Biochemical analysis of elephant foot yam (*Amorphophallus paeoniifolius*) lacto-pickle with probiotic *Lactobacillus plantarum*

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Abstract

Lacto-pickles are tangy, crisp, and definitely not intimidating to eat. We previously standardized fermentations of elephant foot yam (EFY) into lacto-pickle using different fermentation specifications. The aims of this work were to perform the effect of starter culture strain *Lactobacillus plantarum* (MTCC-1325) on chemical and biochemical properties of EFY lacto-pickle. In this study, we conducted the preprocessing of blanched EFY blocks/cubes in brine (NaCl, 8%, w/v) and inoculated with the starter culture (*L. plantarum* MTCC-1325). The impact of *L. plantarum* (MTCC-1325) during lacto-pickle fermentation and its effect on the antioxidant property, structural characterization (scanning electron microscopy (SEM) and infrared spectroscopy (FTIR)), sensory analysis, and volatilome profile (gas chromatography and mass spectrometry (GC-MS)) of EFY lacto-pickle were determined. Inoculated samples showed significantly higher acidification of the brine, reaching a pH of 2.67 and titratable acidity (TA) of 2.8 g/L within 42 days of fermentation, suggesting potential antioxidant activity. The FTIR technique revealed the changes/deformation of functional groups and SEM study suggested the interaction/adhesion between starter culture *L. plantarum* (MTCC 1325) on EFY lacto-pickle. The starter culture used clearly influenced volatile organic compounds (VOCs) profiles of EFY lacto-pickle. Moreover, EFY lacto-pickle produced with the starter culture significantly perceived a positive response in the overall acceptance. Overall results indicated the successful and accelerated EFY lacto-pickle was achieved when using *L. plantarum* (MTCC-1325) as starter culture and thus the possible use of this fortified product by the consumers in their diet.

Keywords Submerged fermentation · Elephant foot yam lacto-pickle · Proximate composition · Sensory evaluation

Introduction

Elephant foot yam (EFY) (*Amorphophallus paeoniifolius* Densst.) Nicolson (*Araceae*) is a highly salubrious but underutilized tropical tuber crop. The tuber is rich in nutrients and is an adequate source of mineral, protein, nutrients, and

starch (Behera and Ray 2016). EFY also forms an integral part of the staple food system for a population of around 500 million people globally (Muniz et al. 2008). The rural and tribal people mostly consume the tuber crop either in raw, boiled, or baked form (Di Cagno et al. 2013). EFY lacto-pickling is a novel process to pickle the tuber without any addition of oil and spices; in turn, pickling is done organically with lactic acid bacteria (LAB) (Sivakumar et al. 2010; Ray and Behera 2016). The LAB is a group of organisms (gram-positive and non-sporulating rods or cocci) that ferment sugar (i.e., glucose) to lactic acid (LA). *Lactobacillus plantarum* is an efficient LAB microorganism which can be used as a starter culture for preparation of EFY lacto-pickle. This species is known to improve the intestinal microflora and inhibit the growth of pathogens, (i.e., *Escherichia coli*, *Salmonella*, and *Staphylococcus*). Further, the group of LAB are probiotics in nature and is often experienced/encountered in human gastrointestinal tracts that confers various health benefits to consumers (Reddy et al. 2014; Behera et al. 2018a).

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In our previous work, we have a standardized (response surface methodology (RSM) technique) the submerged fermentations (SmF) of EFY into lacto-pickle using different fermentation specifications (Behera et al. 2018). In continuation of the above-mentioned study, the current study focuses on analytical/biochemical (pH, titratable acidity (TA), LA, starch, and total sugar) and proximate composition (organic matter, ash, fat, crude protein, gross energy, moisture, and dietary fiber) of EFY lacto-pickle. The antioxidant property and sensory analysis/attributes (aroma, texture, flavor, color/appearance, taste, and after taste) of the EFY lacto-pickle was judged. Analysis of volatile compounds (GC-MS analysis) and structural characterization (SEM and FTIR) of the EFY lacto-pickle was also conducted for evaluating the adhesion and interaction studies.

Materials and methods

EFY roots

EFY roots/tubers (cultivar: IGAM-1) from the experiential field/farm of Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, Chhattisgarh (CG) (located center-east of India), were used in experiments. Freshly harvested and unbruised EFY roots were collected during the season/month of November 2016 with night and day temperature were 14 ± 2 °C and 28 ± 2 °C, respectively. The roots/tubers were used in a period of 24 h later harvest.

Propagation of starter culture

Lactobacillus plantarum (MTCC-1325) was maintained on the Man, Rogosa, and Sharpe (MRS) agar (Merck KGaA, Darmstadt, Germany) inclines/slants at 4 °C in a refrigerator (Panda and Ray 2008). The *L. plantarum* (MTCC-1325) culture was the stock culture (viable) of the Institute of Microbial Technology (IMTEC), Chandigarh (a union territory of India). Sweet grapes (var. Bangalore blue) (100 g) of good quality was chosen and cleaned thoroughly by running tap water to discard the impurities. A mixer-grinder (Bajaj, India) was used to mash the grapes. The extracted juice (by use of juice squeezer) was sieved using a cheese cotton cloth and a mixture with a ratio of 1:1 (v/v) of extracted juice and water was prepared. A hot plate was used to boil the mixture at 28 ± 2 °C for a duration of 10–15 min and was immediately let to cool at room temperature. An inoculum consisting of *L. plantarum* (MTCC-1325) culture was inoculated to the grape juice and was made up in an incubator (Beautex Instruments, India) at 30 °C for 24 h. This culture was used as a starter culture for conducting the proceeding experiments.

Lactic acid fermentation of EFY

EFY roots were finally washed for the elimination of the surface dirt and were grated/peeled and carefully chopped into small blocks/cubes (nearly $1 \times 1 \times 1$ cm³). Blanching (cooking) of the cubes was conducted by incubation of these cubes in baking (boiling) water for 10–15 min at 70 °C. Plastic jars (500 mL) (Polypet®, Bombay, India) were used to dispense the blanched EFY cubes (140 g). Further, 300 mL of brine solution (NaCl, 2–10% w/v) was added to each bottle. Three replicates/repeats were maintained for each salt concentration and the info from the biochemical analyses was determined as the mean of three repeats. Each bottle was inoculated with 10 mL of starter culture (1×10^7 CFU/mL) lidded securely. The EFY brine solutions were incubated (30 ± 2 °C) and were left for fermentation process (42 days).

Biochemical and proximate analysis

Physical (pH) and biochemical, i.e., lactic acid (LA), titratable acidity (TA), starch, and total sugar constituents of EFY lacto-pickle were evaluated. A digital pH meter with the electrode (type-335, Systronic, India, Pvt. Ltd.) was used (standardized with a buffer solution of pH 7.0 and 9.2) to determine the pH value of EFY lacto-pickle. TA was determined by the titration method (Amerine and Ough 1980) and an ultraviolet-visible spectrophotometer (Model Cecil CE-7250; Cecil Instruments Ltd., Cambridge, UK) was used to determine the contents of LA. The starch and total sugar (constituents) contents were detected (method described by Mahadevan and Sridhar 1998) and the data was revealed as the equivalent of grams per kilogram EFY lacto-pickle and grams per liter lacto-pickle brine solution.

The proximate compositions of EFY lacto-pickle, such as organic matter was measured by the procedure described in USDA (1984) and ash content was estimated by a muffle furnace method. The fat content (ether extract) was evaluated by continuous Soxhlet extraction (organic solvent like hexane) techniques (Socs Plus, SCS 4, Pelican Equipments, Chennai, Tamil Nadu, India), protein (nitrogen content, $N \times 6.25$) content was determined by Kjeldahl digestion/method (Hoover 2001). Gross energy (kcal/g EFY lacto-pickle) was determined by using an adiabatic bomb calorimeter (Parr Instrument Company, Moline, IL, USA) (USDA 1984). Moisture was determined by vacuum oven (Lindberg/Blue M™), and crude fiber was evaluated according to the enzymatic-gravimetric method (Prosky et al. 1984) using Fibra Plus Fiber Tech apparatus (Pelican Equipments, T Nagar, Chennai, India). Protein, fat, and ash contents were reported as grams per kilogram EFY lacto-pickle.

Effect of shelf-life on cell viability and stability of EFY lacto-pickle

EFY lacto-pickle (75 mL) formed after 72 h of fermentation were allowed to ferment (42 days) and preserved at room temperature for 6 months. Samples (EFY lacto-pickle for 8% equilibrated salt concentration) were taken at weekly intervals and counted from the Petri plates (100 mm × 18 mm) containing MRS (Mann-Rogosa-Sharpe) agar medium (Tominaga and Sato 1996) and incubated (30 ± 2 °C) under microaerophilic (low oxygen) conditions for 24 h. After incubation, colonies with distinct morphology were picked and subcultured individually. The viability of *L. plantarum* (MTCC-1325) culture and shelf-life of the EFY lacto-pickle was determined and expressed as colony-forming unit (CFU) per milliliter of lacto-pickle. The bacteria (other than LAB) was carried out by using Gram's staining method, colony appearance (sizes and shapes), and growing on nutrient agar. EFY lacto-pickle (75 mL) sample (in triplicates for enumeration of bacteria, fungi, and yeasts) was filtered by using a filtered disc (0.45 µm) and a suction pump. The discs were plated on nutrient agar, potato dextrose agar (PDA), and wort agar plates for enumeration of bacteria, fungus, and yeasts, respectively (Dubey and Maheshwari 2004). The colonies formed on the agar plates (nutrient, PDA, wort) were then used to estimate the numbers of bacteria, fungus, and yeasts in the original sample. Colonies developing on plates from the last positive dilution (10^{-1} to 10^{-6}) were first grouped by their macroscopic and microscopic morphotypes.

Morphological (SEM) analysis

The sample (EFY lacto-pickle) topography and interaction between *L. plantarum* (MTCC 1325) and EFY lacto-pickle was studied using scanning electron microscopy (SEM, JEOL, USA) according to a previously described method (Bernet et al. 1994) with some modifications. A drop of fermented EFY was fixed in 2.5% (v/v) glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 h at room temperature. Following, the fixed EFY lacto-pickle were freeze-dried in a lyophilizer (Labconco, USA) and was taken on aluminum SEM pin stub/holder testing carbon conductive tape (double-sided adhesive) with an acceleration voltage of 10 kV (50-µm magnification) in low vacuum mode.

Structural analysis of EFY lacto-pickle

The bond level characterization of developed EFY lacto-pickle was recorded on an attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) spectrophotometer (Alpha ATR-FTIR, Bruker, Germany). ATR-FTIR was used for analysis of the bond variations between the fresh EFY (i.e., unfermented EFY) and the final product

(i.e., lacto-pickle). The bond vibrations were measured by putting a sample with 128 scans and 2/cm resolution in the range of 4000–500/cm on the diamond crystal.

GC-MS analysis

Quantitative examination of the volatile compound profiles present in EFY lacto-pickle was determined using GC/MS-QP 2010 SHIMADZU with flame ionization and mass spectrometry detection. The GC-MS system was fitted with VF-NAX MAS capillary wax column ($L = 30$ m and $D = 0.25$ mm). Injection of the specimen into the column was enjoined with a carrier gas (He, 99.99% purity) at the flow/drift rate of 1.5 mL/min. The commencing oven/kiln temperature was kept at 90 °C for 2 min with an increase of up to 220 °C. The volatile organic compounds (VOCs) present in the sample (EFY lacto-pickle) were ionized at 70 eV ionization energy, 230 °C ion source temperature and were analyzed over a mass electron (m/z) range 0–500. The data collected in the form of chromatograms at changed retention time and corresponding mass spectra for different VOCs were plotted/framed and compared against the spectral info/data with the NIST repository.

Antioxidative property

The 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical scavenging activity of EFY lacto-pickle was determined by spectrophotometric (UV-1800 spectrophotometer, Shimadzu, Japan) method (Marxen et al. 2007). The N, N-dimethyl-p-phenylenediamine (DMPD) assay was performed according to the method described by Fogliano et al. (1999) and ferric-reducing ability of plasma (FRAP) assay was performed as described by Benzie and Strain (1996).

Sensory evaluation assay

Sensory attributes of EFY lacto-pickle were assessed using a 5-point hedonic scale (wherein, maximum score/point, i.e., “five” fitted to “like extremely”, and minimum score/point, i.e., “one” suited for “dislike extremely” and score “0” indicated as an in-consumable product). One hundred panelists (gender 43 women and 57 men; age group 30–45), chosen from the faculty, staff, and local inhabitants of many agronomical (botanical) departments who habitually consume pickles and other lactic-fermented commodities. Polypropylene transparent cups (tagged with 3-digit haphazard numbers) were used for serving the samples to the people. Provisions were made for questionnaires (public-opinion poll) and water for mouth rinsing within each tasting session. In order to familiarize panelists with the product, a session was conducted prior to evaluation in each step. To avoid any misinterpretation of the attributes, a thorough reading of the

questionnaires and the gist of each attribute was recommended for the panelists. The sensory assessment score was identified as the mean of the panelists' count. The statistical significance of the variations checked among the scores of the two tests was evaluated using a standard *t* test.

Statistical methods

The experimental data were subjected to evaluation of analytical and proximate results using factorial analysis of variance (ANOVA) through the general linear model (GLM) program. The Fischer's least significant difference (LSD) multiple testing/comparison test was used to correlate the determinant/factor level differences when significant (fitting) differences/deviation in ANOVA ($P < 0.05$) were observed. An α -level was fixed a priori. The multivariate analysis (MVA), such as multiple variable (correlations) analysis and principal component analysis (PCA)/factor analysis was applied for scrutiny of the combined behavior of different variables. SPSS statistics (Windows 19.0, SPSS Inc., Chicago, IL, USA) were used for analysis of the data (input) design/matrix of the variables.

Results and discussion

In our previous work, we have standardized (RSM technique) the submerged fermentations (SmF) of EFY into Lacto-pickle using different fermentation specifications, i.e., salt concentration (NaCl, 2–10%, w/v), incubation period/duration (2–42 days), and inoculum capacity/volume (1–20%, v/v). However, 8% (w/v) salt concentration was found to be the most acceptable among all, in terms of organoleptic attributes. Moreover, 10% (v/v) inoculum volume (*L. plantarum* MTCC 1325) and 22 days of fermentation period found to be a maximum yield of fermented-/lacto-pickle (Behera et al. 2018). Lacto-pickles are usually made from the fresh ripened subtropical and tropical crops like mango, papaya, citrus, guava, and sapota, basically due to their high water and sugar content, suitable taste, flavor, and seasonal availability (Muniz et al. 2008). The analytical and proximate variations during the LA fermentation of EFY lacto-pickling development are reported afterward. In the present study, the mass of salt, if any, which might have opposed while expressing the quality of starch, LA, TA, etc., in grams per kilogram EFY lacto-pickle has been ignored. This is because, unlike surface and solid-state fermentation, SmF (in which the present study is conducted) does not tend to adhere or let much amount of salt to penetrate into the pickled surface and hence restrict alterations for the general trend of the data.

Biochemical analysis

The changes in acidity (pH, TA, and LA) and carbohydrates (starch and total sugar) in the fermented product (cubes) during the course of fermentation are discussed below.

Variations in pH during the EFY lacto-pickle fermentation

pH plays an influential factor in the development of aroma and flavor of brewed/fermented vegetables and fruits (Liu 2003). It was observed that on the 7th day of fermentation, there was a rapid decline in the pH (i.e., 6.4 on the 0th day to 4.88 on 7th day) (Table 1). The rapid decrease in pH might have occurred due to the presence of salt/brine solution and incubation time of fermentation. ANOVA result showed that the days of fermentation ($F(4, 17) = 127.34$, $P < 0.001$) and salt concentration ($F(5, 17) = 37.66$, $P < 0.001$) significantly affected the pH. This finding corroborated with other similar fermented products, such as LA fermented cucumbers, Irish potato, table green olives, etc. (Ogunjobi et al. 2005). In the brew of *togwa* (a Tanzanian fermented cuisine) during the first 24 h of fermentation (carrying starter cultures of yeasts and LAB), the pH reported a drop from 5.87 to 3.24 (Mugula et al. 2003). Further, it has been reported that a quick drop in pH significantly affected the quality and texture of fermented products (Ogunjobi et al. 2005). The pH values were measured in time intervals of 7 days, (i.e., on 14th, 21st, 28th, 35th, and 42nd) of fermentation. However, there are no variations observed and the trends were found similar to the end of the pickling/fermentation process (42 days). Nevertheless, increasing the salt (NaCl) concentrations (0–10%, w/v) resulted in preventing the loss/decline in pH, possibly due to less accumulation of LA. The “post-hoc” analysis/tests also revealed that the salt concentration of 0–4% (w/v) did not differ significantly in comparison to other treatment means of salt concentrations (6–10%, w/v) ($P < 0.05$; LSD). This was more evident after 21 days of fermentation at 8% (w/v) salt concentration. Most of the LAB were able to grow at acidic pH (optimum at 4.4–4.8) and, not surprisingly, were tolerant to NaCl concentrations within a range of 15 to 20 g/L over the course of fermentation (Montet et al. 2006). Tolerating salts at higher concentrations is an advantageous property of LAB as this restricts the growth of non-desirable organisms and discourages proteolytic and pectinolytic enzyme activity, that is responsible for softening of vegetables. In our study, the strain *L. plantarum* (MTCC-1325) was able to tolerate up to 8–10% salt concentration which resulted in the comfortable EFY pickling process. However, earlier studies have reported higher salt tolerance capacities (up to 14%, w/v) for this bacterium (Di Cagno et al. 2013).

Table 1 pH values of EFY lacto-pickle during LA fermentation

Salt concentration (%)	7 days	14 days	21 days	28 days	35 days	42 days
0	4.88 ± 0.13b	3.80 ± 0.06c	2.89 ± 0.10b	2.78 ± 0.10b	2.72 ± 0.08b	2.70 ± 0.09c
2	4.86 ± 0.12b	3.76 ± 0.06b	2.86 ± 0.11b	2.76 ± 0.10c	2.76 ± 0.10c	2.69 ± 0.08c
4	4.87 ± 0.14b	3.77 ± 0.08c	2.82 ± 0.09b	2.77 ± 0.14b	2.67 ± 0.12b	2.64 ± 0.08c
6	4.82 ± 0.13b	3.82 ± 0.09c	2.82 ± 0.13a	2.75 ± 0.13b	2.72 ± 0.11b	2.68 ± 0.09c
8	4.83 ± 0.13b	3.80 ± 0.07a	2.85 ± 0.12a	2.76 ± 0.13a	2.69 ± 0.10b	2.67 ± 0.10c
10	4.88 ± 0.15a	3.81 ± 0.10a	2.89 ± 0.15b	2.78 ± 0.15a	2.68 ± 0.13a	2.66 ± 0.11a

Significant at $P < 0.001$. Different letters in the same row indicate significant differences ($P < 0.05$; LSD)

Initial (0 day) pH values of EFY cubes was $6.4 \pm \text{SD}$

Variations in TA during the EFY lacto-pickle fermentation

Initial (0 day) titratable acidity of EFY cubes was 0.70 g/kg and for brine, it was observed to be considerably low (i.e., 0.18 g/L). However, throughout the passage of fermentation (0–42 days), the variations were significant (Table 2). Interestingly, the rise in TA production showed an inversely proportional relationship to the increase in NaCl concentration in the brine solution. ANOVA analysis of cube samples showed the factors, such as the salt content and fermentation time significantly affected the TA levels, i.e., ($F(5, 17) = 270.64$, $P < 0.001$) and ($F(4, 17) = 187.49$, $P < 0.001$). Similar results were obtained for saline/brine samples, i.e., TA was significantly affected with differences in salt concentration ($F(5, 17) = 210.66$, $P < 0.001$). It seems that an increase of TA in brine is probably due to an appreciable quantity of organic acid, which might have seeped out of the EFY onto brine, mostly because of higher osmotic pressure generated by the salt concentration (Panda and Ray 2007).

Variations in LA during the EFY lacto-pickle fermentation

Lactic fermentation by *Lactobacilli* mostly produces LA as the prime and essential organic acid (Gardner et al. 2001). In the present study, *L. plantarum* (MTCC-1325) was used as a starter culture, to harvest predominately L (+) LA (Liu 2003). Initial observations revealed nearly fewer amounts of LA were found in EFY blocks/cubes (0.50 g/kg) and in a saline/brine solution (0 g/L). However, a significant increase in LA concentration (1.3–3.5 g/kg) in the fermentation medium was observed after 7 days of inoculation of *L. plantarum* (Table 3). The sharp increase in the LA concentration might have been owing to the transformation of fermentable sugars into LA by the sportive action of *L. plantarum* (Di Cagno et al. 2013). Further, the results also confirmed a reduction of LA concentration in EFY pickles as compared to brine. Hence, it is supposed that the lion's share of salt supplement, resided in brine while a small amount penetrated into EFY lacto-pickles. Further, this might have led to a change in osmotic pressure

Table 2 Titratable acidity (TA) concentration of EFY lacto-pickles (g/kg cubes) and brine (g/L) during fermentation

Salt concentration (%)	7 days	14 days	21 days	28 days	35 days	42 days
0	5.2 ± 0.4b (5.4 ± 0.3)c	5.4 ± 0.3b (5.5 ± 0.4)c	5.6 ± 0.3c (5.9 ± 0.1)b	5.0 ± 0.2c (6.2 ± 0.3)c	4.1 ± 0.2b (4.7 ± 0.3)c	3.8 ± 0.3b (4.4 ± 0.3)c
2	4.8 ± 0.3c (5.0 ± 0.1)c	4.9 ± 0.3b (5.5 ± 0.3)b	5.3 ± 0.1b (5.8 ± 0.1)b	4.8 ± 0.2b (5.5 ± 0.3)b	3.9 ± 0.4c (4.4 ± 0.2)c	3.6 ± 0.2b (4.2 ± 0.3)b
4	4.0 ± 0.4b (4.3 ± 0.1)b	4.3 ± 0.2a (4.8 ± 0.3)a	5.1 ± 0.1b (5.4 ± 0.2)b	4.5 ± 0.4c (5.0 ± 0.3)a	3.6 ± 0.2b (4.0 ± 0.3)b	3.2 ± 0.3b (3.6 ± 0.2)b
6	3.8 ± 0.3b (4.1 ± 0.3)b	3.9 ± 0.1b (4.3 ± 0.3)b	4.5 ± 0.2b (4.9 ± 0.3)a	4.2 ± 0.3a (4.8 ± 0.3)b	3.2 ± 0.3b (3.8 ± 0.1)b	2.9 ± 0.1b (3.2 ± 0.2)b
8	3.4 ± 0.2a (3.9 ± 0.2)a	3.7 ± 0.1a (4.1 ± 0.2)a	3.9 ± 0.3c (4.9 ± 0.2)a	3.5 ± 0.1c (4.5 ± 0.2)a	2.7 ± 0.4a (3.2 ± 0.2)a	2.6 ± 0.3c (2.8 ± 0.1)c
10	0.9 ± 0.2a (1.1 ± 0.1)b	1.7 ± 0.1 a (1.9 ± 0.1)a	3.3 ± 0.3 a (3.7 ± 0.1)b	3.0 ± 0.1 a (3.4 ± 0.3)b	2.6 ± 0.2 a (2.9 ± 0.3)b	1.9 ± 0.3a (2.2 ± 0.3)b

Significant at $P < 0.001$. Different letters in the same row indicate significant differences ($P < 0.05$; LSD)

Initial (0 day) titratable acidity of EFY cubes was 0.70 g/kg and for brine was 0.18 g/L

Figures in parentheses indicate the corresponding values for lacto-pickle in brine ± SD

within cubes and brine elicit the extraction/loss of a considerable volume of LA from cubes into the brine to retain equilibrium in fermentation medium (Montet et al. 2006). Experimental results further revealed that the LA concentration was significantly more in cubes treated with 0% and 2% NaCl as compared to those treated with high (8–10%) salt concentrations. A possible explanation for this might be the increased salt concentrations could repress the growth of *L. plantarum* (Zhang et al. 2000; Montet et al. 2006). ANOVA analysis showed that the salt concentration ($F(5, 17) = 66.78, P < 0.001$) and frothing/fermentation period ($F(4, 17) = 269.88, P < 0.001$) had significantly affected the production of LA in EFY lacto-pickle in brine samples. Further, the post hoc tests confirmed, that means of both 8 and 10% salt concentrations (w/v) and days of fermentation significantly varied, in respect to LA production; however, 8% (w/v) salt concentration varied most significantly ($P < 0.05$; LSD).

Variations in starch during the EFY lacto-pickle fermentation

EFY roots are rich in starch, which serves as a nutrient source for amylolytic organisms (organisms converting starch into sugar/saccharose) (Sharma and Satyanarayana 2013). Strains of *L. plantarum* (LMG-18053, NCIM-2084, MTCC-1407, and MTCC-1325) have been made known to be amylolytic (Reddy et al. 2008) and hence, in the present study, variations in starch content after inoculation of *L. plantarum* (MTCC-1325) is studied. The experimental results showed a marginal decrease in the starch content (148 g/kg in 0 days to 145.0–135.0 g/kg) in the fermented EFY lacto-pickle after 7 days of fermentation in EFY cubes (Table 4). Further, with different

salt concentrations in the brine and with an increase in fermentation time (42 days), a steep decrease of starch content (94.7–68.0 g/kg) in the EFY lacto-pickle was observed. This reduction in starch content is attributed to the amylolytic response of *L. plantarum* (MTCC-1325) that utilized the starch in the EFY lacto-pickle. However, during fermentation, the starch content in brine was found less as compared to EFY lacto-pickle. A rational reason for this might be the ability of the starch to dissolve into organic acids as compared to cold water. Panda and Ray (2007) reported that the α -amylase activity of *L. plantarum* (MTCC-1407) was responsible for the conversion of starch into sugar in preparation of sweet-potato lacto-pickle. ANOVA analysis showed that the incubation time of fermentation, ($F(4, 17) = 1534.68, P < 0.001$) and salt concentration, ($F(5, 17) = 638.44, P < 0.001$) was significantly affected by starch contents in EFY lacto-pickle of brine samples. Moreover, the post hoc tests/analysis showed that salt concentrations of 8% and 10% had significantly affected the days (0, 7, 14, and 21) of fermentation; however, 8% salt concentration varied significantly in terms of variation of starch in EFY lacto-pickle ($P < 0.001$; LSD).

Variations in total sugar

The variations observed in the total sugar content about the fermented EFY lacto-pickle were analogous to that of starch as represented in “Variations in starch during the EFY lacto-pickle fermentation” section. With the rise in the incubation time of fermentation, (i.e., from 7 to 42 days), sugar concentration continually decreased (Table 5). It was apparent that the amylolytic behavior of the inoculant microorganisms

Table 3 Lactic acid (LA) concentration of EFY lacto-pickles (g/kg cubes) and brine (g/L) during fermentation

Salt concentration (%)	7 days	14 days	21 days	28 days	35 days	42 days
0	3.5 ± 0.2a (4.1 ± 0.1)b	3.9 ± 0.1a (5.3 ± 0.3)b	4.2 ± 0.2b (5.2 ± 0.3)b	3.8 ± 0.3b (5.0 ± 0.2)b	3.2 ± 0.1c (4.8 ± 0.2)c	3.1 ± 0.3c (4.5 ± 0.3)c
2	2.8 ± 0.3b (3.9 ± 0.1)b	3.7 ± 0.1a (5.1 ± 0.2)c	4.0 ± 0.2b (5.1 ± 0.2)a	3.4 ± 0.2b (4.5 ± 0.2)c	3.0 ± 0.3c (4.3 ± 0.2)c	2.6 ± 0.3c (4.0 ± 0.2)c
4	2.6 ± 0.2b (3.3 ± 0.1)b	3.3 ± 0.3a (4.3 ± 0.2)b	3.8 ± 0.2a (5.0 ± 0.1)a	3.3 ± 0.2b (4.0 ± 0.3)c	2.8 ± 0.2c (4.0 ± 0.3)c	2.2 ± 0.1c (3.6 ± 0.3)c
6	2.0 ± 0.1b (3.0 ± 0.2)c	2.5 ± 0.3a (3.8 ± 0.2)b	3.5 ± 0.2b (4.7 ± 0.3)b	3.2 ± 0.2b (3.8 ± 0.3)c	2.4 ± 0.1b (3.7 ± 0.2)c	1.9 ± 0.2c (3.3 ± 0.1)c
8	1.6 ± 0.2a (2.8 ± 0.2)a	1.7 ± 0.2a (3.3 ± 0.4)a	3.0 ± 0.2b (4.3 ± 0.3)a	2.5 ± 0.2b (3.5 ± 0.1)a	2.0 ± 0.4b (3.2 ± 0.2)c	1.6 ± 0.2b (2.7 ± 0.2)c
10	1.3 ± 0.2a (1.6 ± 0.1)a	1.9 ± 0.1a (2.7 ± 0.2)a	2.8 ± 0.2a (3.9 ± 0.2)a	2.0 ± 0.2a (3.0 ± 0.3)b	1.6 ± 0.2b (2.6 ± 0.3)b	1.3 ± 0.3b (2.3 ± 0.2)b

Significant at $P < 0.001$. Different letters in the same row indicate significant differences ($P < 0.05$; LSD)

Initial (0 day) LA of EFY cubes was 0.50 g/kg and for brine was 0 g/L.

Figures in parentheses indicate the corresponding values for lacto-pickle in brine ± SD

Table 4 The starch concentration of EFY lacto-pickles (g/kg cubes) and brine (g/L) during fermentation

Salt concentration (%)	7 days	14 days	21 days	28 days	35 days	42 days
0	145.0 ± 6.4 ^c (24.2 ± 0.3) ^c	138.8 ± 4.6 ^b (22.4 ± 0.1) ^b	130.7 ± 4.2 ^c (18.0 ± 0.1) ^b	118.7 ± 4.0 ^c (15.3 ± 0.3) ^c	108.3 ± 3.7 ^c (12.8 ± 0.2) ^b	94.7 ± 3.2 ^c (12.0 ± 0.2) ^b
2	143.4 ± 5.8 ^c (21.3 ± 0.5) ^c	133.2 ± 3.8 ^b (17.2 ± 0.3) ^b	128.2 ± 4.7 ^b (15.6 ± 0.3) ^b	115.5 ± 3.5 ^b (13.2 ± 0.6) ^b	103.4 ± 3.8 ^c (11.3 ± 0.4) ^c	83.1 ± 3.9 ^c (10.6 ± 0.3) ^c
4	141.3 ± 6.6 ^b (18.2 ± 0.4) ^b	132.7 ± 5.3 ^b (15.5 ± 0.5) ^c	118.4 ± 5.6 ^b (13.3 ± 0.2) ^c	102.6 ± 3.3 ^b (12.0 ± 0.4) ^c	100.2 ± 4.4 ^b (9.4 ± 0.5) ^c	70.3 ± 5.3 ^c (10.0 ± 0.5) ^b
6	141.0 ± 5.4 ^b (15.7 ± 0.2) ^b	130.0 ± 5.0 ^b (13.3 ± 0.4) ^b	116.7 ± 4.7 ^c (12.0 ± 0.6) ^c	100.3 ± 5.0 ^b (10.6 ± 0.3) ^b	92.5 ± 3.9 ^c (8.6 ± 0.4) ^c	69.7 ± 4.0 ^b (9.2 ± 0.3) ^b
8	139.0 ± 2.6 ^a (14.9 ± 0.3) ^a	124.0 ± 2.3 ^a (12.0 ± 0.5) ^a	112.0 ± 2.7 ^c (10.3 ± 0.5) ^a	98.0 ± 1.9 ^a (8.5 ± 0.4) ^a	84.0 ± 2.0 ^a (7.3 ± 0.4) ^a	55.0 ± 2.8 ^a (8.8 ± 0.3) ^a
10	135.0 ± 2.0 ^a (12.6 ± 0.6) ^a	120.0 ± 2.9 ^a (10.2 ± 0.3) ^a	117.0 ± 2.1 ^c (8.4 ± 0.4) ^a	108.0 ± 2.7 ^a (7.0 ± 0.6) ^a	91.0 ± 2.2 ^a (6.2 ± 0.3) ^a	68.0 ± 2.3 ^a (8.4 ± 0.2) ^a

*Significant at $P < 0.001$. Different letters in the same row indicate significant differences ($P < 0.05$; LSD)

Initial (0 day) starch of EFY cubes was 148 ± 2.1 g/Kg and for brine was 0 g/L.

*Figures in parentheses indicate the corresponding values for Lacto-pickle in brine $\pm$ SD

(*L. plantarum* MTCC-1325), was the responsible factor which ultimately led to LA during organic acid metabolism. Similar observations were stated by Joshi and Somesh (2010) while fermentation of vegetables (radish, carrot, and cucumber) using *L. plantarum* (NCDC-020) as a starter. However, in the present study, the total amount of fermentable sugar generated during the fermentation process was not converted into LA. This might have resulted because of the intake of some fraction of sugars for the metabolic process of the inoculum (*L. plantarum*) (Montet et al. 2006). ANOVA results for NaCl concentration and days of fermentation for brine samples showed significant differences, i.e., (F (5, 17) = 667.48,

$P < 0.001$), and (F (4, 17) = 355.821, $P < 0.001$) in terms of variation in total sugar contents in EFY lacto-pickle. Post hoc analysis showed the days of fermentation (i.e., 7, 14, and 21) and both brine samples (8% and 10%) had a significant effect on the variation of total sugar content; however, 8% brine solution varied most significantly with respect to variation of total sugar content in EFY lacto-pickle ($P < 0.001$; LSD).

Proximate analysis

From the preliminary experimental results, it was evident that EFY lacto-pickle with 8% brine solutions was acceptable both

Table 5 Total sugar concentration of EFY Lacto-pickles (g/Kg cubes) and brine* (g/L) during fermentation

Salt concentration (%)	7 days	14 days	21 days	28 days	35 days	42 days
0	12.0 ± 2.8b (9.5 ± 2.2)b	11.8 ± 2.3a (8.3 ± 0.2)a	10.2 ± 2.2a (7.0 ± 0.2)a	8.0 ± 1.2c (6.0 ± 0.1)a	6.6 ± 0.7a (5.6 ± 0.4)a	5.3 ± 0.2c (4.0 ± 0.3)c
2	11.6 ± 2.0c (10.0 ± 1.2)b	11.3 ± 1.1b (9.4 ± 0.9)b	10.0 ± 0.8a (8.5 ± 0.9)a	7.8 ± 0.2b (7.3 ± 0.1)a	5.8 ± 0.6b (5.2 ± 0.2)b	4.1 ± 0.3b (3.8 ± 0.1)b
4	11.1 ± 1.4b (10.2 ± 0.8)c	11.0 ± 1.3c (9.0 ± 0.7)c	9.2 ± 0.2a (8.0 ± 0.5)c	6.7 ± 0.3c (6.0 ± 0.8)a	4.6 ± 0.5b (3.5 ± 0.2)c	3.0 ± 0.2c (2.2 ± 0.3)c
6	10.9 ± 1.2b (8.9 ± 0.3)b	10.4 ± 0.6b (7.7 ± 0.4)a	8.9 ± 0.6 b (6.8 ± 0.5)a	5.3 ± 0.2a (4.0 ± 0.3)b	3.3 ± 0.4 a (3.0 ± 0.1)a	2.6 ± 0.3a (1.8 ± 0.1)b
8	10.8 ± 0.4a (8.5 ± 0.3)a	8.3 ± 0.5a (6.3 ± 0.2)a	6.4 ± 0.3 a (4.8 ± 0.4)a	4.1 ± 0.2a (3.6 ± 0.1)a	2.5 ± 0.3a (2.0 ± 0.3)a	1.3 ± 0.1a (1.7 ± 0.3)a
10	9.2 ± 0.2a (6.9 ± 0.1)a	6.6 ± 0.2a (5.7 ± 0.3)b	4.8 ± 0.1b (3.5 ± 0.2)b	3.1 ± 0.1a (2.8 ± 0.1)a	2.5 ± 0.2a (1.9 ± 0.2)a	1.6 ± 0.1b (1.5 ± 0.1)a

Significant at $P < 0.001$. Different letters in the same row indicate significant differences ($P < 0.05$; LSD)

Initial (0 day) total sugar of EFY cubes was 12.8 ± 0.6 g/kg and for brine was 0 g/L.

Figures in parentheses indicate the corresponding values for lacto-pickle in brine $\pm$ SD

organoleptically and taste-wise. Hence, proceeding experiment was conducted with EFY fermented with saline/brine of the above mentioned (8%, w/v) NaCl concentrations. The relative/proximate proportion of EFY lacto-pickle is depicted in Table 6. With increase in fermentation time, subsequent decrease in ash, protein, and organic loads were observed, with no significant alterations in calorific value and fat content. The obtained results were in agreement to Ogunjobi et al. (2005) who proclaimed decrease in ash quantity (8.01 to 4.08 mg/g) of Irish potato (*Solanum tuberosum*) in brine solution (2%) during spontaneous fermentation process. The decline in total sugar (carbohydrate content) is an additional choice/advantage, more specifically, for diabetic patients (Arva et al. 2013). ANOVA results showed that all periods of fermentation, (i.e., from 0 to 21 days) had significant result on 8% the salt concentration. For example, organic matter ($F(6, 14) = 623.06, P < 0.05$); ash ($F(6, 14) = 384.33, P < 0.05$); fat ($F(6, 14) = 208.4, P < 0.05$); crude protein ($F(6, 14) = 256.22, P < 0.05$); etc. Further, ANOVA reveals that there was no significant effect of incubation time during the fermentation on the gross energy, ($F(6, 14) = 3.25, P < 0.032$).

Effect of shelf-life on cell viability and stability of EFY lacto-pickle

Microscopic observation revealed that the starter culture (*L. plantarum* MTCC-1325) were Gram-positive and long slender rod-shaped bacillus. The *L. plantarum* (MTCC-1325) counts at 7 days of fermentation (NaCl, 8%, w/v) was in the range of 1.4×10^7 CFU/mL which decreased (0.8×10^7 CFU/mL) up to 42 days of fermentation (Table 7). Following it, a static phase was observed during 6 months of storage. *Lactobacillus plantarum* (MTCC-1325)

are known to readily grow on food substrates with lower pH, even up to a point where other competing organisms strive to sustain. Reasonably, at higher salt concentrations (8%, w/v), the growth and activity of other microbes (except salt-tolerant strains of *L. plantarum* (MTCC-1325) (Sivakumar et al. 2010) might have been inhibited. Further, the quality and taste of EFY lacto-pickle was stable for 6 months without any deterioration. Enumeration of fungus and yeast cells in the EFY lacto-pickle by potato dextrose agar (PDA) and wort agar plates showed the presence of 1–2 CFU/75 mL of EFY lacto-pickle, which was negligible. No other microorganisms were detected in the EFY lacto-pickle samples which ensured its sterility.

Morphological characteristics

The morphology and adhesion of *L. plantarum* (MTCC-1325) on EFY lacto-pickle was observed by SEM image. This observation is similar to those of other workers who have studied interactions of *Lactobacillus* and fermented roots and tubers (Wang et al. 2010). SEM was conducted at 10 kV to visualize the cross section and attachment of *L. plantarum* (MTCC-1325) to fermented EFY lacto pickle. The photograph shows the strong attachment of rod-shaped *L. plantarum* (MTCC-1325) to the surface of EFY pickle (Fig. 1). This is the evidence of fermented EFY as a carrier of benevolent microbial strain along with the inherent nutritional properties of EFY.

Structural analysis of EFY lacto-pickle

The infrared spectra used to detect the vibrational frequencies/bond length between the atoms of study samples. The spectra (4000–500/cm) were inferred by using the guidelines of

Table 6 Proximate composition of EFY Lacto-pickles during LA fermentation

Equilibrated salt concentration (%)	Days	Organic matter (g/kg)	Ash (g/kg)	Fat (g/kg)	Crude protein (g/kg)	Gross fiber energy (kCal/g)	Moisture (%)	Dietary (g/kg)
8	0	208.7 ± 11.3b	839.80 ± 38.5c	32.7 ± 0.3b	52.7 ± 0.6a	4.2 ± 10.8 b 18.9 ± 3.1a	75.5 ± 4.8b	
	7	204.4 ± 15.5b	815.0 ± 33.5c	30.8 ± 0.2b	49.8 ± 0.3a	3.08 ± 0.7b 18.5 ± 2.9a	75.5 ± 4.8b	
	14	201.0 ± 10.5a	794.0 ± 27.5c	29.3 ± 0.5a	47.3 ± 0.3a	3.07 ± 0.7b 17.6 ± 2.0a	74.6 ± 4.7a	
	21	185.5 ± 18.5a	766.0 ± 19.0b	28.6 ± 0.1a	45.6 ± 0.1b	3.05 ± 0.5a 17.2 ± 1.8a	72.2 ± 4.2a	
	28	183.0 ± 17.6a	630.0 ± 13.0c	25.2 ± 0.1a	44.2 ± 0.2b	3.00 ± 0.5a 16.8 ± 1.5b	68.0 ± 3.4a	
	35	179.5 ± 20.0b	602.9 ± 11.5b	23.7 ± 0.2a	40.7 ± 0.3b	2.78 ± 0.5a 15.5 ± 1.2b	66.5 ± 3.5a	
	42	176.5 ± 13.6b	600.5 ± 10.0b	22.5 ± 0.3b	37.9 ± 0.1b	2.63 ± 0.5a 13.8 ± 1.9b	63.8 ± 4.5a	

± SD

*Significant at $P < 0.001$. Different letters in the same row indicate significant differences ($P < 0.05$; LSD)

Table 7 *Lactobacillus* counts (10^7 CFU/mL) of EFY lacto-pickle

Equilibrated salt concentration (%)	Fermentation (days)					
	7	14	21	42	28	35
0	1.0	1.0	0.9	0.8	0.7	0.4
8	1.4	1.2	1.1	0.9	0.8	0.8
10	1.2	1.0	1.0	0.7	0.7	0.6

Genkawa et al. (2012). The main absorption peaks at 3390, 2931, 2500, 1670, 1167, and 899/cm in IR spectroscopy were generated in the fresh EFY lacto-pickle (Supplementary Fig. 1). The wider bands at 3390/cm can be ascribed to hydrogen bonded –O–H– stretching vibration and at 2931/cm might be asymmetric stretching of alkyl –C–H–. Similarly, the peaks at 2500/cm are attributed to carboxylic acid (–O–H). The bands above 1670/cm exhibited strong coordination to one oxygen of each carboxyl (C (=O) OH) group coordinated to facial sites and two C=O bonds directing opposite from the surface (Panda et al. 2017). The peaks around 1167 and 899/cm were assigned to the vibration of acyl or phenyl C–O and aromatic C–H group, respectively. However, changes in fundamental regions of IR spectra of the lacto-pickle (after fermentation) were detected with new absorption peaks at 3650 and 2088/cm indicating the occupancy of added organic functional groups. The formation of organic acids is ascribed to the substantial LA fermentation by *L. plantarum* (MTCC-1325). The peaks at 2945, 2847, and 887/cm might be due to the presence

of aromatic carboxyl, hydroxyl, and C–H groups. However, weak peaks of oxalate for EFY lacto-pickle confirm the successful decrease in anti-nutritional factor (acidity) due to LA fermentation.

Analysis of volatile organic compounds

GC-MS analysis is an accurate and time efficient technique for analysis of volatile compounds and has been used for detection of LA by other researchers (Panda et al. 2017). The distinctive flavor to lacto-pickle is mostly due to the presence of volatile compounds and hence, GC-MS was used for detection of the same. In total, 26 volatile compounds, including acids, alcohols, esters, and other compounds were detected in the sample (Tables 8 and 9; Supplementary Fig. 2). The compound phenol-2,4-bis(1,1-dimethylethyl), was detected with a maximum peak area up to 31.26% ($m/z = 206.20$), whereas lactic acid was found with a peak area of 1.29% ($m/z = 83.10$). However, the presence of other VOCs (e.g., bis(2-ethylhexyl) phthalate, 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, phenol, 4-nitro-2-diphenylphosphino-, 2,5-di-tert-butyl-1,4-benzoquinone, etc.) was negligible in the sample. Although in uncooked/pre-fermented EFY tuber contain some toxic anti-nutrients such as raphides (composed of calcium oxalate crystals) (Behera and Ray 2016), none of the particular hazardous organic variety was detected in the EFY lacto-pickle. These results clearly indicate the modification or disintegration of these risky organic compounds during the progression of SmF fermentation.

Fig. 1 SEM photograph shows the topography and attachment of rod-shaped *L. plantarum* (MTCC-1325) to the surface of EFY lacto-pickle during the process of fermentation (on 21st day). Circle with arrows = *L. plantarum* (MTCC-1325) attachment on the sample (EFY lacto-pickle)

Table 8 Sensory evaluation of the EFY lacto-pickle in different salt concentrations

Attributes	Equilibrated salt concentrations (%)				
	2	4	6	8	10
Texture	1.6 ± 0.3c	1.8 ± 0.2b	2.9 ± 0.4c	3.5 ± 0.5b	3.9 ± 0.7a
Aroma	1.3 ± 0.3b	1.5 ± 0.4b	3.4 ± 0.2c	4.0 ± 0.3b	4.7 ± 0.5a
Taste	1.8 ± 0.6b	1.9 ± 0.4b	2.8 ± 0.3	3.4 ± 0.6a	4.3 ± 0.3b
Color/appearance	1.4 ± 0.3b	1.8 ± 0.4c	2.6 ± 0.3b	3.5 ± 0.5a	3.7 ± 0.6a
Flavor	1.4 ± 0.4c	1.7 ± 0.3b	2.7 ± 0.3b	3.8 ± 0.5a	4.5 ± 0.7a
After taste	1.4 ± 0.3c	1.7 ± 0.4c	2.8 ± 0.3b	3.6 ± 0.6b	4.0 ± 0.7b

Significant at $P < 0.001$. Different letters in the same row indicate significant differences ($P < 0.05$; LSD)

± SD

 n (no. of persons) = 100

Values are means of the panelist's scores

1 dislike extremely, 2 like moderately, 3 like much, 4 like very much

Antioxidant activity

DPPH activity is frequently used for analysis of antioxidant property of foodstuff and plant extracts. In the present study, EFY lacto-pickle was observed to have a DPPH scavenging activity of $64.44 \pm 0.34\%$, at a dose of 100 μL . The DPPH scavenging activities of EFY lacto-pickle was increased along with the fermentation period and was the duration-dependent manner up to 22nd day fermentation using *L. plantarum* (MTCC-1325) (Fig. 2a). EFY lacto-pickle was found to be an effective DMPD^{•+} radical scavenger in a concentration-

dependent manner. It was able to scavenge DMPD^{•+} radicals by 66% at 10 μL and 18% at 50 μL of EFY lacto-pickle (Fig. 2b). The reducing power (FRAP assay) of the fermented pickle also increased with dose-dependent manner and found to be 0.716 and 1.132 for 100 and 300 μL , respectively, of EFY lacto-pickle (Fig. 2c).

Sensory evaluation

Consumer panel is a popular technique for assessing the acceptability of food products (Sivakumar et al. 2010). The sensory

Table 9 Compounds identified EFY lacto-pickle by GC-MS analysis

No	RT (min)	Compounds	RA (%)
1	11.350	Lactic acid	1.29
2	12.935	Phenol, 2,4-bis (1,1-dimethylethyl)-	31.26
3	13.828	9-Hexadecene, (E)-	2.83
4	16.060	1-Octadecene	3.78
5	17.487	2,5-di-tert-Butyl-1,4-benzoquinone	2.65
6	17.819	n-Hexadecanoic acid	7.98
7	18.083	1-Nonadecene	3.42
8	18.260	Thiazolidine-2, 4-dione, 3-methyl-5-(3,4-methylenedioxybenzylideno)-	4.13
9	19.930	Behenic alcohol	2.49
10	21.446	Phenol, 4-nitro-2-diphenylphosphino	4.05
11	21.632	Cyclotetrasiloxane	2.31
12	22.919	Bis (2-ethylhexyl) phthalate	0.89
13	22.976	Triphenylphosphine oxide	0.37
14	23.205	9-Nonadecene	3.38
15	23.661	Silane, dimethyldicyclohexyloxyhexadecyloxy-	4.88
16	23.729	Triphenylphosphine sulfide	1.79
17	24.668	Octacosanol	2.21
18	24.958	5-Methyl-2-trimethylsilyloxy-acetophenone	0.50
19	26.032	Tris (tert-butyl)dimethylsilyloxy) arsane	1.39
20	26.359	Trimethyl[4-(2-methyl-4-oxo-2-pentyl) phenoxy] silane	0.26
21	26.515	Cyclotrisiloxane, hexamethyl-	0.96
22	26.603	1,4-Bis (trimethylsilyl) benzene	0.43
23	26.681	Silicic acid, diethyl bis(trimethylsilyl) ester	0.58
24	27.023	5-Methyl-2-trimethylsilyloxy-acetophenone	8.77
25	27.065	1,2-Bis (trimethylsilyl) benzene	5.98
26	27.807	1,2-Benzisothiazol-3-amine tbdms	1.41

No. compound number in order of elution, RT retention time (min), RA relative area

Fig. 2 Antioxidant activities of EFY lacto-pickles **(a)** 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical scavenging assay shows increase of scavenging activities with the fermentation period up to 22nd day of fermentation. **b** N, N-dimethyl-p-phenylenediamine (DMPD) scavenging capacity shows effective radical (DMPD⁺) scavenger in a concentration-dependent manner of sample. **c** ferric reducing ability of plasma (FRAP) assay shows increase in reducing power with dose-dependent manner of sample

evaluation scores of EFY lacto-pickle with salt concentrations of 2–10% (w/v) were delivered to one-way analysis of variance (ANOVA). The reports showed a significant effect of salt (8%,

w/v) concentrations on texture/smoothness ($F(4, 10) = 568.5$, $P < 0.001$); odor ($F(4, 10) = 717.9$, $P < 0.001$); taste ($F(4, 10) = 311.1$, $P < 0.001$); color ($F(4, 10) = 422.6$, $P < 0.001$);

Fig. 3 Sensory evaluation shows the comparison of sensory scores of EFY lacto-pickles of various salt concentrations (NaCl, 2–10%, w/v). The figure sights a significant effect of salt (NaCl, 8%, w/v) concentrations on texture/smoothness, odor, taste, color, flavor, and after taste of sample (EFY lacto-pickle). No. of persons = 100; Values are means of the panelist's scores; 1 = dislike extremely, 2 = like moderately, 3 = like much, 4 = like very much, 5 = like extremely

flavor ($F(4, 10) = 423.9, P < 0.001$); and after taste ($F(4, 10) = 358.6, P < 0.001$) of EFY lacto-pickle (Table 8 and Fig. 3). Post hoc analysis exhibited that, except for 2% and 4% of salt concentration, all other combinations were significantly factious/variant from each other ($P < 0.05$; LSD). Furthermore, lacto-

pickles processed in 8 and 10% saline/brine though were similar in some conditions, but aftertaste and taste wise pickles framed in 8% saline/brine was more preferable.

The correlation analysis was used for the measurement of the linear association between variables. The Pearson's

Fig. 4 The component plot in rotated space shows the graphical representation of principal components (50.9%, PC1 and 44.9%, PC2) of analytical and proximate variables using varimax rotation with loading plot

correlation coefficients (r^2) among the analytical and proximate variables are presented in Supplementary Table 1. A significant correlation was observed between all the parameters. For instance, pH was observed to be significantly coordinated with LA and TA with the other important correlations are, such as starch-sugar (0.955), organic matter-gross energy (0.897), starch-protein (0.970), and contents of EFY lacto-pickle. Adopting PCA, the 12 original variables were shortened to two principal components/elementals (PC1 and PC2), which had eigenvalues more than 1 and kept for rotation. The variations observed were 50.9% and 44.9% for PC1 and PC2, respectively. The combination of PC1 and PC2 in coexisting reported for 94% of the overall variations. Further, the varimax method was used to rotate the factor pattern which was helpful for interpretation of dimensions. All the variables were loaded heavily on two dimensions. Five variants/variables, i.e., pH (+ve), TA (+ve), starch (+ve), sugar (+ve), and dietary fiber (+ve) were loaded heavily on PC2, showing strong correlations with these traits/attributes (Supplementary Table 2). Hence, the unite of these traits/variables loading on PC2 may be widely assigned to as *nutrient axis* or *taste axis* as these components are developing on major supplements/nutrients in EFY lacto-pickle. A more detailed understanding informs that the factor loadings of organic matter (+ve), crude protein (+ve), gross energy (+ve), and moisture (+ve) may be annotated as *carbon axis*. Figure 4 depicts the plotting of PC1 against PC2 which highlights a similar trend. It is noteworthy that, till date, only a few reports have been published for application of PCA in the estimation of lacto-fermented food-stuff analysis (Panda et al. 2009, 2017).

Conclusions

The present investigation caters adequate scientific evidence for enhancement of the functional aspects and simultaneous preservation/storage of EFY lacto-pickle for an extended time period. Consumption of EFY lacto-pickle formed from SMF fermentation of blanched cubes in common salt (NaCl, 8%, w/v) solution can be a rich source of nutrients and beneficial attributes. Further, reports suggest the production of antimicrobial substances such as H_2O_2 , LA, alcohols (–OH), and alternative metabolites from EFY lacto-pickle, which may inhibit unsolicited pathogens in the human intestine. Additionally, the EFY lacto-pickle is a novel functional and fermented product containing nutritious and antioxidant factors. The biochemical and sensory attributes of EFY lacto-pickle also had encouraging results among the consumers and hence carry worthy anticipation for commercialization among small and cottage-scale industries. The overall results obtained in this study warrant further investigation of probiotic properties of *L. plantarum* (MTCC-1325) for enhancing functional properties of EFY lacto-pickles.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

- Amerine MA, Ough CS (1980) Methods for analysis of musts and wine. Wiley, New York
- Arva HR, Bhaskar JJ, Salimath PV, Mallikarjuna AS (2013) Anti-diabetic effect of elephant-foot yam (*Amorphophallus paeoniifolius* (Dennst.) Nicolson) in Streptozotocin-induced diabetic rats. *Int J Biomed Pharm Sci* 7:1–6
- Behera SS, Ray RC (2016) Konjac glucomannan, a promising polysaccharide of *Amorphophallus konjac* K. Koch in health care. *Int J Biol Macromol* 92:942–956
- Behera SS, Panda SH, Mohapatra S, Kumar A (2018) Statistical optimization of elephant foot yam (*Amorphophallus paeoniifolius*) lacto-pickle for maximal yield of lactic acid. *LWT Food Sci Technol* 87: 342–350
- Behera SS, Ray RC, Zdolec N (2018a) *Lactobacillus plantarum* with functional properties: an approach to increase safety and shelf-life of fermented foods. *Biomed Res Int*. <https://doi.org/10.1155/2018/9361614>
- Benzie IFF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Anal Biochem* 239:70–76
- Bernet MF, Brassart D, Neeser JR, Servin AL (1994) *Lactobacillus acidophilus* LA 1 binds to cultured human intestinal cell lines and inhibits cell attachment and cell invasion by enterovirulent bacteria. *Gut* 35:483–489
- Di Cagno R, Coda R, De Angelis M, Gobbetti M (2013) Exploitation of vegetables and fruits through lactic acid fermentation. *Food Microbiol* 33:1–10
- Dubey RC, Maheshwari DK (2004) Practical manual of microbiology. Nirali publication, Pune
- Fogliano V, Verde V, Randazzo G, Rittieni A (1999) Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. *J Agric Food Chem* 47:1035–1040
- Gardner NJ, Savard T, Obermeier P, Caldwell G, Champagne CP (2001) Selection and characterization of mixed starter cultures for lactic acid fermentation of carrot, cabbage, beet and onion vegetable mixtures. *Int J Food Microbiol* 64:261–275
- Genkawa T, Watari M, Nishii T, Ozaki Y (2012) Development of a near-infrared/mid-infrared dual-region spectrometer for online process analysis. *Appl Spectrosc* 66:773–781
- Hoover R (2001) Composition, molecular structure, and physicochemical properties of tuber and root starches: a review. *Carbohydr Polym* 45: 253–267
- Joshi VK, Somesh S (2010) Preparation and evaluation of sauces from lactic acid fermented vegetables. *J Food Sci Technol* 47:214–218
- Liu SQ (2003) Practical implications of lactose and pyruvate metabolism by lactic acid bacteria in food and beverage fermentations. *Int J Food Microbiol* 83:115–131

- Mahadevan A, Sridhar R (1998) Methods in physiological plant pathology, 5th edn. Sivakami Publication, Madras
- Marxen K, Vanselow KH, Lippemeier S, Hintze R, Ruser A, Hansen UP (2007) Determination of DPPH radical oxidation caused by methanolic extracts of some microalgal species by linear regression analysis of spectrophotometric measurements. *Sensors* 7:2080–2095
- Montet D, Loiseau G, Zakhia-Rozis N (2006) Microbial technology of fermented vegetables. In: Ray RC, Ward OP (eds) *Microbial biotechnology in horticulture*, Volume 1. Science Publishers Inc., Enfield, pp 309–343
- Mugula JK, Narvhus JA, Sørhaug T (2003) Use of starter cultures of lactic acid bacteria and yeasts in the preparation of togwa, a Tanzanian fermented food. *Int J Food Microbiol* 83:307–318
- Muniz CR, Borges MF, Freire FCO (2008) Tropical and subtropical fruit fermented beverages. In: Ray RC, Ward OP (eds) *Microbial biotechnology in horticulture*, vol 3. Science Publishers, New Hampshire, pp 35–70
- Ogunjobi AA, Adebayo-Tayo BC, Ogunshe AA (2005) Microbiological, proximate analysis and sensory evaluation of processed Irish potato fermented in brine solution. *Afr J Biotechnol* 4:1409–1412
- Panda SH, Ray RC (2007) Lactic acid fermentation of β -carotene rich sweet potato (*Ipomoea batatas* L.) into lacto- juice. *Plant Food Hum Nutr* 62:65–70
- Panda SH, Ray RC (2008) Direct conversion of raw starch to lactic acid by *Lactobacillus plantarum* MTCC 1407 in semi-solid fermentation using sweet potato (*Ipomoea batatas* L.) flour. *J Sci Ind Res* 67:531–537
- Panda SH, Panda S, Shiva Kumar PS, Ray RC (2009) Anthocyanin-rich sweet potato lacto-pickle: production, nutritional and proximate composition. *Int J Food Sci Technol* 44:445–455
- Panda SK, Behera SK, Qaku XW, Sekar S, Ndinteh DT, Nanjundaswamy HM, Ray RC, Kayitesi E (2017) Quality enhancement of prickly pears (*Opuntia* sp.) juice through probiotic fermentation using *Lactobacillus fermentum*-ATCC 9338. *LWT Food Sci Technol* 75:453–459
- Prosky L, Asp NG, Furda I, DeVries JW, Schweizer TF, Harland BF (1984) Determination of total dietary fiber in foods and food products: collaborative study. *J Assoc Off Anal Chem* 68:677–679
- Ray RC, Behera SK (2016) *Amorphophallus*: Technological interventions. In: Sharma HK (ed) *Tropical tuber crops: technological interventions*. Wiley, pp 591–612
- Reddy G, Altaf MD, Naveena BJ, Venkateshwar M, Kumar EV (2008) Amylolytic bacterial lactic acid fermentation—a review. *Biotechnol Adv* 26:22–34
- Reddy CK, Haripriya S, Mohamed AN, Suriya M (2014) Preparation and characterization of resistant starch III from elephant foot yam (*Amorphophallus paeonifolius*) starch. *Food Chem* 155:38–44
- Sharma A, Satyanarayana T (2013) Microbial acid-stable α -amylases: characteristics, genetic engineering and applications. *Process Biochem* 48:201–211
- Sivakumar PS, Panda SH, Ray RC, Naskar SK, Bharathi LK (2010) Consumer acceptance of lactic acid fermented sweet potato pickle. *J Sens Stud* 25:706–719
- Tominaga M, Sato K (1996) Lactic acid fermentation of saccharified solution from rice flour. *J Food Sci* 61:627–631
- USDA (1984) Composition of foods, vegetables and vegetable products raw processed, prepared. *Agricultural hand book*, Department of agriculture, Washington, DC
- Wang CY, Lin PR, Ng CC, Shyu YT (2010) Probiotic properties of *Lactobacillus* strains isolated from the feces of breast-fed infants and Taiwanese pickled cabbage. *Anaerobe* 16:578–585
- Zhang JH, Hu F, Chen HY (2000) Processing technique of vegetable juice beverage of *Sechium edule* Swartz and fermentation beverage of *Cucurbita moschata*. *Duch J Shanghai Agric Coll* 18:114–117

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