. 2. DH was higher in case of papain treated sauce samples as compared to LAB treated samples. DH of papain treated and untreated samples were found to 21.51 and 2.34 % after 120 d of storage from the original 12.01 and 2.38% respectively. Whereas, the LAB inoculated and control samples were showed 14.39 and 7.52% of DH from the original 6.53 and 4.74%, respectively. The increase in DH of LAB fermented sample compared to the control may be due to the acid producing ability and proteolytic activity of the LAB employed in the fermentation.

The changes in the total acid content of fermented fish sauce are presented in the Table 2. There was 3 fold increase in the total acids was found in the sauce produced by papain treatment after 120 d of storage when compared to the control. This was probably due to the hydrolysed proteins in the papain treated samples, which serve as the ingre-

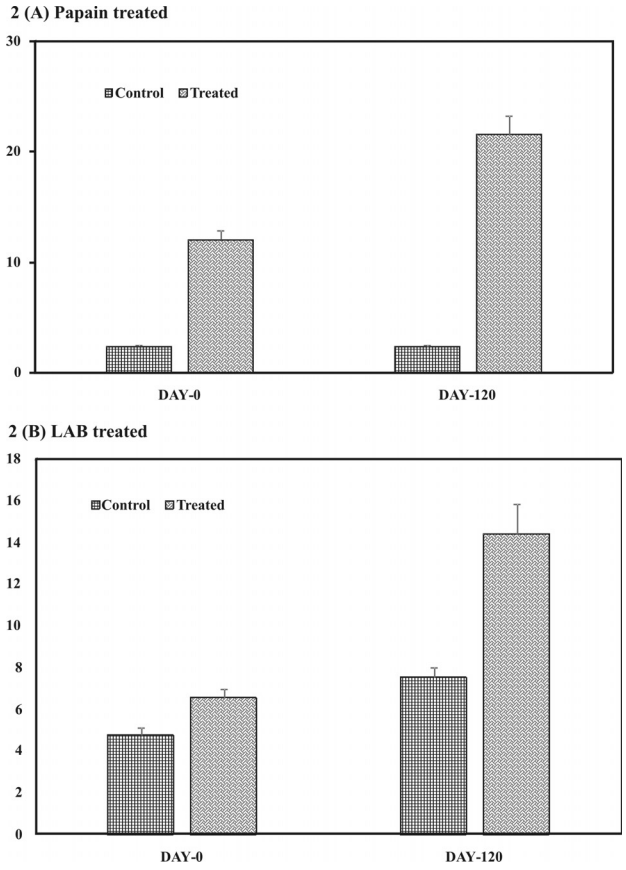

Fig. 2. Degree of hydrolysis (%) of papain treaded (A) and LAB treated (B) rohu head sauce

dients for the fermenting indigenous microbes. They inturn produce organic acids as secondary metobolite during storage. The same trend was enhanced in the LAB treated samples. The total acids content of LAB treated sample was 1.1 g 100 ml^{-1} which was 5 times higher after 120 d from the original (0.2 g 100 ml^{-1}). These values were in agreement with the findings of Xu et al. (2008). Changes in TVB-N, TSN and NPN contents are presented in the Table 2. The measurement of TVB-N indicates the degree of protein degradation in samples by spoilage bacteria, autolytic enzymes, deamination and nucleotide catabolites (FDA 2004). The increased content of TVB-N was observed in untreated control than in papain and LAB treated samples. Comparatively lower content of TVB-N was found in the LAB treated sauce (53.46 mg 100 g^{-1}) than papain treated sauce (64.64 mg 100 g^{-1}). The TVB-N values were within the acceptable range (14.1-338.6 mg 100 ml^{-1}) which were found in most Southeast Asian fish sauce (Cho et al., 2000).

The total soluble nitrogen (TSN) content of the sauce samples increased after 120 d fermentation. Higher content of TSN was observed in papain treated samples than the LAB treated sample. The changes of NPN were found to be similar to the changes in TSN. The increases of TSN and NPN content during processing of fish sauce could be attributed to the combined effect of autolysis, enzyme activity and microbial degradation of the rohu head muscle. Udomsil et al. (2015) reported that the total nitrogen content (1.89 g 100 g^{-1}**)** in the fish sauce inoculated with *Staphylococcus* sp. CMS5-7-5 and incubated at 35°C for 180d. The total nitrogen content of enzyme treated fish sauce exceeded the minimum value for second grade fish sauce (1.5-2.0%) set by the Thai Industrial Standards Institute after 120 d. The total nitrogen content reported for different types of fish sauce has been variable and may be based on the raw material or the processing condition. Total nitrogen content was in the range of 1.176 to 1.316 g 100 g^{-1} in the fish sauce prepared using low salt, solid state fermentation with anchovy by-products (Yu et al., 2014). As shown in Table 2, the NPN levels of papain and LAB treated samples increased significantly from the original 0.246 g 100 g^{-1} and 0.157 g 100 g^{-1} to 0.350 g 100 g^{-1} and 0.223 g 100

Table 2. Biochemical properties of fermented rohu head sauce

Parameter		TA (g%)		TVB-N (mg%)		TSN (g%)		NPN (g%)		Non-enzymatic browning	
Treatment	Day	C	T	C	T	C	T	C	T	C	T
Papain treated	0	0.1	0.3	11.73	11.67	0.361	1.29	0.068	0.246	0.016	0.008
	120	0.3	0.9	71.30	64.65	0.380	1.54	0.058	0.350	0.058	0.105
LAB treated	0	0.2	0.2	11.67	11.80	0.641	0.84	0.125	0.157	0.035	0.033
	120	0.4	1.1	64.64	53.46	0.654	1.08	0.145	0.223	0.204	0.203

C-control, T-treated

g^{-1} respectively after 120 d of fermentation. NPN level increased after 12 h of fermentation from the original 0.06 g 100 g^{-1} to 0.69 g 100 g^{-1} after 48 h of fermentation in fermented surimi with *Actinomucor elegans* XH-22 as starter (Zhou et al., 2014).

Non-enzymatic browning index of fermented rohu head sauce is showed in the Table 2. An increase in browning was observed in the samples obtained after 120 d of fermentation. Greater browning was found in the LAB treated and untreated samples after 120 d compared to original. Higher browning was observed in the papain treated sample than control at the end of 120 d of storage. According to Klomklao et al. (2006), the increase in browning depends on the concentration of salt, highest browning was observed in fish sauce produced with a low salt concentration. Peptides and amino acid release during proteolysis served as substrates for Maillard browning reaction (Yongsawtdigul et al., 2007). The Maillard browning reaction contributes brown colour in fish sauce yu-lu (Lopetcharat et al., 2001). The result of the present study was in correlation with the findings of Klomklao et al., (2006) and Yongsawtdigul et al. (2007).

In vitro antioxidant activities of lyophilized powder against different radicals are presented in the Table 3. Sauce samples inoculated with LAB (*P. pentosaceus* FSBP4-40) had better TAO activity as compared to the papain treated samples. Papain treated sauce samples showed high scavenging activity (37.73±0.78, 56.55±2.67 and 98.82±0.84%) whereas, the untreated control exhibit decreased scavenging activity (18.05±0.47, 23.30±1.25 and 68.93±1.26%) towards DPPH, superoxide and ABTS radicals respectively, after 120 d of storage. The LAB treated samples showed increased DPPH, superoxide and ABTS radical scavenging as compared to the control after 120 d of fermentation. Ferric chloride reducing power of both papain and LAB treated and untreated samples are given in the Fig. 3. It is well understood that, the LAB treated samples exhibited high ferric reducing power than the papain treated samples. Overall, the antioxidant activities of enzyme (papain) treated samples were higher compared to LAB treated samples except TAO. Peralta et al. (2008) reported that the Philippine's salt fermented shrimp paste showed 24.3–61.5% of DPPH radical scavenging activity in 80% ethanolic extract. The peptides from fermented fish products have been reported to act as antioxidants (Majumdar et al., 2015). The proteins present in the raw material are hydrolysed into peptides and amino acids during fermentation. These protein hydrolysates in the sauce might be responsible for antioxidant activity.

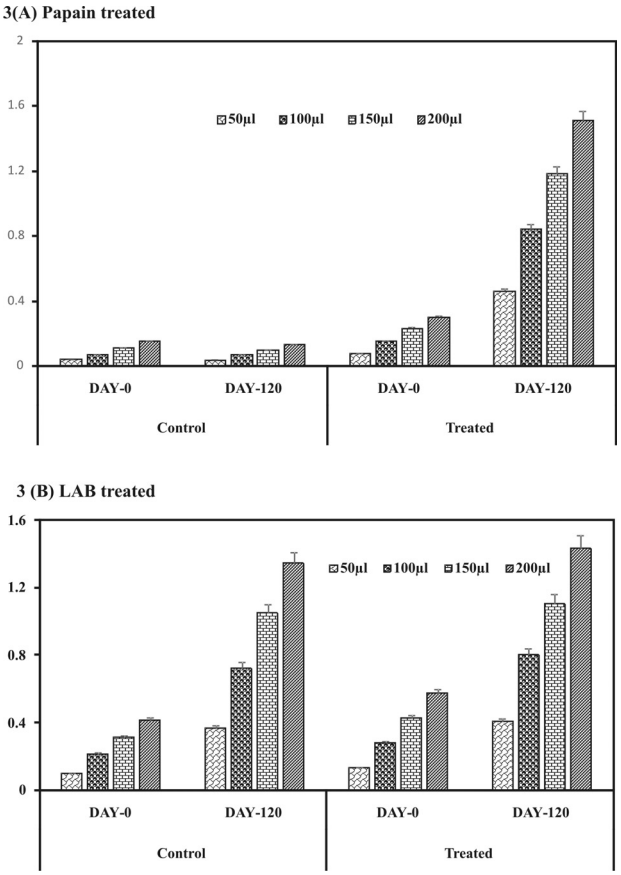

Fig. 3. Reducing power assay of papain treated (A) and LAB treated (B) rohu head sauce

Rohu head sauce obtained from different treatment had different colour characteristics (Table 4). The colour of the sauce samples intensed gradually as the fermentation time increased. Generally, all the samples had decreased L^* values and increased a^* and b^* values after 120 d of fermentation. From the result, the sauce samples treated with papain and LAB were showed decreased L^* values than untreated control. Papain treated sample had slightly greater a^* value as compared to LAB treated sample after 120 d of storage. Papain treated and untreated samples were showed increase in the b^* values but, the LAB treated and control samples had decreased b^* values after 120 d of fermentation. This could be probably due to the decrease in the added sugar content in the fermented media, most of the small peptides and amino acids in the fish sauce

Table 3. Antioxidant activity of fermented rohu head sauce

Parameter		TAO*		DPPH (%)		SOS (%)		ABTS (%)	
Treatment	Day	C	T	C	T	C	T	C	T
Papain treated	0	310.00 ±7.07	744.37± ±7.95	18.87 ±0.54	21.34 ±0.16	32.49 ±2.14	40.43 ±1.96	77.81 ±1.26	89.05 ±0.42
	120	246.25 ±8.84	1070.63 ±11.49	18.05 ±0.47	37.73 ±0.78	23.30 ±1.25	56.55 ±2.67	68.93 ±1.26	98.82 ±0.84
LAB treated	0	485.63 ±4.42	612.50 ±1591	16.45 ±0.86	21.40 ±1.01	27.96 ±2.49	34.38 ±1.60	89.05 ±1.26	97.34 ±0.42
	120	1037.50 ±14.14	1236.25 ±7.07	26.40 ±1.09	27.39 ±2.02	32.62 ±0.89	36.83 ±3.03	93.79 ±3.77	97.63 ±1.67

*Expressed as ascorbic acid equivalents in μg g^{-1} of sample
Values in column are mean±SD, C-control, T-treated

Table 4. Hunter colour values of fermented rohu head sauce

Parameter		L*		a*		b*		dE*ab	
Treatment	Day	C	T	C	T	C	T	C	T
Papain treated	0	63.66	57.33	3.68	7.72	3.04	14.31	36.52	45.45
	120	49.87	14.24	7.72	18.33	15.84	23.94	53.14	90.91
LAB treated	0	43.86	37.32	7.13	8.92	36.57	35.63	67.38	72.50
	120	18.35	16.75	18.80	17.68	27.18	28.00	88.09	89.60

C-control, T-treated

contribute to the brown colour development (Lopetcharat et al., 2001). The colour intensity of salt fermented fish sauce changes according to fermentation period and also affected by the maturity of the product (Mueda, 2015). The study revealed that the papain treated and LAB fermented fish sauce samples after 120 d were ripened. It is based on the description that fish sauce is a clear brown liquid sauce, with straw yellow to amber in colour (PNS 413:1993).

Lipolysis played a major role in flavour formation during ripening of fermented rohu head sauce. Fatty acid profile of papain treated and LAB fermented fish sauces are presented in Table 5 and 6 respectively. Overall composition of the fatty acids after 120 d of storage was reduced in both papain and LAB treated samples. Amongst saturated fatty acids, palmitic acid (C16:0) was found to be dominant in papain treated and untreated samples than the LAB treated and untreated samples. But, the vice versa was observed in case of palmitoleic acid (C16:1) concentration. Among the monoenoic fatty acid, oleic acid (C18:1n-9c) increased two times in LAB treated and control samples than papain treated samples. The saturated fatty acid (SFA) was highest on day-0 and had been decreased after 120 d in both papain and LAB treated and untreated sauce samples. Unsaturated fatty acid (USFA) concentration in enzyme treated sample was high (35.00%) as compared to control (31.19%) and also LAB inoculated samples showed increased (33.74%) concentration than the control (30.39%) after 120 d of storage. EPA in papain and LAB treated samples were 1.19% and 0.61% respectively.

Changes in microbiological count (log cfu g^{-1}) of all the sauce samples are presented in Table 7. Initial counts of aerobic bacteria in papain treated and control samples were 6.18±0.16 and 6.12±0.30 and it has been decreased to 1.77±0.27 and 2.21±0.10 respectively. Lactic acid bacteria count of papain treated and untreated samples were 4.03±0.57 and 4.58±0.06 on day-0 and the values declined to 1.64±0.19 and 1.46±0.32, respectively after 120 d. Bacterial colonies exhibited clear zone on MRS agar

Table 5. Fatty acid composition (% w/w of total lipids) of papain treated rohu head sauce

Name of Fatty acid methyl ester	DAY-0		DAY-120	
	C	T	C	T
Lauric acid (C12:0)	1.403	0.613	0.742	0.631
Myristic acid (C14:0)	5.157	4.820	4.808	3.924
Myristoleic acid (C14:1)	1.206	0.514	1.748	1.118
Pentadecanoic acid (C15:0)	1.280	0.946	1.293	0.969
Palmitic acid (C16:0)	34.443	32.012	30.900	26.667
Palmitoleic acid (C16:1)	12.042	13.000	11.090	11.659
Hepta decanoic acid (C17:0)	1.897	1.606	0.628	0.631
Cis-10 Hepta decanoic acid (C17:1)	-	0.689	-	0.785
Stearic acid (C18:0)	6.389	4.996	5.628	4.365
Oleic acid (C18:1n-9c)	9.601	9.925	9.302	9.349
Elaidic acid (C18:1n9t)	4.093	3.996	4.555	3.998
Linoleic acid (C18:2n-6c)	2.358	5.132	2.374	5.573
ã- Linolenic acid (C18:3n6)	-	-	1.613	0.422
á- Linolenic acid (C18:3n3)	1.416	4.992	-	-
Arachidic acid (C20:0)	0.662	0.458	0.647	0.473
Eicosatrienoic acid (C20:3n6)	-	0.758	-	0.932
Eicosapentanoic acid (C20:5n3)	-	0.495	0.505	1.194
SFA	51.231	45.451	44.646	37.66
USFA	30.716	39.501	31.187	35.003
Unidentified	18.053	15.048	24.167	27.337

C-control, T-treated

containing 0.3% (w/v) $CaCO_3$ were considered for total LAB count. $CaCO_3$ was used as an indicator for acid-producing LAB strains since it dissolved when interact with acid then a clear zone is observed around the colony (Onda et al., 2002). *Escherichia coli*, staphylococci, *salmonella-shigella*, halophilic bacteria and yeast and mould in papain treated and control samples were not detected except, 4.3±0.12 of halophilic bacteria in enzyme treated sample after 120 d of storage. The LAB treated and untreated sauce samples showed counts for all the microbiological parameters initially except halophilic bacteria but, after 120 d of fermentation the counts were not detected for all except lactic acid bacteria (3.48±0.26). Paludan-Muller et al. (2002) have reported that the halotolerant bacteria will grow and propagate after 5 days of fermentation and these halophiles mostly were LAB and yeasts. The probable reason for decrease in microbiological counts was depletion in the nutrition ingredients and accumulation of metabolites with progress of fermentation.

The study suggested that rohu heads, an edible fish by-product could be fermented in 120 days into fish sauce with acceptable quality in terms of nutrition. In addition, the commercially available papain and native halotolerant, proteolytic lactic acid bacteria were suitable agents for the acceleration of fermentation. Although the papain treated fish sauce samples appeared to have slightly better biochemical quality than the sauce prepared from LAB inoculated one, these fish sauces showed better microbiologically quality. Results of physicochemical properties such as, yield, pH and fat in the sauce inoculated with *P. pentosaceus* FSBP4-40 were supe-

Table 6. Fatty acid composition (% w/w of total lipids) of LAB treated rohu head sauce

Name of Fatty acid methyl ester	DAY-0		DAY-120	
	C	T	C	T
Lauric acid (C12:0)	1.139	0.661	1.043	1.421
Tridecanoic acid (C13:0)	0.628	0.456	0.452	1.064
Myristic acid (C14:0)	2.057	2.280	2.694	2.269
Myristoleic acid (C14:1)	0.627	0.803	0.687	1.995
Pentadecanoic acid (C15:0)	0.713	0.813	0.741	0.893
Palmitic acid (C16:0)	27.582	29.785	22.537	25.641
Palmitoleic acid (C16:1)	4.151	4.641	3.212	3.647
Hepta decanoic acid (C17:0)	1.410	1.648	0.619	0.448
Stearic acid (C18:0)	5.689	6.272	4.986	5.639
Oleic acid (C18:1n-9c)	21.525	23.638	20.255	19.593
Elaidic acid (C18:1n9t)	3.076	3.022	3.012	2.206
Linoleic acid (C18:2n-6c)	4.107	6.698	2.108	3.903
ã- Linolenic acid (C18:3n6)	1.026	1.519	1.115	1.171
á- Linolenic acid (C18:3n3)	-	2.028	-	0.619
Arachidic acid (C20:0)	2.261	2.441	2.070	2.322
Eicosatrienoic acid (C20:3n6)	-	0.563	-	-
Eicosapentanoic acid (C20:5n3)	-	0.363	-	0.605
Behenic acid (C22:0)	0.622	0.515	0.678	0.739
SFA	42.101	44.871	35.82	40.436
USFA	34.512	43.275	30.389	33.739
Unidentified	23.387	11.854	33.791	25.825

C-control, T-treated

Table 7. Microbiological characteristics of fermented rohu head sauce

Parameter	Papain treated				LAB treated			
	DAY-0		DAY-120		DAY-0		DAY-120	
	C	T	C	T	C	T	C	T
Total plate count	6.12±0.30	6.18±0.16	2.21±0.10	1.77±0.27	6.71±0.14	6.84±0.12	ND	ND
Escherichia coli count	4.27±0.16	4.18±0.16	ND	ND	3.39±0.20	3.19±0.02	ND	ND
LAB count	4.58±0.06	4.03±0.57	1.46±0.32	1.64±0.19	6.20±0.19	6.95±0.15	ND	3.48±0.26
Staphylococci count	5.22±0.46	5.71±0.17	ND	ND	5.73±0.18	4.87±0.06	ND	ND
Salmonella-Shigella count	2.47±0.20	2.32±0.08	ND	ND	4.83±0.11	4.16±0.06	ND	ND
Halophile count	4.66±0.22	3.59±0.11	ND	4.30±0.12	ND	ND	ND	ND
Yeast and mold count	1.34±0.13	1.29±0.19	ND	ND	3.87±0.07	3.80±0.13	ND	ND

C-control, T-treated, ND-not detected
Values in column are mean±SD for triplicates and expressed in log cfu ml^{-1} of sample

rior than the papain treated sauce. Total antioxidant activity and ferric chloride reducing potential in the LAB treated sauce was excellent than in papain treated sauce. In summary, addition of papain and LAB reduces the fermentation time in the effective utilization of fish by-products and the developed product should also applied as flavouring condiment in wide variety of sea-foods.

Acknowledgments

GSS thanks the University Grants Commission and Department of Collegiate Education for the Teacher Fellowship for his doctoral program. Dr. S. G. and Dr. N. B. are thankful to University of Mysore, Mysuru and CSIR-CFTRI, Mysuru respectively, for the permission to collaborate on this work. The work forms part of the doctoral studies of GSS.

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