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Effect of different pretreatment on the microbial diversity of fermented potato revealed by high-throughput sequencing

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Abstract

To unravel prospective lactic acid bacteria (LAB) and yeasts with potential application in potato-based products, the microbial diversity of fermented potato was studied. High-throughput sequencing revealed that the 5 samples differ in microbial compositions resulting from nutrients modification by the pretreatment methods adopted. *Weisella* and *Saccharomyces cerevisiae* were found to be the dominant LAB and yeast in fermented potato respectively. The high abundance of *Weisella* (35.84%) *and Gluconobacter* (20.80%) in steamed fermented potato (SFP) resulted in lowering of pH (4.39 ± 0.09) and inhibition of undesirable bacteria and fungi. Fermentation increased protein and dietary fiber contents of boiled fermented potato (BFP) and SFP, with highest protein content (8.18 ± 0.22%) recorded in BFP. The dietary fiber contents of BFP and SFP are 9.68 ± 0.68% and 9.48 ± 0.37% respectively with no observable significant difference. This study provides information on microorganisms with potential benefits in quality enhancement of potato-based products.

Keywords: High-throughput sequencing; microorganisms; fermented potato; physicochemical composition

Chemical compounds studied in this paper:

Hydrochloric acid (PubChem CID:313); Sodium hydroxide (PubChem CID:14798); Potassium sulfate (PubChem CID:24507); Sulfuric acid (PubChem CID:1118); Nitric acid (PubChem CID: 944); Petroleum ether (PubChem CID:8900); Ethanol (PubChem CID:702).

1. Introduction

Potato (*Solanum tuberosum L.*) is the fourth most important crop worldwide after rice, wheat, and maize (Bártová, Bárta, Brabcová, Zdráhal, and Horáčková, 2015). Potato is considered as nutritious vegetable because it is rich in nutrients such as starch, dietary fiber, amino acids, minerals, vitamins and phytochemicals (Bártová et al., 2015; Ezekiel, Singh, Sharma, and Kaur, 2013). Potato protein contains balanced amino acid which supersedes cereal proteins (Ezekiel et al., 2013). Aside from a cuisine, potatoes are now used in the production of different food products such as bread, steamed bread, dumplings, pancakes biscuits, pizza, and noodles (Liu, Mu, Sun, Zhang, and Chen, 2016).

Processing of potato requires innovation for preservation, conservation and enhancement of its nutrient composition. Fermentation is a proven biotechnology approach of food preservation that could find application in potato processing. It involves slow decomposition of organic substances into simpler compounds (such as organic acids, alcohol, carbon dioxide etc.) by microorganisms especially lactic acid bacteria (LAB) and yeast. A wide range of fermented foods are produced from grains, meat, fruits and vegetable, dairy products as well as tuberous crops like cassava and yam which are, to some extent, similar to potatoes (He et al., 2017). Aside preserving food, fermentation also helps in improving the nutritional, functional, textural, sensory quality, and the bioactive compounds of food (He et al., 2017). However, pretreatments prior to fermentation are required for easy hydrolysis of carbohydrate and protein by LAB, and minimizing undesirable microorganisms that might compete with the LAB during early stage of fermentation (Swain, Anandharaj, Ray, and Rani, 2014). Both pretreatments and fermentation would play significant role in preventing enzymatic browning that may hinder consumers' acceptability of potato-based products.

Cultural and non-cultural techniques have been used to reveal the microbial diversity of fermented foods. Although cultural methods have been long used and still in use but its inability to capture wide range viable but not culturable microorganisms of significant use has led to the use of molecular methods in identifying microorganisms. On the other hand, molecular-based methods are more accurate and reliable for the identification of culture-dependent and culture-independent microorganisms in foods. High-throughput sequencing technique (HTST) is the commonest molecular-based method for profiling microorganisms. In

recent times, HTST have been used to unravel vast range of microorganisms in fermented products (He et al., 2017; Sun et al., 2014).

The genus *Lactobacillus, Carnobacterium, Pediococcus, Leuconostoc* and *Weisella* have been identified in traditional Peruvian fermented potato using HTST (Jiménez et al., 2018) while Liu et al. (2015) reported yeasts which include *Saccharomyces, Pichia, Galactomyces, Vanderwaltozyma, Wickerhamomyces* and *Cordyceps* in naturally fermented Mongolian cow milk using HTST. In spite of the application of fermentation in many food products, fermentation of potato for application in staple food products has not been explored. The report of Adegunloye and Oparinde (2017), which suggests increase in protein, ash and fat contents of potato peels following fermentation, provides insights that the fermentation can promote effective utilization of potatoes by improving the nutritional and functional properties.

Therefore, in this present study, fresh potatoes were pretreated by boiling, steaming and high hydrostatic pressure (HHP) respectively with subsequent fermentation for 48h. The microbial composition of the fermented and unfermented samples was revealed by HTST. In addition, the effect of fermentation on the physical properties (pH and total titratable acidity) and nutritional quality (proximate and mineral composition, total starch and total sugar contents) of potato were evaluated. Our results could provide useful information for effective utilization of fermented potato in the food industry.

2. Materials and Methods

2.1 Sample preparation

Potato (Cultivar: Favorita) was purchased from a market in Beijing, China. A 2kg of potato samples were processed for each of the treatments. The samples were washed, peeled, sliced into cubes prior to processing. Thereafter, they were treated by boiling and steaming at 100˚C for 30min, and high hydrostatic pressure (HHP) (300MPa, 400MPa, 500MPa and 600MPa respectively) for 15min at room temperature. The sample for HHP treatment were packed in vacuum bag in ratio 1:1 w/v of sliced potato sample and sterile distilled water. The HHP treatments were carried out using high pressure device (Model HHP.L3-600/0.6; Tianjin Huatai Senmiao Engineering and Technique Co.Ltd, Tianjin, China). Raw potato samples were also processed and unprocessed raw potato was used as the control. All the treated samples were fermented using submerge method in ratio

(Sample: distilled water 1:2 w/v) in a fermenting machine at 30˚C and 80% relative humidity for 48h. The samples were freeze dried, milled into flour and then evaluated.

2.2 The pH and total titratable acidity

The pH and total titratable acidity (TTA) were determined according to the method described by Li, Li, Deng, Bian, and Liu (2014). The samples (10g) were homogenized with 90mL of sterile distilled water and the pH was measured with a pH meter (**OHAUS*,** STARTER 3100). The TTA was determined by titration with 0.1M NaOH until a pH of 8.5 is attained. Acidity was expressed in mL of 0.1M of NaOH used to neutralize the sample.

2.3 Proximate composition

Crude protein, moisture, crude fat, dietary fiber and ash were determined in accordance to AOAC method (Association of Analytical Chemists, 2003). Protein was determined by Kjeldahl method with nitrogen conversion factor of 6.25 (AOAC 955.04), moisture (AOAC 935.29), crude fat (AOAC 920.39), total dietary fiber (AOAC 991.43) and ash (AOAC 923.03). Carbohydrate content was obtained by difference method as described by Abeshu and Kefale (2017).

2.4 Total starch content

Total starch content was determined according to AOAC method (Association of Analytical Chemists, 2003)**.** Absorbance was read for each sample and the D-glucose control at 510 nm against the reagent blank using a UV1101 spectrophotometer (Hitachi, Japan). Total starch content was expressed as mg /100g DW basis.

2.5 Total sugar content

The total sugar content was determined according to the 3,5-dinitrosalicyclic acid method described by Menezes et al. (2016) with slight modification. The absorbance of the samples were measured at 540nm using a UV1101 spectrophotometer (Hitachi, Japan).

2.6 Mineral composition

Mineral composition analysis was performed according to AOAC (2003). The samples were digested in concentrated HNO_3. The digest was transferred to a 25mL volumetric flask, with volume adjusted to 25mL with deionized water. A blank digest was prepared in a similar manner. Mineral content was determined using

inductively coupled plasma atomic emission spectrometry (ICAP6000, Thermo Fisher Scientific, Waltham, MA, USA) and the values were expressed as mg/100g DW of flour.

2.7 High-throughput sequencing analysis

Total genome DNA extraction was done according to the manufacturer's instructions using OMEGA DNA isolation kit (Omega, D5625-01, USA). The 16S rRNA V3 + V4 region and ITS region were amplified for bacteria and fungi respectively. Appropriately diluted (10ng) DNA was used as template for the V3 + V4 region of 16S rRNA gene polymerase chain reaction (PCR). The reaction was carried out in a final volume of 20μL containing 4 μL 5 X FastPfu buffer, 2 μL 2.5 mM dNTP, 1.0 U of Taq polymerase, 0.8 μL of each primer 5 μM, 0.2 μL BSA(Bovine serum albumin), and distilled water. The DNA was denatured at 95˚C for 3min. Reactions were run for 27cycles of 95˚C for 30s, 55˚C for 30s and 72˚C for 45s for 16SrRNA and 30cycles for ITS region and finally extended at 72˚C for 10min.

2.8 Biodiversity analysis

The PCR products were sequenced using Illumina Highseq protocol in accordance to Biomarker Technologies, Beijing, China procedures. The data of the double ended sequences obtained from Hiseq sequencing were merged, filtered to obtained high quality sequences which were later clustered into different operational taxonomical units (OTUs) based on 97% similarity using UCLUST of QIIME (Version 1.8.0) software and they were aligned with the Silva (http://www.arb-silva.de/) and UNITE (http://unite.ut.ee/index.php) reference gene data base for bacteria and fungi respectively.

Alpha diversity which consists of (Chao1 and ACE species abundance estimator, Shannon and Simpson species diversity indices, and rarefaction curve) reflects the richness and diversity of a single sample species. Alpha diversity analysis was carried out using Mothur (V.1.11.0) software. Beta diversity analysis conducted by Bray-Curtis was used to compare the difference of all samples (Hu et al., 2018). Principal component analysis (PCA) was carried out to assess the bacterial and fungal composition of samples. Venn diagram was designed to illustrate the similarity and difference between the bacterial and fungal communities respectively in the five samples.

2.9 Statistical Analysis

All experiments were performed in triplicate with values expressed as the mean ± standard deviation (SD) of the data obtained. Statistical analyses were done using SPSS 16.0 (SPSS Inc., Chicago, USA) software. Data were subjected to one – way ANOVA analysis of variance and Duncan's multiple range test was used for comparison of group means with statistical significance at $p < 0.05$.

3. Results and Discussion

3.1 Changes in pH and TTA during fermentation

The results of the changes in pH and TTA during fermentation are reported in Table S1. The pH value of all samples decreased significantly ($p<0.05$) throughout the fermentation period. Among all samples, at 0h raw fermented potato (RFP) recorded the highest pH (6.23 ± 0.06) while lowest pH was recorded in 500MPa HHP treated fermented potato (HFP_{500}) (5.87 ± 0.04).

Elfnesh, Tekalign, and Solomon (2011) also reported pH of raw potato to be in the range of 6.0. During fermentation, the decrease in pH could be attributed to the hydrolysis of carbohydrate into simple sugar with further conversion to lactic acid by microorganisms dominating the fermenting medium. The decreased in pH observed in this study correlates with the report of Adegunloye and Oparinde (2017) on the fermentation of Irish potato and sweet potato peels.

A concomitant increase was observed in the TTA of all samples during fermentation. TTA in fermentation is a measure of total organic acid in foods, as a result of acidification. There was significant increase in TTA values of all the samples as fermentation progresses. The lowest pH and highest TTA was observed in SFP after 48h of fermentation. Bello and Akinyele. (2007) stated that pH decrease is accompanied by increase in acidity as microbial activities cause accumulation of lactic acid.

3.2 Proximate composition

Table 1 shows the proximate composition of the samples. Ash content reflects the mineral contents of foods. Ash content value of the control (raw potato RP) 3.77 ± 0.01% was significantly higher ($p< 0.05$) than all fermented samples. Among the fermented samples SFP (2.80 ± 0.00%) and RFP (2.80 ± 0.00%) had the highest ash content and were not significantly different. The decrease in ash content value was more pronounced in HHP treated fermented samples, with the lowest value found in 300MPa high hydrostatic

pressure treated fermented potato (HFP_{300}) (0.94 ± 0.03%). However, the ash content tends to increase as pressure increases. Our result conforms with the report of Abeshu and Kefale (2017) that recorded a higher ash content value in unprocessed lupin beans and lower ash content value in fermented, cooked and soaked beans. The reduction in ash content can be attributed to leakage of soluble minerals into the soaking and cooking water (Kavitha and Parimalavalli, 2014). The reduction in ash content may also be due to its utilization by microorganisms for metabolic activities.

Fat enhances the taste, aroma and texture of food. It also serves as source of energy and essential fatty acid (linoleic and linolenic acid) needed by body. High fat content value was recorded in all HHP treated fermented samples and SFP with no significant difference observed. The lowest fat content was observed in boiled fermented potato sample (BFP) 0.11 ± 0.03% and RFP 0.14 ± 0.01% without significant difference. Ndidi et al. (2014) have also reported a similar reduction in fat content of *Sphenostylis stenocarpa* seeds during boiling. Microorganisms oxidize fat to yield substantial amount of energy required for their growth thus causing reduction in fat content (Babalola and Giwa, 2012).

The protein content showed significant difference with values ranging from 5.59 ± 0.24% in 600MPa high hydrostatic pressure treated fermented potato (HFP_{600}) to 8.18 ± 0.22% in BFP. Protein is essential in food because it plays a significant role in human growth and development. Kaur and Aggarwal (2015) reported that the protein content of steamed and water cooked potato were not significantly different from that of raw potato tuber. Our study recorded that the protein contents of BFP and SFP were significantly higher than those of the control and other samples. The increase in protein content of BFP and SFP might be caused by loss of dry matter especially carbohydrate. The reduction in carbon ratio causes an increase in nitrogen concentration thus leading to protein increase (Offiah, Ariahu, and Igyor, 2016). The HHP treated samples and RFP recorded lower protein content with HFP_{600} (5.59±0.24%) having the lowest value and all showed significant difference. The decrease can be attributed to hydrolysis of complex and soluble protein into simple forms by microorganisms and leaching out of soluble protein into the fermenting medium (El-Adawy, Rahma, El-Bedawy, and Sobihah, 2000).

Dietary fiber is known as carbohydrate that cannot be metabolized by human digestive enzymes. It plays an important role in human diets and can help in combating life threatening diseases such as diabetes, cancers,

gastrointestinal diseases, constipation etc. (Otles and Ozgoz, 2014). Potato is known to contain reasonable amount of dietary fiber, thus increase daily intake of potato should be recommended. Raw potato dietary fiber content had been previously reported by Otles and Ozgoz (2014). The value of dietary fiber of raw potato in our report is higher as compared to the report of Mallillin, Trinidad, Raterta, Dagbay, and Loyola (2008) and lower as compared to the report of Otles and Ozgoz (2014). The dietary fiber contents of BFP (9.68 ±0.68%) and SFP (9.48±0.37) were significantly greater than that of other samples and not significantly different from each other. Previous studies have also reported that cooking treatments increase total dietary fiber of potato (Zhao, Andersson, and Andersson, 2018b; Dhingra, Michael, Rajput, and Patil, 2012). The increase in total dietary fiber in these samples might be due to the formation of complexes between polysaccharides and other compounds such as protein and lipid present in food (Dhingra et al., 2012). Samples HFP_{300} (5.32 ± 0.06%), HFP_{500} (5.93 ± 0.41%) and RFP (5.66 ± 0.03%) recorded low dietary fiber and show no significant difference. Also 400MPa high hydrostatic pressure treated fermented potato (HFP_{400}), HFP_{600} and RP were not significantly different. The reduction in dietary fiber caused by fermentation might be due to increase in metabolic activity of microorganisms that lead to hydrolysis of dietary fiber components and conversion of insoluble dietary fibre to soluble dietary fiber (Rodríguez, Jiménez, Fernández-Bolaños, Guillén, and Heredia, 2006).

The HHP treated samples showed high carbohydrate content with higest value recorded in HFP_{300} (86.44 ± 0.09%). The high content of carbohydrate in the HHP fermented potato may be due to hydrolysis of complex oligosaccharides by alpha galatosidase found in the microorganisms involved in the feremntation. Moreso, the increase in carbohydrate content might be attributed to decrease in moisture content (Igbabul, Amove, and Twadue, 2014). The lowest carbohydrate content was recorded in SFP (67.82±0.32%). The decrease in carbohydrate content can be linked to the ability of microorganisms to metabolize and use carbohydrate as energy source.

3.3 Total starch and total sugar content

Total starch and total sugar contents results are shown in Table 1. The total starch contents ranged from 48.28± 1.12 mg/100g (RFP) to 61.67 ± 0.00 mg/100g (HFP_{500}). Samples BFP and HFP_{400} are not significantly different from RP (control). Also HFP_{300}, HFP_{600} and SFP were not significantly different. The

reduction in total starch content may be due to break down of starch molecules into sugars, used as carbon sources by LAB for the synthesis of desirable metabolites (Gunawan et al., 2015).

The total sugar content ranged from 8.64 ± 0.13 mg/100g (SFP) to 11.79 ± 0.13 mg/100g (RP). Sample SFP had the lowest value which is not significantly different from the value recorded for RFP. The low sugar contents correlates with the low pH observed in these samples. This indicates that LAB metabolize these sugars to produce organic acids. The sugar content of HFP_{500} is not significantly different from that of the control. The increase in total starch and total sugar contents might be attributed to the reduction in dietary fiber that indirectly causes an increase in carbohydrate content.

3.4 Mineral composition

Potato is an important source of minerals as it contains relatively low amount of phytic acid. Mineral composition of the samples is shown in Table 2. K, P, and Mg are the major elements with high contents in this study. Potato has been previously reported as good source of K, P, and Mg (Kita et al.,2013). K and Na are important dietary minerals and electrolytes required for water balance and acid-base balance in the blood and tissues. K has been reported to be the major cation in potato. Fermentation significantly reduced the contents of Na, K, Ca, Mg, and P in all the fermented samples when compared with the unfermented sample. Similar reduction in Na and P content after fermentation had earlier been reported by Digbeu et al. (2013). The Zn and Mn contents decreased significantly in all the fermented samples except RFP. The Zn content of RFP show no significant difference from the control but the Mn content of RFP was significantly higher than the control. This shows that fermentation of potato without any pretreatment can help preserve Zn and Mn content of potato. Zn is an important trace element that plays a vital role in immune system regulation. Manganese is needed for diverse metabolic functions such as skeletal system developments, nervous system and immunological systems functions etc. The reduction in Zn, Ca and Mg contents in this study might be due be due to utilization of these minerals for growth by microorganisms. Fe is a mineral that is vital to human health and it is known to perform many functions in the body. It is a component of hemoglobin. Fermentation improved the Fe content of the steamed and raw fermented potato, with SFP significantly greater than RFP and other samples. Low Fe value was recorded in all HHP treated fermented samples and BFP. Samples HFP_{300}, HFP_{400}, HFP_{600} and BFP were not significantly different. The lowest Fe content was recorded in HFP_{500}. The

reduction in Fe content of BFP might be due to its leaching out during boiling (Iheke, Oshodi, Omoboye, and Ogunlalu, 2017). Fermentation increased the Cu content in all the samples except for HFP_{500} and SFP that are not significantly different from the control. Cu is an essential trace element that plays numerous functions in the body. Examples include iron absorption and its works in conjunction with Fe to form red blood cells. Among the fermented samples, RFP and SFP have the highest mineral contents and this can be attributed to the high ash content observed in these samples. Since microorganisms require minerals for growth and development, mineral reduction might occur in the fermented samples (Iheke et al., 2017).

3.5 Richness and diversity analysis of bacterial and fungal communities

A total of 1,199,840 pair-end reads for bacterial communities and 1,199,434 pair-end reads for fungal communities were produced from the 5 samples. After filtering the raw tags, 1,038,703 and 1,088,352 clean tags were obtained for bacterial and fungal communities respectively. Each samples generated an average of 207,741 and 217,670 clean tags respectively. The clean tags were allocated to 121 and 128 OTUs based on 97% similarity for bacterial and fungal, respectively.

3.5.1 Rarefaction analysis

The bacterial and fungal communities rarefaction curves based on OTUs 97% similarity are shown in Fig. 4Ai and 4Aii, respectively. The result for both bacterial and fungal diversity showed that the rarefaction curves reached the saturation plateau (i.e. rarefaction showed approximation to an asymptote). It can be presumed from these results that the recovered sequences commendably reflected the bacterial and fungal communities in the five samples.

3.5.2 Bacterial and fungal alpha diversity

Chao1 and ACE were used to calculate the number of bacterial and fungal species while Shannon diversity and Simpson diversity indices are used to estimate the diversity. Bacterial alpha diversity (Table S2) revealed that sample BFP had the highest number of OTUs, ACE, Chao1, and Shannon Index with lowest Simpson. It has been previously reported that the lower the Simpson index of a sample the higher the microbial diversity. The lowest Simpson index obtained in sample BFP indicates that the sample is rich in diversity as compared to other samples. Sample RP (control) had the highest Simpson index which indicates that the species richness,

evenness and diversity are low in this sample. Although it has higher Chao1 and ACE values (high numbers of species) as compared to SFP and RFP but these species occur in low percentage.

Fungal alpha diversity (Table S2) showed that sample RP had the highest number of OTUs, ACE, Chao1, and Shannon Index with lowest Simpson index value. While sample SFP has the lowest number of OTUs, ACE, Chao1, and Shannon Index with highest Simpson index value. This result indicates that RP is rich in fungal species and diversity as compared to other samples, while sample SFP has the lowest fungal diversity. The high acidity reported in sample SFP might be responsible for the low number of fungal species and low diversity recorded in this samples, this is in line with the report of Zhao, Li, Jiang, and Deng (2018a).

3.6 Venn diagram analysis of bacterial and fungal diversity

The Venn diagrams are shown in Fig.S1 A and B for bacterial and fungal diversity respectively. The Venn diagrams were used to evaluate the distribution of OTUs among the different samples. The total numbers of OTUs obtained are 109, 107, 44, 103, and 40 from samples BFP, HFP_{600}, RFP, RP and SFP respectively for bacterial diversity. The samples shared 22 OTUs in common. The shared OTUs accounted for 85.07% of the total sequence and 7% of the shared OTUs were unclassified. The OTUs common to the samples belong to the genera *Acetobacter, Lactobacillus, Gluconobacter, Bacillus, Klebsiella, Weisella, Streptococcus, Staphylococcus, Bacteriodes Sphingomonas, Enterococcus, Gluconobacter, pseudomonas and Nicotiana otophora* at the genus level. Only sample BFP was found to have 3 unique OTUs belonging to the genus *Desulfovibrio, Branchybacterium* and *Parasutterella* and these OTUs accounted for 0.43% of the total sequences.

The total number of OTUs obtained from BFP, HFP_{600}, RFP, RP and SFP are 103, 105, 70, 109, and 38 respectively as shown in Fig.S1B. The Venn diagram of fungal diversity revealed that 30 OTUs were common to the 5 samples. The shared OTUs contain 92.42% of the total sequences, 46.64% and 27.76% of the shared OTUs were unassigned and unclassified respectively. The fungi of the shared OTUs belong to the genus *Trichosporon, Cladosporium, Alternaria, Wickerhamomyces, Malassezia, Penicillium,* and *Saccharomyces.* Samples BFP, HFP_{600}, RFP, and RP were found to contain 1, 4, 5, and 3 unique OTUs respectively while sample SFP has no unique OTU. The unique OTUs belong to the genus (*Kernia*), (*Lauriomyces, Clonostachys, Alternariaster,* and *Nadosonia*), (*Purpureocillium, Chrysosporium, Erythrobasidium, Mucor* and

Lecanicillium), (*Paramycosphaerella*, *Lophiostoma* and *Myrmecridium*) for samples BFP, HFP_{600}, RFP and RP respectively.

3.7 Relative abundance of bacterial and fungal communities

Fig.1Ai and Aii show the relative abundance of bacterial and fungal communities at phylum and genus level respectively. A total of 15 bacteria phyla were identified in this study but only the phyla with top relative abundance value ≥ 0.01% are displayed in Fig. 2Ai. The identified bacteria phyla are Fusobacteria, Deferribacteres, Bacteriodetes, Actinobacteria, Proteobacteria, Firmicutes and Cyanobacteria. Others with low relative abundance include, Cloacimonetes, Acidobacteria, Saccaribacteria, Spirochaetae, Synergistetes, Tenericutes, Verrucomicrobia and WS6. Cyanobacteria was found to be the predominant phylum in RP, HFP_{600} and RFP accounting for 90.51% , 44.77%, and 59.96% of the sequences of sample RP, HFP_{600} and RFP, respectively. Cyanobacteria are known to be ubiquitous in nature and are found in environment such as soil, aquatic habitat, rock etc. The high amount of the phylum in HFP_{600} and RFP reflects that they are able to survival pH below 5 this is contrary to previous report of Rai and Rajashekhar (2014) that optimal growth of cyanobacteria ranged from pH 7.5 and above. They recorded lower growth at pH of 6.5. Kallah and Castenholz (1982) had earlier mentioned that the optimal pH for most cyanobacteria are between 7.5 and 10 with few that are able to grow at pH 4 to 5. Our report shows that bacteria in the phylum cyanobacteria can grow over a wide range of pH.

In addition to the phylum Cyanobacteria, Firmicute and Proteobacteria were also found in sample RP (0.12% and 4.38%), HFP_{600} (30.87% and 23.45%) and RFP (26.95% and 13.08%), respectively. Our report showed that phylum Firmicutes occurred in low amount in RP. In this study both sample SFP and BFP recorded 0.46% of the phylum Cyanobacteria. This indicates that combination of thermal treatment and fermentation significantly reduced the amount of this phylum. Rai and Rajashekhar (2014) studied the growth of species of cyanobacteria under different temperature and found that they grew best at 20 ˚C and 30 ˚C and growth ceased at 40 ˚C and 50 ˚C. The dominant phyla in Sample BFP and SFP are Firmicutes (59.65% and 50.65) and Proteobacteria (39.88% and 42.40%) for BFP and SFP respectively. Firmicutes were found to be more dominant in these samples. Kõiv et al. (2015) have earlier reported the phylum Firmicutes, Proteobacteria, Actinobacteria, bacteriodetes as part of the resident microflora of potato tuber. Firmicutes,

Proteobacteria, Actinobacteria and bacteriodetes have also been previously identified in fermented foods by other authors and the phylum Firmicutes has been reported as the most predominant in fermented foods. Sun et al. (2014) reported Firmicutes, Proteobacteria, Actinobacteria, bacteriodetes in fermented tarag collected from different regions of Mongolia and China and found phylum Firmicutes as the predominant phylum

A total of 157 genera were reported in the 5 samples. The bacteria genera in the top abundance level are Acetobacter, Leuconostoc, *Aeromonas, Lactobacillus, Lactococcus, Gluconobacter, Bacillus, Klebsiella, Weisella* and *Nicotaiana otophora*. The genus with low abundance values ≤ 0.001% was merged together as others. *Nicotiana otophora* was the dominant genus in RP, HFP_{600} and RFP accounting for 88.80%, 44.06% and 59.82% respectively of the total sequences in these samples. The genera *Leuconostoc*, *Aeromonas* and *Lactococcus* were absent in SFP. *Aeromonas* was also absent in sample RFP. The lower pH recorded in RFP (4.44) and SFP (4.39) might be responsible for the absence of these genera. Mari et al. (2010) also reported *Lactococcus* and *Leuconostoc* at the early stage of fermentation of traditional Mongolian fermented milk. They found that *Lactococcus* and *Leuconostoc* were unable to tolerate the low pH of the final products. The optimum pH for *Aeromonas* is 5.5 to 9, although they are capable of resisting pH 4.5 to 9 (Igbinosa, Igumbor, Aghdasi, Tom, and Okoh, 2012). The genus *Lactobacillus* occurred in low abundance in RP, HFP_{600}, BFP, SFP and RFP (0.01%, 4.91%, 1.31% , 3.11% and 3.95% respectively). Our studies indicate that the genus *Lactobacillus* is not the predominant LAB in fermented potato. *Lactococcus* (11.66%) was the predominant LAB in HFP_{600}. While *Weisella* was the predominant LAB in BFP (27.78%), SFP (35.84%) and RFP(19.02%). *Weisella spp* have occurred in boundless number of habitats such as the skin, milk and feces of animal, breast milk, sourdough, and fermented foods. They have been isolated from plants and vegetables. Some species of *Weisella* are capable of producing dextran which can find potential use in bakery products quality especially gluten-free bread made from gluten-free flour like potato flour (Fusco et al., 2015). In addition, some species can produce bacterocin capable of inhibiting the growth of undesirable microorganisms. The high abundance of *Weisella* might have contributed to the absence and low abundance of other bacteria species in sample SFP. *Gluconobacter* was found in high amount in sample SFP (20.80%) while other samples recorded significantly low amount. It recorded 52.16% of proteobacteria in sample SFP. This indicates that the sample contains nutrients that favour the growth of LAB and acetic acid bacteria since *Gluconobacter* are

classified as acetic acid bacteria. Sample BFP (30.76% and 14.22%) and SFP (13.49% and 19.87%) recorded relatively high number of *Klebsiella* and *Bacillus,* respectively, as compared to other fermented samples. *Klebsiella* accounted for the highest relative abundance of genus in sample BFP. The presence of these two genera in BFP and SFP might be due to contamination from the environment during processing.

Fig.1Bi and Bii showed the relative abundance of fungal communities at phylum and at genus level, respectively. As shown in Fig.1Bi, total of 3 phyla (Mortierellomycota, Basidiomycota and Ascomycota) were identified from the samples while others were categorized as unclassified and unassigned. The sequences that could not be classified into any known group were assigned as unclassified and sequences that have not been adequately characterized were grouped as unassigned. It means that the sequences cannot be classified based on the current available taxonomical reference data. The phyla relative abundance differ in all the samples. An unassigned phylum was the predominant phyla in RP (91.27%), HFP_{600} (85.13%) and RFP (44.88%). While the predominant phylum in BFP and SFP are Basidiomycota and Ascomycota for BFP and SFP respectively. The relative abundance of the phylum Basidiomycota in BFP is 73.915% and Ascomycota relative abundance in SFP is 99.17%. The phylum Mortierellomycota was absent in BFP and SFP, but found in low abundance in RP (0.003%), HFP_{600} (0.002%) and RFP (0.01%).

A total of 11 genera were displayed on the bar chart based on relative abundance > 0.01%, genera with abundance < 0.01% were classified as others. The genera are *Alternaria, Ramichloridium, Cladosporium, Aureobasidium, Candida, Colletotrichum, Sarocladium, Saccharomyces, Trichosporon,* unclassified and unassigned. Sample RP (91.27%), HFP_{600} (85.13%) and RFP (44.88%) were dominated by the genus grouped as unassigned. Sample SFP contains 98.78% unclassified genera while the genus *Trichosporon* was found to be the predominant genus in sample BFP. This genus makes up 99.43% of the phylum Basidiomycota found in sample BFP. Sun et al. (2014) have previously reported high amount of the genus *Trichosporon* in tarag and they attributed the high abundance of this genus to poor hygiene. The unclassified genera contributed 99.60% of the phylum Ascomycota recorded in sample SFP.

3.8 Bacterial and fungal community comparisons

The heat map analysis and multiple – sample similarity tree constructed based on Bray-Curtis were used to compare the differences and similarities of the 5 samples microbial communities' structure. Fig.2A and 3A

showed the heat map analysis of both bacterial and fungal communities at genus level. The colour intensity represents the genus relative abundance with colour gradient from blue to red indicating the relative abundance from low to high. The heat map analysis also confirms the dominance of *Nicotiana otophora* in RP, RFP and HFP_{600}, and it low abundance in BFP and SFP. The result is consistent with the relative abundance analysis as shown in Fig.1Aii. The result also showed that sample SFP and RFP contained low number of bacteria species while BFP has high number of bacteria species and diversity. The result correlates with alpha diversity analysis shown in Table S2. *Lactococcus* was the most abundance LAB in HFP_{600}, as compared to *Weisella* which dominates BFP, SFP and RFP. *Gluconobacte*r belonging to acetic acid bacteria group was also dominant in SFP as compared to other samples. The result showed that SFP contained lactic acid and gluconic acid in sufficient amount thus leading to low species abundance recorded in this sample. As shown in Table 1 SFP recorded the lowest sugar content, *Gluconobacter* might have also contributed to this reduction as they are capable of oxidizing sugar to produce gluconic acid (Mamlouk and Gullo, 2013).

Fungal communities' heat map analysis showed that RFP and SFP contain low number of fungal species. The result is consistent with relative abundance report as shown in Fig.1Bii. The low pH (Table S1) observed in these samples might be responsible for the low species abundance. Saccharomyces was the most abundance yeast genus in the fermented samples with highest abundance in sample HFP_{600}.

Hierarchical cluster tree based on bray-curtis was used to evaluate the similarities in species diversity between different samples. It was observed that BFP and SFP, RFP and RP shareed similar bacterial composition while HFP_{600} existed as a unique sample (Fig. 2B). Also BFP and RFP, HFP and RP were grouped together based on fungal composition closeness, while SFP existed as a unique sample (Fig. 3B). This result correlates with relative abundance analysis for both bacterial and fungal communities.

Principal component analysis (PCA) for bacterial and fungal communities is shown Fig. 4Bi and 4Bii respectively. The result revealed a notable separation of the bacterial and fungal communities in different samples. The first and second axes showed values of cumulative percentage variance of species equal to (89.64% and 6.43%) for bacteria and (62.30% and 32.37%) fungi respectively. In total, 95.89% and 94.67% were explained by the two axes for bacterial and fungal communities. Bacterial communities PCA analysis revealed similarity between RP and RFP while others existed separately. The high abundance of *Lactococcus*,

Klebsiella and *Gluconobacter* in HFP_{600}, BFP and SFP respectively is responsible for this clear separation. The PCA analysis indicated that fungal communities were similar between RFP and BFP, RP and HFP_{600} with SFP clearly separated. PCA analyses for the samples fungal communities follow the same trend with the Hierarchical cluster tree. BFP and RFP both contain high abundance of *Trichosporon*, RP and HFP_{600} were both dominated with unassigned genus and also had *Aureobasidium* which was absent in other samples. SFP was unique because of its low genus abundance and diversity, which was mostly dominated by unclassified genus. The difference in bacterial and fungal communities among the different samples might be due to the different pretreatment the samples were subjected to prior to fermentation. Samples modification through pretreatments might have affected nutrient composition hence causing variation in the microbial composition. Dhingra, Michael, Rajput, and Patil (2012) had earlier stated that some simple pretreatment methods such as soaking and cooking could modify the composition and availability of nutrient. The flour composition is one of the most important factor during fermentation because the microorganisms derived nutrients needed for optimum activities from the flour (Hammes et al., 2005)

4. Conclusion

For the first time, our study revealed the microbial composition of potato using high-throughput sequencing, and the physicochemical properties of fermented potato. The different processing methods had significant effects on the nutrient availability and microbial composition. Our results suggest that SFP had the least bacterial species and least fungi diversity due to the dominance of the phylum Firmicutes. The high abundance of *Weisella* correlates with the low sugar content and pH recorded in this sample. Also, the sample contains 98.78% of unclassified genus in the phylum Ascomycota which implies that some useful yeast genus awaits identification. Steaming prior to fermentation can be a successful way to isolate the most dominant LAB and yeast with technological properties in potato. We conclude that further insight is required to deeply unravel the unassigned and unclassified fungal that constitutes large percentage of the fungal communities of potato. We recommend that SFP could be used for potato-based foods as most nutrients especially dietary fiber and iron were conserved using this method. Also, its successful use can significantly impact consumers' health owing to the abundance of *Weisella* sp, which is capable of producing EPS which reportedly has positive impact on human health.

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Conflict of interest

The authors have no conflicts of interest to declare.

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Figure legends

Fig. 1. Relative abundance of bacterial (Ai and Aii) and fungal community (Bi and Bii) at phylum and genus level based on 97% sequence similarity.

Fig. 2. Bacterial community heat map analysis at the genus level (A). Hierarchical cluster tree for bacteria (B).

Fig.3. Fungal community heat map at genus level (A). Hierarchical cluster tree for fungi (B).

Fig. 4. The rarefaction curves from V3 + V4 region of 16s rRNA (Ai) and ITS1 region of the ITS (Aii) sequences of fermented and unfermented potato samples, Multiple samples of PCA analysis according to bacterial diversity (Bi) and fungal diversity (Bii).

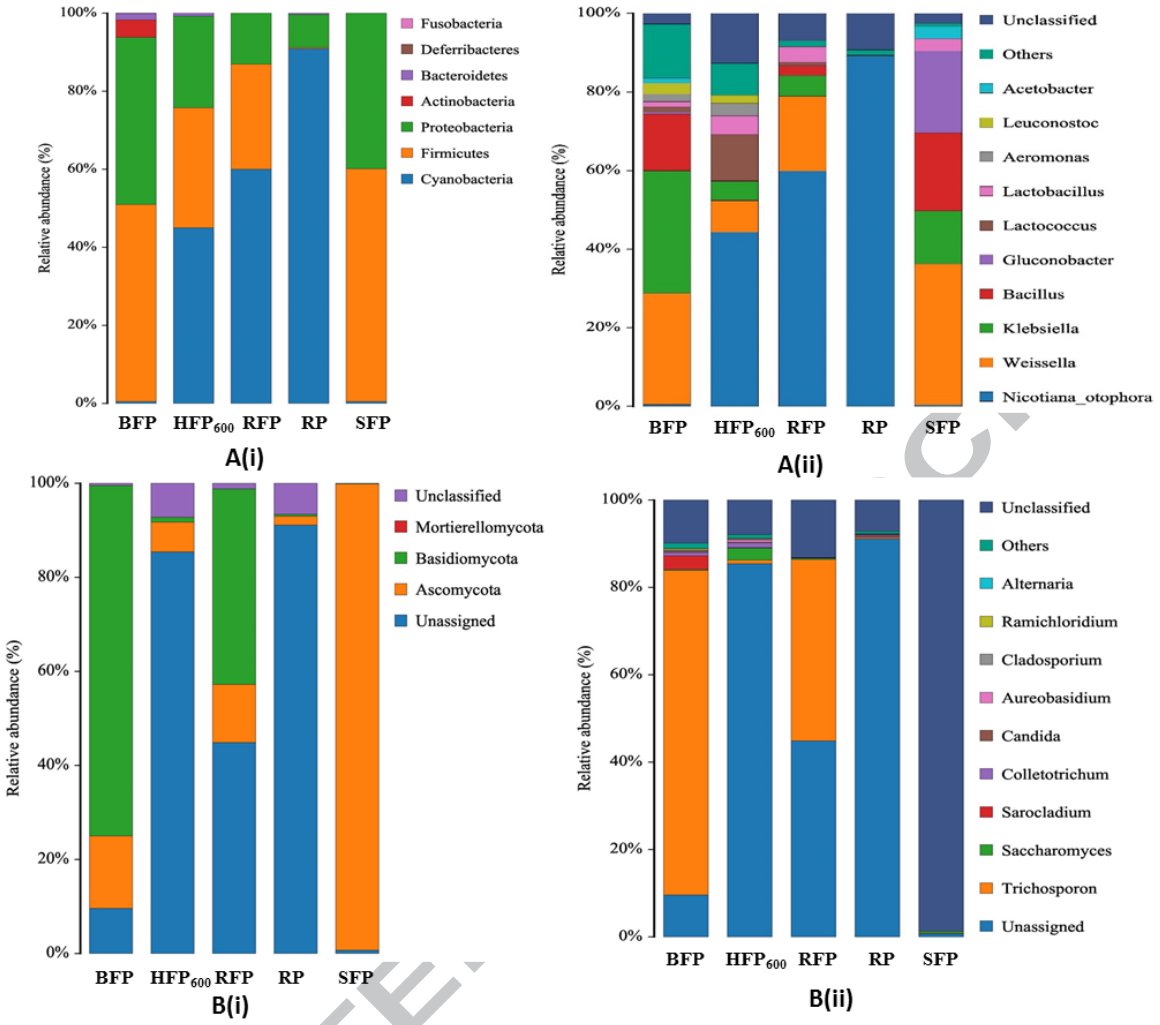

Fig. 1.

BFP represents boiled fermented potato, HFP_{600} represent 600MPa high hydrostatic pressure fermented potato, RFP represents raw fermented potato, RP represents raw unfermented potato, and SFP represents steamed fermented potato.

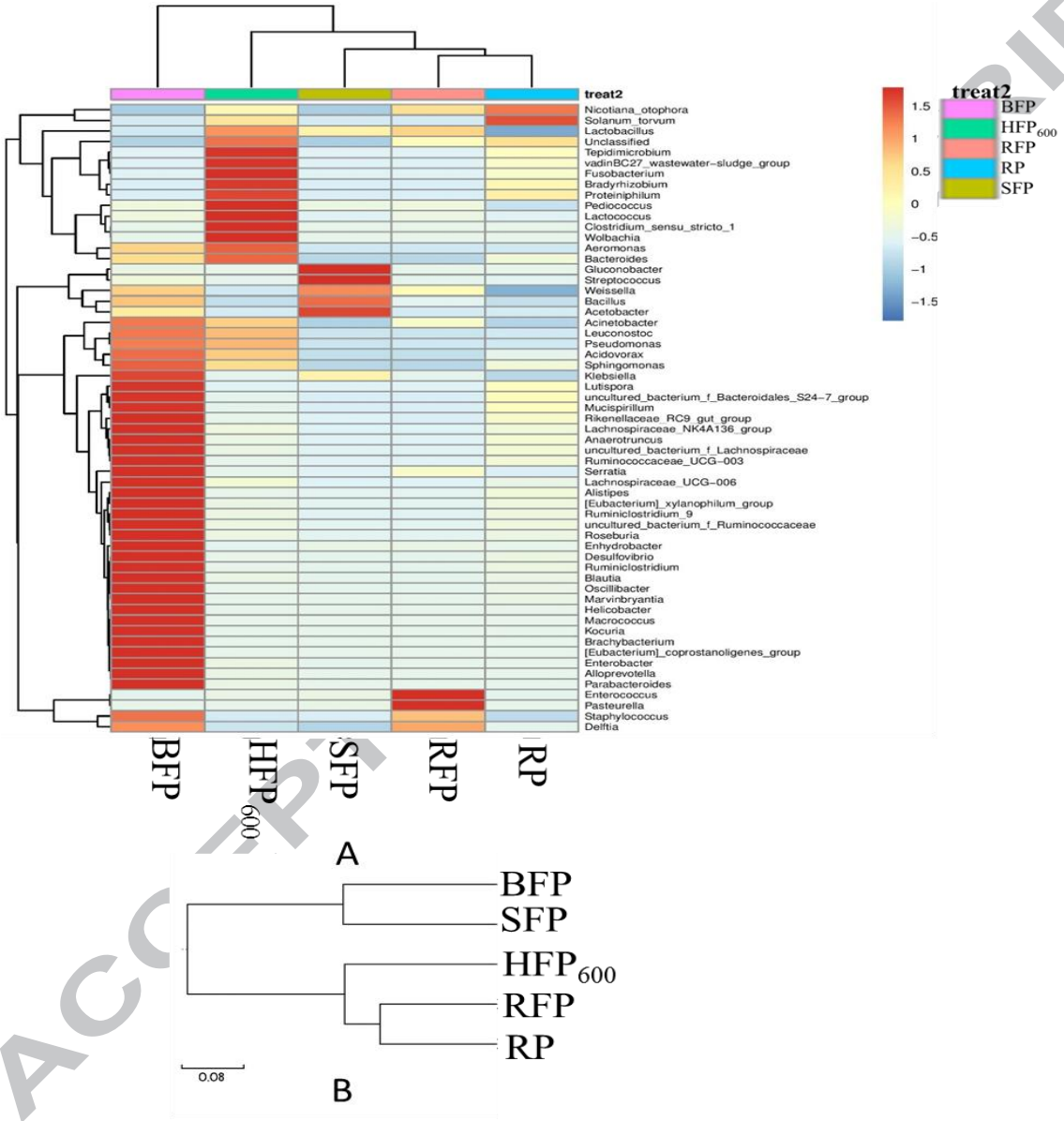

Fig. 2.

BFP represents boiled fermented potato, HFP_{600} represent 600MPa high hydrostatic pressure fermented potato, SFP represents steamed fermented potato, RFP represents raw fermented potato, and RP represents raw unfermented potato.

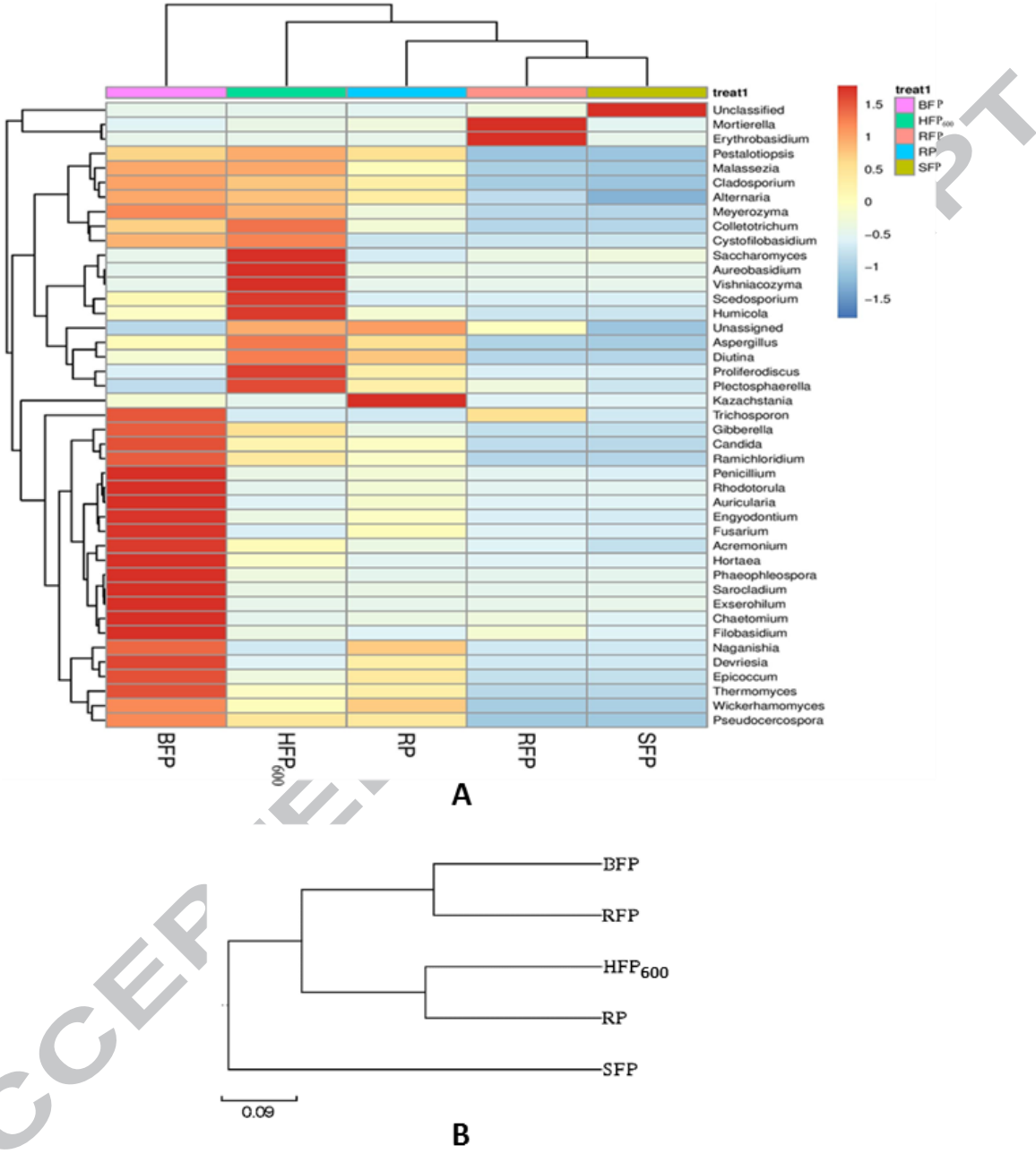

Fig. 3.

BFP represents boiled fermented potato, HFP_{600} represent 600MPa high hydrostatic pressure fermented potato, RP represents raw unfermented potato, RFP represents raw fermented potato, and SFP represents steamed fermented potato.

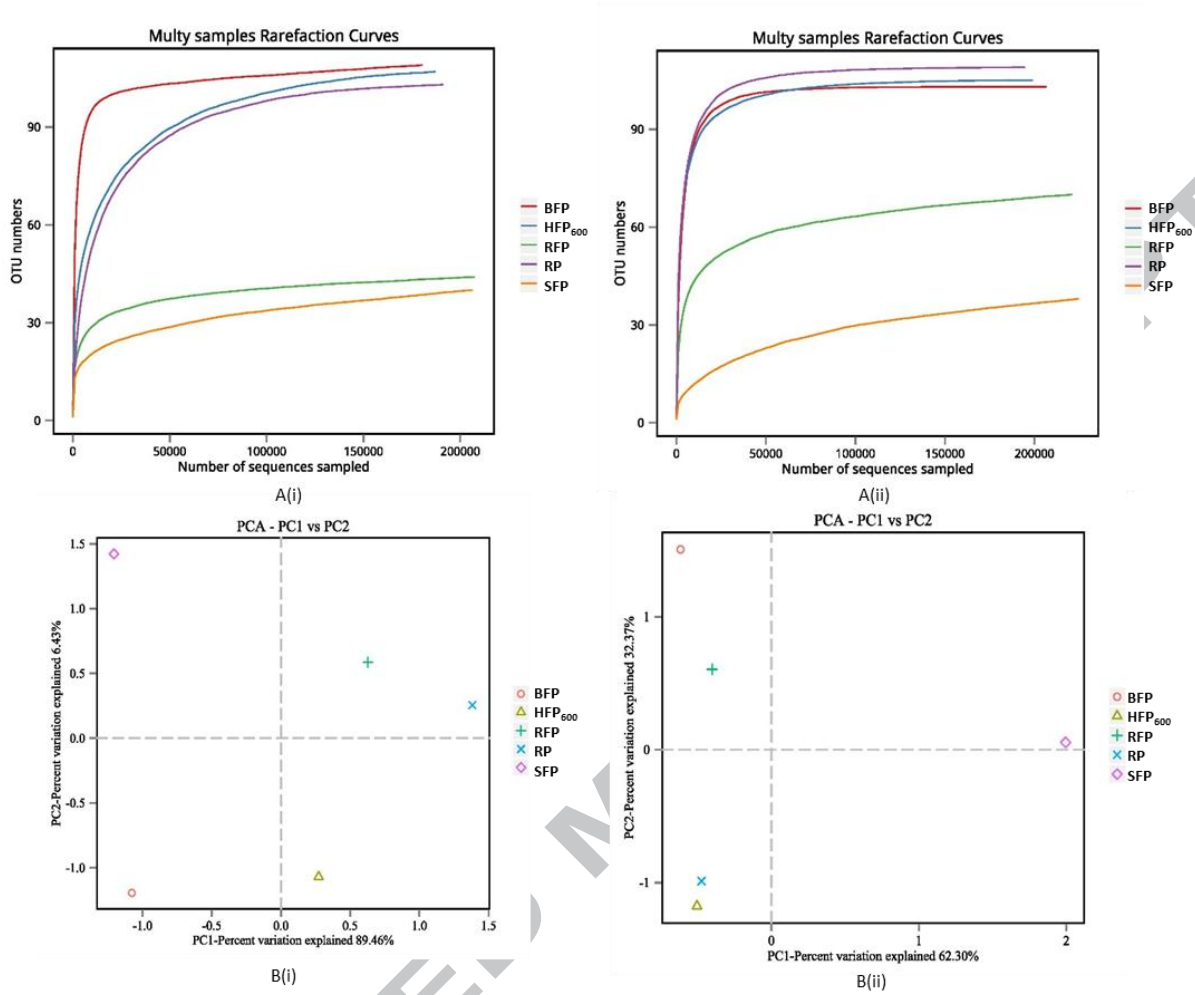

Fig. 4.

BFP represents boiled fermented potato, HFP_{600} represent 600MPa high hydrostatic pressure fermented potato, RFP represents raw fermented potato, RP represents raw unfermented potato, and SFP represents steamed fermented potato.

Table legends

Table 1. Chemical composition of fermented and unfermented potato

Table 2. Mineral composition of fermented and unfermented potato (mg/100g DW)

1 Table 1

Samples	Ash (%DW)	Fat (% DW)	Protein (% DW)	Dietary fiber (%DW)	Carbohydrate (% DW)	Total starch (mg/100g DW)	Total sugar (mg/100g DW)
RP	3.77 ± 0.01^{a}	0.2 ± 0.01^{b}	7.12±0.00^{c}	7.14±0.34^{b}	72.83±0.50^{f}	54.63 ± 0.56^{b}	11.79 ± 0.13^{a}
HFP_{300}	0.94 ± 0.03^{g}	0.24 ± 0.01^{a}	6.09±0.01^{f}	5.32±0.06^{c}	86.44±0.09^{a}	51.45 ±0.01^{bc}	10.86 ± 0.01^{ab}
HFP_{400}	1.13 ± 0.00^{f}	0.26 ± 0.01^{a}	6.83±0.01^{cd}	7.71±0.06^{b}	82.08±0.02^{c}	55.11 ± 0.53^{b}	10.77 ± 0.01^{ab}
HFP_{500}	1.42 ± 0.01^{e}	0.25 ± 0.00^{a}	6.41±0.00^{e}	5.93±0.41^{c}	84.68±0.40^{b}	61.67 ± 0.00^{a}	11.37 ± 0.05^{a}
HFP_{600}	1.68 ± 0.02^{d}	0.24 ± 0.01^{a}	5.59±0.24^{g}	7.17±0.10^{b}	81.03±0.18^{d}	53.17 ± 0.53^{bc}	9.48 ± 0.02^{cd}
BFP	2.51 ± 0.07^{c}	0.11 ± 0.03^{c}	8.18±0.22^{a}	9.68 ±0.68^{a}	70.54±0.25^{g}	54.59 ± 5.28^{b}	10.02 ± 0.07^{bc}
RFP	2.80 ± 0.00^{b}	0.14 ± 0.01^{c}	6.80±0.18^{d}	5.66±0.03^{c}	78.75±0.32^{e}	48.28± 1.12^{c}	8.77 ± 1.24^{d}
SFP	2.80 ± 0.00^{b}	0.26 ± 0.01^{a}	7.69±0.04^{b}	9.48±0.37^{a}	67.82±0.32^{h}	50.96± 3.37^{bc}	8.64 ± 0.134^{d}

2 RP represents raw potato (control); HFP_{300}, HFP_{400}, HFP_{500}, and HFP_{600} represent (300MPa, 400MPa, 500MPa and 600MPa high hydrostatic pressure
3 fermented potato)**;** BFP represents boiled fermented potato**;** RFP represents raw fermented potato; SFP represents steamed fermented potato. Values are
4 expressed as mean ± SD. Different letters in the same column represents statistical results $p < 0.05$.

5

Table 2

Sample	Na	K	Ca	Mg	P	Zn	Fe	Cu	Mn
RP	10.70± 0.14^{a}	1681.30± 6.21^{a}	33.10± 0.76^{a}	77.95± 1.00^{a}	238.32± 10.18^{a}	0.90±0.01^{a}	2.08±0.08^{bc}	0.19±0.00^{e}	0.72±0.01^{b}
HFP_{300}	4.65± 0.07^{f}	545.45± 1.91^{h}	23.10± 0.12^{d}	37.74± 0.23^{f}	111.43± 2.56^{e}	0.43±0.03^{de}	1.44±0.08^{cd}	0.14±0.00^{f}	0.06±0.00^{g}
HFP_{400}	5.80± 0.14^{e}	625.55± 0.64^{g}	20.54± 1.00^{e}	40.32± 0.84^{ef}	105.86± 6.74^{e}	0.46±0.02^{cd}	1.28±0.08^{cd}	0.22±0.01^{d}	0.09±0.01^{de}
HFP_{500}	7.45± 0.21^{c}	685.91± 2.88^{e}	25.93± 0.17^{bc}	43.07± 0.15^{e}	106.42± 9.33^{e}	0.46±0.02^{cd}	1.21±0.05^{d}	0.19±0.01^{e}	0.07±0.14^{fg}
HFP_{600}	6.30± 0.00^{d}	661.86± 6.75^{f}	28.01± 2.01^{b}	41.48± 0.93^{e}	98.23± 3.72^{e}	0.51±0.03^{c}	1.71±0.03^{cd}	0.24±0.01^{c}	0.08±0.00^{ef}
BFP	8.40± 0.14^{b}	876.80± 1.62^{d}	25.31± 1.07^{c}	53.27± 3.96^{d}	125.97± 4.70^{d}	0.77±0.04^{b}	1.38±0.21^{cd}	0.33±0.00^{b}	0.10±0.00^{d}
RFP	3.30± 0.00^{g}	1061.40± 10.19^{c}	19.28± 0.02^{e}	65.03± 0.14^{b}	150.86± 0.465^{c}	0.88±0.01^{a}	2.51±0.02^{b}	0.38±0.00^{a}	0.83±0.01^{a}
SFP	6.30± 0.00^{d}	1130.20± 6.18^{b}	21.37± 0.36^{de}	60.76± 0.93^{c}	199.50± 2.14^{b}	0.38±0.01^{e}	3.28±0.88^{a}	0.19±0.01^{e}	0.13±0.00^{c}

RP represents raw potato (control); HFP_{300}, HFP_{400}, HFP_{500}, and HFP_{600} represent (300MPa, 400MPa, 500MPa and 600MPa high hydrostatic pressure fermented potato)**;** BFP represents boiled fermented potato**;** RFP represents raw fermented potato; SFP represents steamed fermented potato. Values are expressed as mean ± SD. Different letters in the same column represents statistical results $p < 0.05$.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Research highlights

1. The dominant microorganisms are *Nicotiana otophora*, *Weisella*, and *Saccharomyces*.

2. Steaming reduced undesirable microorganisms and promoted dominance of *Weisella*.

3. Fermentation improved protein and dietary fibre contents in steamed and boiled samples.

4. Steaming is recommendable for industrial processing of fermented potato.