ECRONICON OPEN ACCESS

EC MICROBIOLOGY
Research Article

Preliminary Screening for Natural Bioactive Compounds in Potato Peel Fermentation Broth

Abdulbaset M Alayat[1], Farid Sotoudehnia[1], Endalkachew Mengistie[1], Karol Gliniewicz[2] and Armando G McDonald[1]*

[1]Forest and Sustainable Products Program, Department of Forest, Rangeland and Fire Sciences, University of Idaho, Moscow, United States
[2]Department of Biological Sciences and the Institute for Bioinformatics and Evolutionary Studies, University of Idaho, Moscow, United States

***Corresponding Author:** Armando G McDonald, Forest and Sustainable Products Program, Department of Forest, Rangeland and Fire Sciences, University of Idaho, Moscow, United States.

Received: March 01, 2021; **Published:** June 29, 2021

Abstract

Potato is one of the most consumed vegetable in the world which generates a large fraction of peel wastes. Potato peel waste (PPW) has been successfully fermented using mixed microbial culture (MMC) to produce mainly lactic acid. During fermentation, the MMC changes from a very diverse bacterial consortium to mainly (86%) *Lactobacillus* species by 16S rRNA gene sequencing which suggests that antimicrobial and bioactive compounds are produced during fermentation. This preliminary study examined the main compositional changes during fermentation as well as analysis of broth extracts. The PPW fermentation broth were extracted with CH_2Cl_2, n-hexane, $CHCl_3$ and ethyl acetate and the extracts analyzed by gas chromatography-mass spectrometry (GC-MS), electrospray ionization-mass spectrometry and sodium decyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to GC-MS analysis led to the identification of bioactive compounds and some were found to have potential antimicrobial, prophylactic, and other curative activities. The findings of this study indicate that PPW is a promising candidate as a valuable source for future biochemical production research.

Keywords: *Potato Peel Waste; Antimicrobials; Fermentation; Mixed Microbial Culture; Lactic Acid*

Abbreviations

ASTM: American Standard Test Method; ESI-MS: Electrospray Ionization-Mass Spectrometry; FAME: Fatty Acid Methyl Esters; FC: Fixed Carbon; FTIR: Fourier Transform Infrared; GCMS: Gas Chromatography-Mass Spectrometry; HPLC: High Performance Liquid Chromatography; LA: lactic acid; MMC: Mixed Microbial Culture; NIST: National Institute of Standard and Technology; PPW: Potato Peel Waste; SDS-PAGE: Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis; TMS: Trimethylsilyl; VM: Volatile Matter; 16S rRNA: 16S Ribosomal Ribonucleic Acid

Introduction

In the quest for improved sustainability of biofuels and green chemical sources, many studies have focused on the production of biochemical building blocks such as organic acids, alcohols, biofertilizer and antimicrobial compounds prepared from many bio-wastes by

various techniques such as fermentation, extraction, and other treatment strategies [1]. The advantages of using the renewable resource (biomass, plants, organic biowastes, etc.) rather than non-renewable resources (natural gas, coal, oil, etc.) to manufacture biochemical production include opportunities for less pollution, no net increase in CO_2 emissions, more biodegradable and eco-friendly products and in some cases, lower price [2]. Potatoes are one of the most important agricultural products for human consumption (French fries, chips, etc.) in the world after rice, wheat and corn [1,2]. According to data from the National Potato Council, the United States is the fifth largest producer of potatoes in the world with a production over 21.4 x 10^9 kg in 2019 [3]. Peeling generally applied on an industrial scale to potatoes using steam, abrasive and/or lye peeling processes [4]. Potato peel waste (PPW) produced in large quantities during the potato processing plant. Depending on the peeling techniques applied, PPW ranges from 6 - 15% from the total potato [5]. Furthermore, PPW has the potential to be converted into a variety of bioproducts that play important roles in the field of the pharmaceutical, green chemicals, and food industries [1]. Due to its composition, the PPW has been considered by researchers for use in many applications including animal feed, produce alternative fuels (e.g. biogas, ethanol), bioactive compounds (e.g. pharmaceutical ingredient, antioxidant, antimicrobial agent) and industrial chemicals (e.g. organic acids, bioplastic composites, biofertilizers, glycoalkaloids, reducing sugars) [2,6]. For instance, some of these studies have yielded ethanol and lactic acid (LA) from PPW fermentation using a mixed microbial culture (MMC), which is a relatively new research topic that is gaining more attention.

These microorganisms have the potential as an upcoming and promising source to produce various ingredients like bioactive compounds (i.e. antimicrobial compounds) from biowastes with the use of fermentation processes [7]. Among microorganisms, lactic acid producing bacteria (*Lactobacilli*) have two strategies to kill competing bacteria which are generating LA which lowers the pH (microbial killing, inhibition) and producing *Lactobacillus* antimicrobial (LAM) agents (i.e. organic acids), specific antimicrobial proteins (bacteriocins), which pierce the cell wall of competing bacteria and cause death [8,9].

Besides this, studies on biowastes and biomass fermentation have been conducted using single bacterial strains to generate renewable chemicals and other bioproducts [10]. However, the utilization of MMC has recently attracted considerable attention as a potential culture media for alternative bioproducts using biomass, by-products, and biowastes in different fields of biotechnology in food production [13]. Various studies have been conducted on the benefits of utilization of PPW as bio-waste for ethanol and lactic acid production using mixed microbial culture fermentation [11]. Despite large efforts done in this field, no research to the best of our knowledge has been done about the utilization of PPW to produce antimicrobial agents via MMC. Due to multi-drug resistant pathogens, secondary metabolites, including antimicrobial agents based on natural products are currently one of the top health issues currently acknowledged worldwide. In addition, many of natural products with potential antimicrobial activities still await further studies. The initial results of chemical composition on the PPW are promising and encouraging, due to the fact that the PPW is rich starch, cellulose, hemicellulose and fermentable sugars that should be considered as an alternative source for the production of bioactive compounds and secondary metabolites [12].

Aim of the Study

The aims of this study were to (i) characterize PPW, (ii) ferment PPW with MMC to produce lactic acid and (iii) identify potential bioactive compounds in solvent extracted PPW fermentation broth that could potentially be effective against pathogenic microorganisms.

Materials and Methods

Sample preparation and extraction

PPW was provided frozen from a potato processing plant (JR Simplot Company, Nampa, ID, USA). PPW was used as received for the fermentation studies. For characterization, PPW sample was freeze dried and ball milled using ULTRA-TURRAX Tube Drive from IKA.

Characterization of PPW

The moisture content of the PPW samples was determined, in duplicate using an HB 43-S Mettler Toledo moisture analyzer (Ohio, USA). The higher heating value (HHV) of PPW was determined using an oxygen bomb calorimeter; Model 1341 from Parr company (Illinois, USA) according to ASTM D5865-04. C, H, and N contents were determined using a Costech Elemental Analyzer (ESC 4010), and protein content was estimated as N × 6.25. The proximate analysis (ash, volatile matter (VM), and fixed carbon (FC)) of PPW was carried out according to ASTM standard test method E870-82. Ash content value was carried out in a muffle furnace at 600°C for 16h. VM and FC were determined by the method of ignition of the sample at 950°C in a muffle furnace for 7 minutes. The supernatant of PPW fermented broth was freeze dried and analyzed by FTIR spectroscopy (Thermo Nicolet iS5 spectrometer with an attenuated total reflection (iD5 ATR, ZnSe crystal) accessory). The spectra were baseline corrected and averaged the OMNIC v9.0 program (Thermo Nicolet Co.).

The PPW (5.0g) was Soxhlet extracted with CH_2Cl_2 (150 mL) for 22h, in duplicate, to obtain lipid (extractives) content gravimetrically according to ASTM D 1108-9623. The fatty acid composition was quantified as fatty acid methyl esters (FAME) derivatives. Extract (5.0 mg, in duplicate) was weighed into 5 mL reacti-vials to which $CH_3OH/H_2SO_4/CHCl_3$ (1.7:0.3:2.0 v/v/v, 2 mL) was added and heated at 90°C for 90 minutes. $CHCl_3$ contained 1-naphthaleneacetic acid as an internal standard (50 µg/mL). The $CHCl_3$ layer was analyzed by GC-MS (FOCUS-ISQ, Thermo Scientific) with a temperature profile of 40°C (1 min) to 320°C at 5°C min^{-1} and a GC capillary column: (ZB5 ms, 30m, 0.25 mm Ø, Phenomenex). The eluted compounds were identified with authentic standards (C12 to n-C20 fatty acids) and by spectral matching with the NIST 2017 Mass Spectral Library.

Total carbohydrate content (TCC) was determined by the phenol-sulfuric acid colorimetric method [13]. Briefly, extractive free PPW (10 mg) was transferred into a glass tube and mixed with 1 mL of phenol (5%, w/v) and 5 mL of concentrated H_2SO_4 (72 wt %) in a shaking water bath. After an incubation of 10 min, the absorbance was measured at 490 nm using the Bio-mate 5 spectrophotometer (Thermo Electron. Corp). The total starch content was determined using a commercial kit from Megazyme International, Ireland (2019) following the amyloglucosidase/α-amylase method according to AOAC Method 996.11. Total lignin content (acid-soluble lignin + Klson lignin) was determined from an extractive free PPW according to ASTM D 1106 [10]. Extractives free biomass (200 mg, in duplicate) was incubated in 72% H_2SO_4 at 30°C for 1 hr. Then was followed by secondary hydrolysis of 4% H_2SO_4 and it was autoclaved at 121°C for 30 min. Klason lignin was determined gravimetrically whereas acid-soluble lignin was quantitated spectrophotometrically at 205 nm using an absorption coefficient of 110 L g^{-1} cm^{-1} (Biomate 5, Thermo Electron Corp) [10].

Preparation and fermentation of PPW

Fermentation experiments were performed in an environmental chamber (Lab-Line 846 Biotronette Mark III, USA). The batch fermentation experiments of PPW fermented were conducted in 250-mL air-locked glass flasks containing 150 mL of water [11], while the investigation of the antimicrobial compounds was performed in 1L cell culture plastic spinner flasks containing 800 mL of water. All experiments were carried out under the same conditions at the same time, 4.5% (v/v) mixed microbial cultures (MMC) inoculated from the waste activated sludge from a wastewater treatment system in Dr. Erik Coats' laboratory, University of Idaho, and placed in orbital shakers (150 rpm). The fermentation temperature was controlled at 35°C, the feed solids content (SC) was 75 g L^{-1} and the fermentation period was 13 days. The pH and dissolved oxygen (DO) content of the PPW fermenters were determined using an Orion-3-Star DO/PH portable meter (Thermo Fisher Scientific Inc., Waltham MA). All the experiments were conducted at least in triplicate.

Fermentation products analysis

Organic acids composition (lactic, acetic, and succinic acids), glucose, ethanol and methanol were analyzed by HPLC, using a Rezex ROA organic acid column at 65°C (7.8 mm × 30 cm, Phenomenex, Torrance, CA, USA) equipped with differential refractive index detector

(Showa Denko Shodex Rise-61, Tokyo, Japan), on elution with 0.005N H_2SO_4 at 0.5 mL/min. Fermentation samples were filtered (0.45 μm) into vials for analysis in triplicate. Sugars were analyzed by HPLC, using two Rezex RPM columns (7.8 mm × 30 cm, Phenomenex, Torrance, CA, USA) in series at 85°C equipped with differential refractive index detector (Model 2414 Waters Corp., Milford, MA), on elution with water (0.5 mL/min). Hydrolyzed samples were deionized and filtered (0.45 μm) for HPLC analysis in triplicate.

Extraction of the PPW fermentation broth

To identify possible antimicrobial compounds in the fermentation broth, the fermenter was sampled on day 13 and centrifuged PPW fermentation broth (35°C at 75 g L^{-1} solids content) supernatant (10 mL) was centrifuged at 10,000×g for 30 min, filtered, and freeze-dried. Freeze-dried PPW fermentation broth (1 mg) was dispersed in pyridine (50 µL) and then silylated with the addition of N, O-bis(trimethylsilyl)-trifluoro-acetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) (50 µL) after heating at 70°C for 30 min then CH_2Cl_2 added (1 mL, containing internal standard). The prepared trimethylsilyl (TMS) derivatives were analyzed by GC-MS (Trace1300-ISQ7000, ThermoScientific) using a ZB1ms capillary column (30 x 0.25 mm Φ, Phenomenex) and temperature profile of 40°C (1 min) to 305°C (10 min) at 5°C/min. In addition, four solvents (CH_2Cl_2, n-hexane, $CHCl_3$, and ethyl acetate (EtOAc)) were also assessed for the extraction of bioactive compounds in the broth. The supernatant aliquots (100 mL) were each extracted twice with 100 mL of either CH_2Cl_2, n-hexane, $CHCl_3$, or EtOAc. The organic phase was obtained using a separating funnel and concentrated to dryness and yield determined. The extract (1.5 mg) obtained was dissolved in 1 mL of CH_2Cl_2 containing internal standard and analyzed directly by GCMS (Trace 1300-ISQ instrument, ThermoScientific) using a ZB-5 capillary column (30 × 0.25 mm Φ, Phenomenex) with a temperature profile of 40°C (1 min) to 320°C (10 min) at 5 °C/min. Identification of compounds was performed by comparison of the obtained mass spectra with the 2017 National Institute of Standard and Technology (NIST) spectral library.

ESI-MS analysis

Direct ESI-MS experiments were carried out on either an Agilent 6210 ESI-TOF mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) and/or Finnigan LCQ-Deca instrument (ThermoQuest) both equipped with electrospray ionization (ESI) interface. The extract (1 mg/mL in methanol containing 1% acetic acid) was delivered at a flow rate of 10 µL/min via a syringe pump. Agilent operating parameters in positive ion mode were: 300°C gas temperature; 215 V fragmentation voltage; m/z 100 to 1000; data recorded and processed using MassHunter vB.08.00 software. LCQ-Deca operating parameters were: 4.50 kV ion source voltage; 20 V ESI capillary voltage; 275°C capillary temperature; data acquisition and analysis were conducted using the Xcalibur v2.2 software.

Taxonomic characterization of bacterial 16S rRNA genes

Genomics analysis was performed on the MMC seed and PPW fermented material as previously described by Liang., *et al* [12]. Briefly, the samples (250 μL aliquot) were first DNA extracted using a lytic enzyme cocktail, incubated for 24h at 37°C, transferred to bead beating tubes (2100 RPM for 1 min), centrifuged, then bacterial genomic DNA was isolated with a QIAamp DNA Mini kit (Qiagen Inc., USA). The isolated DNA was examined with 1% agarose gel stained with ethidium bromide in 0.5 TAE buffer and quantified with QuantiFluor dsDNA kit (Promega, Inc.) on Turner TBS-380 minifluorometer (Turner BioSystems, USA) and verified with Agilent DNA 1000 kit on Agilent Bioanalyzer 2100. PCR amplification was carried out immediately after DNA extraction of both samples, using primers targeting the V1-V3 hypervariable regions of 16S rRNA genes by using paired-end sequencing on Illumina MiSeq platform (Illumina, San Diego, CA, USA). The amplicons were obtained by PCR using the universal primers that flanked the variable regions 1 and 3 of bacterial 16S rRNA genes (*E. coli* positions 27F-534R). The sequences of the PCR primers are given in [12]. Sequence and taxonomic classification were performed as previously described [12].

Gel electrophoresis

To identify any peptides and proteins in the PPW fermentation broth, SDS-polyacrylamide gel electrophoresis (SDS-PAGE) technique was carried out using an 8 x 12 cm gel (4 - 20% Tris-HCl Ready Gels), Mini-PROTEAN 3 electrophoresis cell (Bio-Rad, Cambridge, MA) equipped with a BioRad PowerPac Basic [13]. The PPW fermentation broth was sampled on day 13 (2 mL), centrifuged (7,000×g for 30 min) and the supernatant filtered (nylon, 25 mm, 0.45 µm). The buffer was prepared by adding 1.0 mL Tris-HCl, (1.0 M and pH 6.8 at 25°C), 0.8 mL 20% (w/v) SDS solution, 0.2 mL β-mercaptoethanol, 1.2 mL 75% (w/v) glycerol and 0.2 mL bromophenol blue to 0.6 mL H_2O. A 20 µL of the sample was mixed with an equal volume (1:1 ratio) of sample buffer and heated at 100°C for 4 min before electrophoresis. During the electrophoresis, a constant voltage of 90 V was applied for 15 min during migration through the stacking gel followed by 120 V for 55 min during migration through the separating gel. Bio-Rad's Precision Plus Protein standards were used for molecular weight calibration.

Results and Discussion

Characterization of PPW

The chemical composition and proximate analysis of dry PPW were determined and the results are given in table 1. PPW had an initial pH of 6.50 ± 0.10. The moisture content of PPW was 5.80 ± 0.33% and ash content was 10.90 ± 0.31%, which was similar to those reported in the literature [5]. The VM and FC content of PPW was 79.6 ± 2.4% and 3.7 ± 0.08%, respectively, and are in agreement with the literature [20]. The C, H, and N contents were 43.78%, 5.96% and 4.06%, respectively. The higher heating value (HHV) of 16.90 MJ kg^{-1} was obtained and was quite similar results reported by Liang., *et al*. (17.37 MJ kg-1) [5]. The total protein content of 25.4% was higher than that reported in the literature [14]. PPW had a starch content of 15.6% and this result was consistent with previous findings (17%) [5]. The total content of carbohydrates in the dried PPW was 34.9 ± 0.9% and comparable to reported values [11]. The neutral sugar analysis showed that PPW contained mainly glucose (18.1 ± 0.4%) followed by galactose (2.1 ± 0.2%) giving a total neutral sugar content of 23.1 ± 1.4%, which is in good agreement with the literature [15], while this value was lower than found in a previous study [1]. Total lignin content was 20.1 ± 0.5% and agrees with reported data [1,5,14]. The PPW lipid content was 2.23% and comparable with reported data [14]. The fatty-acid profile was determined as their FAME derivatives (Table 2). The profile of fatty acids ranged from C12 (lauric acid) to C30 (melissic acid). The main fatty acids found in the extracts were melissic acid (2.34 mg/g), palmitic acid (2.01 mg/g), and hexacosanoic acid (1.45 mg/g), and were comparable to the literature [3,7]. Generally, the differences in the chemical composition of PPW between this study and some previous studies could be attributable to diversity of potato varieties [16]. However, our findings showed that the PPW samples contain a good composition of fatty acids and could be used for microbial production of biochemicals. Consequently, the initial physicochemical properties in this study demonstrated the potential of PPW as a useful source for biofuel and valuable bioproducts, could generate economic gains for the various industries.

Parameters/unit	Dry PPW
Proximal analysis (%)	
Moisture	5.80 ± 0.33
Volatile matter (VM)	79.6 ±2.4
Fixed carbon (FC)	3.7 ±0.08
Ash	10.90 ± 0.31
Higher heating value (HHV) (MJ kg^{-1})	16.90 ± 0.4
CH_2Cl_2 extractives (%)	2.23 ± 0.60
Total carbohydrate (phenol-sulfuric)	34.86 ± 0.92
Starch (g/100g)	15.60

Acid soluble lignin (%)	3.36 ± 0.80
Klason lignin (%)	16.75 ± 0.10
Total lignin (%)	20.11± 0.45
Elemental analysis	
C (%)	43.78 ± 0.15
H (%)	5.96 ± 0.12
N (%)	4.06 ± 0.01
Protein (N to 6.25) (%)	25.37± 0.15
Neutral sugar content (NSC) (%)	
Glucose	18.1 ± 0.4
Xylose	0.83 ± 0.7
Galactose	2.05 ± 0.2
Arabinose	1.47 ± 0.1
Mannose	0.56 ± 0.02
Total Neutral sugar (%)	23.1 ± 1.4

Table 1: *Proximate and chemical characterization of PPW.*

No.	Name of identified compound	RT (min)	M+ (m/z)	Concentration (mg/g PPW)
1	Lauric acid (C12:0)	22.58	214	0.13
2	Myristic acid C14:0	27.91	242	0.42
3	Pentadecanoic acid C15:0)	29.82	256	0.06
4	Palmitic acid (C16:0)	31.82	270	2.01
5	Heptadecanoic acid (C17:0)	33.77	284	0.06
6	Linoleic acid (C18:2)	35.06	294	0.35
7	Linolenic acid (C18:3)	36.69	296	0.05
8	Stearic acid (C18:0)	35.58	298	0.72
9	Eicosanoic acid (C20:0)	39.04	326	0.01
10	Docosanoic acid (C22:0)	42.22	354	0.34
11	Tricosanoic acid (C23:0)	43.72	368	0.10
12	Tetracosanic acid (C24:0)	45.17	382	0.93
13	Pentacosnic acid (C25:0)	46.57	396	0.12
14	Hexacosanoic acid (C26:0)	47.93	410	1.45
15	Montanic acid (C28:0)	50.52	438	0.58
16	Melissic acid (C30:0)	52.94	466	2.34
	Total			9.67

Table 2: *Fatty acid analysis of PPW CH_2Cl_2 extract as FAME derivatives.*

FTIR spectroscopy was used to identify the chemical functional groups present in the solid material before fermentation and fermented freeze-dried PPW broth (Figure 1). Significant differences in the spectra were observed and separated into regions. The absorption bands in the region between 3200, 3600 cm^{-1} for both PPW samples were attributed to (O-H) stretching (e.g. water, alcohol, and phenol) and N-H (amines) stretching vibrations [10]. The broad region 2927 - 2845 cm^{-1} indicated the presence of C- H (CH_3, CH_2) groups of lipids and lignin for both samples, which are in agreement with previous observations [10,17]. The region between 1800 and 1500 cm^{-1} was assigned to mainly proteins and peptides [18]. The band at 1606 cm^{-1} for PPW was attributed to C=O stretching (Amide I), while the band was shifted to 1629 cm^{-1} in the fermented freeze-dried PPW broth. Besides, the absorption band at 1520 cm^{-1} of the fermented freeze-dried PPW broth also shifted to 1534 cm^{-1} for PPW, which can be assigned to (Amide II) of secondary amide in protein [17,19]. The bands at 1629, 1534, and 3377 cm^{-1} indicated the presence of peptide bonds in the fermented freeze-dried PPW broth. The absorption bands in the region between 1500 and 1200 cm^{-1} for both PPW samples were attributed to proteins and fatty acids [18]. The band observed at

1395 cm^{-1} in the fermentation broth were previously assigned by the presence of aryl OH [20]. The close bands at 1005 and 1014 cm^{-1} in PPW and broth were assigned to C-O stretching [7]. The absorption bands in the region of 1120 - 900 cm^{-1} in PPW were attributed to polysaccharides [18]. Other strong bands at 660 - 829 cm^{-1} in the fermented freeze-dried PPW broth were attributed to the presence of substituent groups on the aromatic ring [21]. The results of FTIR analysis confirmed the presence several organic functional groups which are important components of some bioactive compounds.

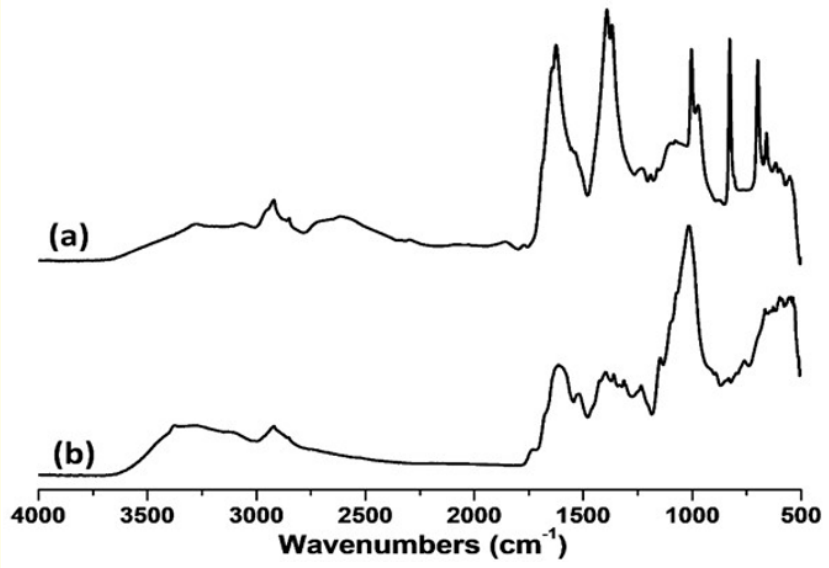

Figure 1: *FTIR spectra of (a) freeze-dried PPW fermentation broth and (b) PPW.*

Wavenumber (cm^{-1})		Band assignment
PPW	**Broth**	
3286	3377	O-H stretching vibration
2921	2921	C-H (CH_3, CH_2) stretching
2851	2849	C-H symmetric stretching
1606	1629	(C=O) stretching of amide I
1520	1534	N-H stretching of amide II
1395	1394	Aryl OH groups
1361	1374	C-H bending
1231	1229	C-N stretching
1014	1005	C-O stretching
--	828	C-H aromatic rings
--	701	C-H aromatic rings

Table 3: *FTIR values and functional groups of fermented broth and PPW.*

Fermentation of PPW

Batch fermentation (250 flask) of PPW (75 g L^{-1}) with MMC at 35°C to lactic acid over 13d was based on previous research [11]. LA, acetic acid, and ethanol were the dominant products of fermentation (Figure 2 and table 4). The highest total lactic acid content on day 7 was 9.8 g L^{-1} (product yield of 0.13 g g^{-1}). The values were comparable to previous work for PPW [11]. While acetic acid content rose to 1.9 g L^{-1} (product yield of 0.02 g g^{-1}) on day 11 and this value was slightly lower to other reports [11]. Ethanol production during the fermentation period of 13 days showed 3.8 g L^{-1} (product yield of 0.06 g g^{-1}) and this result was slightly different from that reported in previous findings (3.2 g L^{-1}) with solid loading of 60 g L^{-1} [11]. The identity of lactic ($[M+H]^+$ = m/z 91; $[M-H]^-$ = m/z 89) and acetic acids ($[M+H]^+$ = m/z 61; $[M-H]^-$ = m/z 59) was also supported by ESI-MS. PPW fermentation was also done at the same time, feed, temperature and MMC seed using a 1 L fermenter to produce a slightly higher concentration of ethanol (4.3 g L^{-1}) and similar lactic acid concentration (9.6 g L^{-1}) on day 13 and both fermenters were similar.

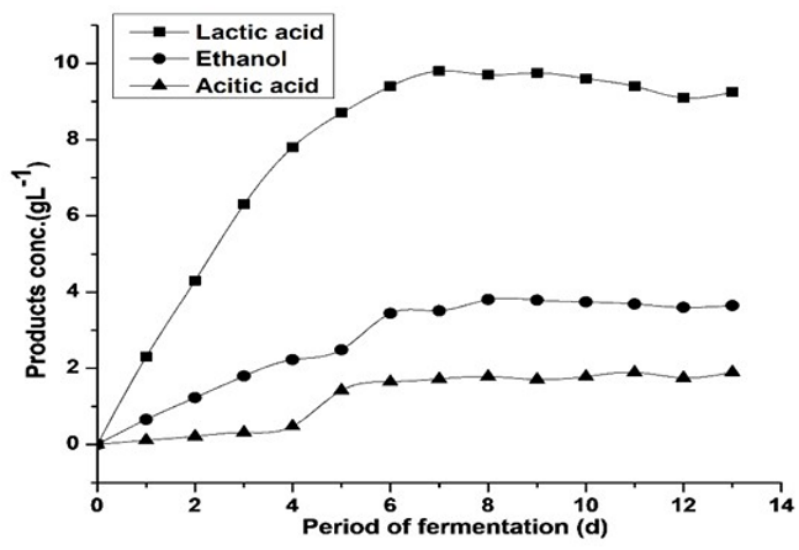

Figure 2: *Lactic acid, acetic acid and ethanol concentration produced during the fermentation of PPW (75 g L-1 at 35 C).*

Identification of fermentation compounds

GC-MS analysis

GC-MS was used for analyzing potential bioactive compounds present from fermentation of PPW. The fermentation broth was initially (i) liquid-liquid CH_2Cl_2 extracted and (ii) freeze dried and these samples converted to their TMS derivatives for analysis. Nineteen compounds were identified by GC-MS for their TMS derivatives. The major bioactive compounds identified in the CH_2Cl_2 extract were nonadecanoic acid, TMS (7.95%), myristic acid, TMS (7.29%), lactic acid-2TMS (4.51%) and tyrosol, 2TMS (2.41%). Other minor bioactive compounds are also listed in table 4. In previous studies, most of these compounds listed in table 4 were found to exhibit therapeutic value due to their biological and pharmacological activities. For example, nonadecanoic and myristic acids have anti-inflammatory and antibacterial activities, respectively [22,23].

In a separate set of experiments, the PPW broth was extracted with 4 different solvents (CH_2Cl_2, n-hexane, $CHCl_3$ and EtOAc) to establish which solvent obtained the highest yields (1.16%, 2.17%, 1.64 and 2.74%, respectively). The highest extract yield was obtained using EtOAc and the least was CH_2Cl_2. The extracts were directly analyzed by GC-MS (Table 5). The direct GC-MS analysis of extractions showed the presence of a series of (C6-C27) various bioactive chemical constituents such as fatty acids, phenols, phthalates, esters, hydrocarbons and alcohols. The prevailing compounds in the CH_2Cl_2 extract were diphenolic acid (17.5%), di-butyl phthalate (4.8%), 4-(1-methyl-1-phenylethyl)phenol (1.6%), dicyclohexyl phthalate (1.2%), N-(aminoacetyl)leucine (0.75%), Frenolicin b (0.6%), and 1-acetylpyrrolidine (0.3%). The most abundant compounds observed in EtOAc extract were diphenolic acid (38.5%), pentanoic acid, 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6methyl (3.4%), 1,3,5-pentanetriol, 3-methyl (2.7%), tetracosane (1.8%), 4-(1-methyl-1-phenylethyl) phenol (1.8%), heptanoic acid (1.6%), 2-methyl-4-pentenoic acid (1.3%), palmitic acid (1.3%) and trequinsin (1.3%). Other minor constituents in the CH_2Cl_2 extract were also identified (Table 5). The $CHCl_3$ extract showed the presence of mainly diphenolic acid (9.7%), diisooctyl adipate (6.3%), dibutyl phthalate, diisooctyl adipate, and octadecane (1.4%). Finally, the n-hexane extract contained mainly ethanone, 2-(4-nitrophenoxy)-1-phenyl (15.4%), 1,2-dimethyl benzene (14.%), meso-2,3-diphenylbutane (9.38%), nonane (7.9%), 1-eicosanol (7.8%), methoxy-phenyl oxime (6.0%), 3-propyl-2,4-pentadien-1-ol (4.2%), 3-pentyl cyclohexene (3.4%), 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazine (1.8%), 1,3diphenylpropanetrione (1.6%), benzyl-2-phenylethylamine (1.4%), and phenyl 2-methylbenzoate (1.1%). The identified compounds were known to have a wide range of biological activities (Table 5). Differences in the chemical profiles between the four solvent extracts was not unexpected due to their polarity.

The GC-MS analysis of the PPW broth extracts indicated the presence of at least 31 potential bioactive compounds, which makes as potential future candidate for biomedical and pharmacological studies. Diphenolic acid was identified in all the extracts and is a bio-based platform compound [24]. The presence of diphenolic acid might be due to the mechanism of acid-catalyzed condensation of phenol [25]. Another study has also reported for the synthesis of diphenolic acid from the condensation of phenol and levulinic acid, a platform chemical from renewable materials [26]. Although there is no evidence that the diphenolic acid has biological activity, this pathway of biosynthesis using MMC may provide new sustainable opportunities for the preparation of diphenolic acid, which is widely used as a chemical intermediate in the polymer industry. Diphenolic acid may originate from plasticware and adhesives. Phthalates were detected and may originate from plasticware [27]. However, dibutyl phthalate, diisooctyl phthalate, di(2-ethylhexyl) phthalate and diisooctyl adipate are natural metabolites and have biological activities (Table 5). Adeyemo., *et al*. showed that some antimicrobial compounds (e.g. dibutyl phthalate, diisooctyl phthalate) were produced from starch casein broth fermentation using co-cultured strains [28]. In addition, Temitope., *et al*. reported that diisooctyl phthalate was extracted from the plant Spondias mombin and showed anti-inflammatory and antimicrobial activity [29]. Several recent studies showed that these phthalates were produced by plants and microorganisms [29,30]. Furthermore, phthalate esters have been detected in activated sludge and are known plasticizers [31].

The identified compounds that are known to have several pharmacological activities. For example, the compound 2,3-dihydro-3,5-dihydroxy-6methyl 4H-pyran-4-one extracted from endophytic fungal extracts has been reported exhibiting antimicrobial, anti-inflammatory and antiproliferative activities [32]. 1,2-Dimethyl benzene, and nonane are known to possess antifungal and antimicrobial activities [33]. Phenylacetic acid and 3-propyl-2,4-pentadien-1-ol are known as antifungal resistance [34,35]. 1-eicosanol has been reported in plant (*Majidea zanguebarica*) extract showing antitumor, antimalarial, antifungal and antioxidant properties [36]. Antifungal, antibacterial, anticancer, and antitumor activities of methoxy-phenyl oxime of neem leaves extract have been reported by Akpuaka., *et al* [35]. In addition to the major bioactive compounds, there are numerous minor compounds with different chemical classes identified have also been reported to have antimicrobial, prophylactic and other curative activities (Table 4 and 5). However, further multipronged research (biologic and pharmacologic) is necessary to evaluating these identified compounds and discover novel bioactive natural products, which would be sustainable approach that address areas of unmet medical needs.

No.	RT (min)	Name of derivative	Molecular formula	M+ (m/z)	Area (%)	Biological activity
1	11.81	Lactic acid-2TMS	$C_9H_{22}O_3Si_2$	234	4.51	Antimicrobial [37]
2	12.07	2,3-Butanediol, 2TMS	$C_{10}H_{26}O_2Si_2$	234	5.34	
3	12.34	Diethylene glycol, 2TMS	$C_{10}H_{26}O_3Si_2$	250	1.15	
4	18.85	Glycerol, 3TMS	$C_{12}H_{32}O_3Si_3$	308	0.39	
5	19.40	Catechol, 2TMS	$C_{12}H_{22}O_2Si_2$	254	0.70	Antibacterial and antifungal agents [38]
6	23.11	Tyrosol, 2TMS	$C_{14}H_{26}O_2Si_2$	282	2.14	anti-oxidative, anti-apoptotic, and anti-inflammatory [39]
7	25.65	Tyrosine, 2TMS	$C_{15}H_{27}NO_3Si_2$	325	0.30	
8	29.07	Pipecolic acid, 2TMS	$C_{12}H_{27}NO_2Si_2$	273	0.17	
9	34.69	Palmitelaidic acid, TMS	$C_{19}H_{38}O_2Si$	326	0.07	
10	35.41	Myristic acid, TMS	$C_{17}H36O_2Si$	300	7.29	Antibacterial [22]
11	38.48	Petroselinic acid, TMS	$C_{21}H_{42}O_2S_i$	354	1.20	Antimicrobial [40]
12	38.89	Stearic acid, TMS	$C_{21}H5_{44}O_2Si$	356	1.89	Cardiovascular diseases drug [41]
13	41.46	Nonadecanoic acid, TMS	$C_{22}H_{46}O_2Si$	370	7.95	Anti-inflammatory [23]
14	42.06	11-Eicosenoic acid, TMS	$C_{23}H_{46}O_2Si$	382	2.32	
15	47.83	Doconexent, TMS	$C_{25}H_{40}O_2Si$	400	0.53	Anti-inflammatory, hypercholesterolemic, anti-neoplastic [42]
16	50.41	10,12-Tricosadiynoic acid, TMS	$C_{26}H_{46}O_2Si$	418	0.81	
17	52.83	Hexacosanoic acid, TMS	$C_{29}H_{60}O_2Si$	468	3.29	
18	54.14	Campesterol, TMS	$C_{31}H_{56}OSi$	472	1.26	Anti-inflammatory [33]
19	55.10	Ursodeoxycholic acid, 3TMS	$C_{33}H_{64}O_4Si_3$	609	1.13	Cholestatic liver disease drug [34]

***Table 4**: Bioactive compounds identified from GC-MS of CH_2Cl_2 extract PPW fermentation broth in the larger scale (1-L) fermenter as their TMS derivatives.*

No.	Identified Compounds	RT (min)	Molecular formula	M+ (m/z)	Solvent				Biological activity or uses
					EtOAc	Hexane	DCM	$CHCl_3$	
					Area (%)				
1	2,3-dihydro-3,5-dihydroxy-6 methyl 4H-pyran-4-one	3.14	$C_6H_{12}O_2$	116	3.39				Antimicrobial, anti-inflammatory, and antiproliferative [32]
2	Heptanoic Acid	4.76	$C_7H_{14}O_2$	130	1.56				
3	2-Methyl-4-pentenoic acid	4.88	$C_6H_{10}O_2$	114	1.35				
4	Formic acid hydrazide	6.02	CH_4N_2O	60	0.30				
5	1,2-dimethyl benzene,	7.68	C_8H_{10}	106		14.91			Antifungal and antimicrobial [35]
6	3-propyl-2,4-Pentadien-1-ol	8.04	$C_8H_{14}O$	126		4.28			Antifungal
7	Nonane	8.15	C_9H_{20}	128		7.94			Antifungal and antibacterial [35]
8	methoxy-phenyl Oxime	8.35	$C_8H_9NO_2$	151		6.02			Antifungal, antibacterial, anti-cancer, and antitumor [20, 35]
9	3-pentyl cyclohexene	8.42	$C_{11}H_{20}$	152		3.39			
10	3-hexyl cyclohexene	8.54	$C_{12}H_{22}$	166		0.80			
11	Benzyl-2-phenylethylamine	8.98	$C_{15}H_{17}N$	211		1.42			
12	Phenyl 2-methylbenzoate	11.60	$C_{14}H_{12}O_2$	212		1.14			
13	3-methyl-1,3,5-Pentanetriol	11.92	$C_6H_{14}O_3$	134	2.67				
14	1,3-Diphenylpropanetrione	12.14	$C_{15}H_{10}O_3$	238		1.63			
15	3-Ethoxypropionic acid ethyl ester	15.51	$C_7H_{14}O_3$	146	0.23				
16	N-Acetylpyrrolidine	15.82	$C_6H_{11}NO$	113			0.32		
17	1-acetylpiperidine	16.73	$C_7H_{13}NO$	127	0.42				
18	N-(Aminoacetyl)leucine	16.77	$C_8H_{16}N_2O_3$				0.75		

19	Meso-2,3-Diphenylbutane	17.20	$C_{16}H_{18}$	210		9.38			
20	Phenylacetic acid	17.96	$C_8H_8O_2$	136	1.22				Antifungal [43]
21	2-(4-nitrophenoxy)-1-phenyl ethanone	18.99	$C_{14}H_{11}NO_4$	257		15.40			
22	1-Eicosanol	19.23	$C_{20}H_{42}O$	298		7.77			Antitumor, antimalarial, antifungal and antioxidant [36]
23	3-Hexenedioic acid	20.40	$C_6H_8O_4$	144	1.25				
24	2,2'-dimethyl 1,1'-Binaphthalene,	23.11	$C_{22}H_{18}$	298		0.53			
25	1-chloro-2-methyl-2-(4-nitrophenyl) propane	26.82	$C_{10}H_{12}ClNO_2$	213				0.88	Muscle relaxant [44]
26	N-(4-Chlorophenyl) acetamide	28.25	C_8H_8ClNO	169	0.23				
27	5,6-Diphenyl-3-(2-pyridyl)-1,2,4-triazine	29.55	$C_{20}H_{14}N_4$	310		1.81			
28	4-Benzyl-2,5-dimethylpyridine	30.96	$C_{14}H_{15}N$	197				0.82	
29	4-(1-methyl-1- phenylethyl) Phenol)	32.68	$C_{15}H_{16}O$	212	1.82		1.64		
30	Palmitic acid	34.44	$C_{16}H_{32}O_2$	256	1.32				Antimicrobial, and antioxidants [19, 45,46]
31	Dibutyl phthalate	34.65	$C_{16}H_{22}O_4$	278	0.95		4.82	3.08	Antimicrobial, and antifungal [19, 49]
32	Diphenolic acid	36.68	$C_{17}H_{18}O_4$	286	38.49		17.48	9.73	
33	Oleic acid	37.83	$C_{18}H_{34}O_2$	282	0.49				Antibacterial [45,50,51]
34	Stearic acid	38.17	$C_{18}H_{36}O_2$	284	0.30				Antimicrobial [45,46,49,52]
35	n-Nonadecane	39.91	$C_{19}H_{40}$	268				1.33	Antimutagenic [53,54]
36	Diisooctyl adipate	41.03	$C_{22}H_{42}O_4$	370				6.34	Antioxidant, antimicrobial, and antiproliferative [17,20]
37	2,3-Bis(acetyloxy)propyl laurate	41.46	$C_{19}H_{34}O_6$	358		0.96			
38	Nor-hexacosane	42.07	$C_{26}H_{54}$	266		0.37			Antibacterial [49]
39	Diisooctyl phthalate	43.11	$C_{24}H_{38}O_4$	390				1.41	Antifungal and antibacterial [20, 51, 32,49]
40	Dicyclohexyl phthalate	44.34	$C_{20}H_{26}O_4$	330	0.26		1.25		
41	3-Ethyl-5-(2-ethylbutyl)- octadecane	44.55	$C_{26}H_{54}$	366				0.45	Antimicrobial and antifungal [20]
42	Eicosane, 2-methyl	45.99	$C_{21}H_{44}$	296				0.97	Antioxidant [55]
43	Heptadecane, 9-octyl	46.69	$C_{25}H_{52}$	352	0.32				
44	10-Octadecenal	47.37	$C_{18}H_{34}O$	296				0.42	Antimicrobial, anti-inflammatory [52]
45	2-Octadecoxyethano	48.70	$C_{20}H_{42}O_2$	314	0.25			0.51	Antimicrobial [56]
46	Trequinsin	48.82	$C_{24}H_{27}N_3O_3$	405	1.34				Antihypertensive vasodilator and antithrombotic [57]
47	Nor-Tetracosane	49.46	$C_{24}H_{50}$	338	1.86				Cytotoxic activity against gastric cancer AGS cell [58]
48	Desulphosinigrin	50.10	$C_{10}H_{17}NO_6S$	279				0.22	
49	Ethyl iso-allocholate	50.88	$C_{26}H_{44}O5$	436				0.32	Antimicrobial, diuretic, anti-inflammatory, and antiasthma [59,60]
50	2-(Acetyloxy)-1-[(octadecyloxy) methyl] ethyl acetate.	51.83	$C_{25}H_{48}O_5$	428		0.40			
51	Frenolicin b	53.28	$C_{18}H_{16}O_6$	328	1.00		0.60		Antiparasitic, and anti-inflammatory [61]
52	3-hydroxy Spirost-8-en-11-one	54.22	$C_{27}H_{40}O4$	428				0.30	Progestogen [44]

***Table 5**: Bioactive compounds identified from GC-MS of PPW fermentation broth in the larger scale (1-L) fermenter using different solvents (CH_2Cl_2, EtOAc, $CHCl_3$, hexane extract).*

ESI-MS analysis

Further identification of bioactive compounds in PPW extract was using ESI-MS technique. The (+) ESI-MS spectrum of PPW fermentation broth extended from m/z 150 to 1000. However, ESI-MS scan was performed, as well, but no molecular ion species could be detected. The (+) ESI -MS showed significant molecular ion peaks $[M+H]^+$ with m/z values of 212.8964, 430.5846, 528.5759, and 626.4854 (Figure 4). On the basis of ESI-MS spectral data, the molecular formula was established to be C_5HCl_3NS (Calcd. for 212.8968) chlorpyriphos-methyl, $C_{27}H_{43}NO_3$ (Calcd. for 430.3316) myxalamid K [62], $C_{32}H_{69}N_3O_2$ (Calcd. for 528.5463) unknown, and $C_{34}H_{59}N_9O_2$ (Calcd. for 626.4864) unknown, respectively. Among these identified compounds, myxalamid K has been reported for its antimicrobial properties [63]. Nevertheless, unknown peaks which remain unidentified in the (+) ESI-MS spectrum were m/z 234.74, 316.69, 332.68, 392.75 and 702.48 (Figure 3).

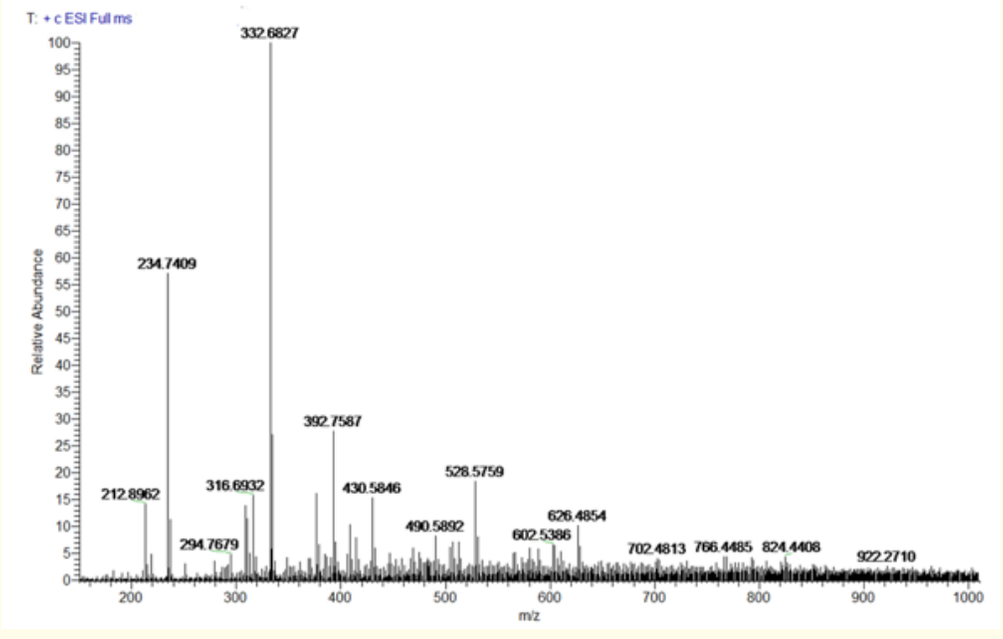

Figure 3: *The (+) ESI-MS spectra obtained from CH^2Cl^2 extract PPW fermentation broth in the larger scale (1-L) fermenter.*

Strain identification by 16S rRNA sequencing

A total of 8962 and 195945 sequence reads were obtained from the seed and PPW-FR samples, respectively using barcoded pyrosequencing analysis of the 16S rRNA gene. For the overall bacterial community, 18 bacterial genera were detected during the seed sample and 8 were detected during the 13h fermentation period. The abundances of the most abundant genera in seed samples were *Albidiferax ferrireducens* (32%), *Acidovorax* (14%) and *Saprospiraceae* (7%) (Figure 4a). These findings were quite different from the results reported by other researchers [16,17]. On the one hand, the abundance of *Lactobacillus delbrueckii* (45.3%) and another different five *Lactobacillus* species (*Lactobacillus* (13.09%), *Lactobacillus fermentum* (11.72%), *Lactobacillus parabuchneri* (8.67%), *Lactobacillus parafarraginis* (5.15%) and *Lactobacillus curvatus* (2.01%) were detected with a total abundance of 41% (Figure 4b). The results of the analysis on the PPW fermented sample showed that only seven bacteria were found during the 13h fermentation period. Among these organisms, *Lactobacillus* species in PPW fermented sample was the dominant genus (86%), suggesting these bacteria are the best adapted to produce lactic acid at 13h from the fermentation of PPW with undefined mixed microbial cultures (MMC) inoculated from the waste activated sludge from the wastewater treatment system.

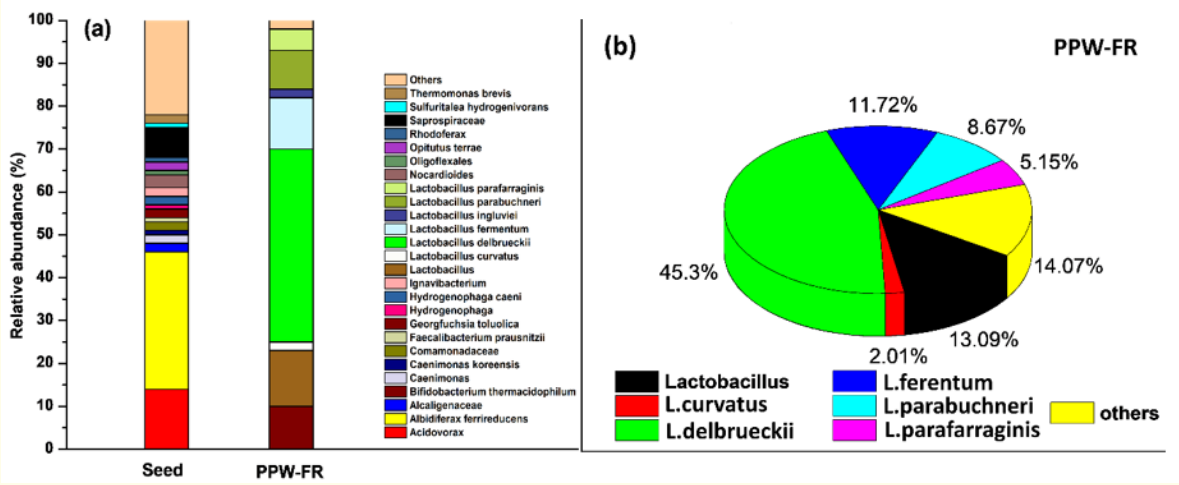

Figure 4: *(a) The relative abundances of major phyla in the seed sample and PPW fermenter and (b) the relative abundance of different lactobacillus species in PPW fermenter determined by 16S rRNA sequencing.*

The SDS-PAGE profile of PPW fermented is presented in figure 5. The pattern of the gel showed two widening bands with apparent molecular masses of approximately 35 and 12 kDa. This has provided evidence that the PPW fermented contained some proteins/peptides, which could be antimicrobial agents. However, further studies need to be carried out to separate peptides by using a separation technique (e.g. HPLC) and then test the effect of the individual peptide on some microorganisms and its toxicological analysis.

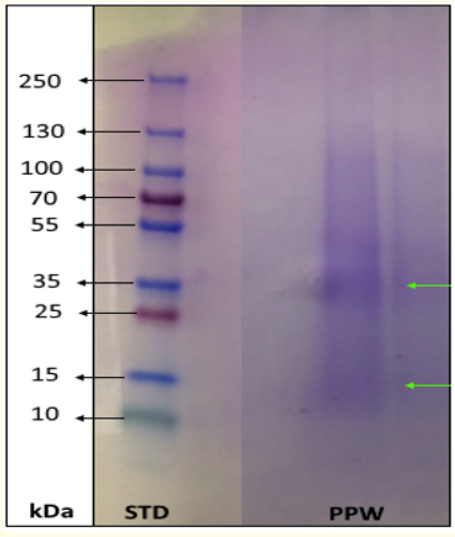

Figure 5: *Electrophoretic pattern in SDS-PAGE of proteins from PPW fermented.*

Conclusion

The results obtained in the present this study suggest that the PPW fermentation broth using a mixed microbial culture (MMC) are rich in the bioactive compounds that are having effective antimicrobial activity. Although there are numerous kinds of organic compounds with different molecular formulas in the PPW fermentation broth, these compounds are promising and encouraging for more antimicrobial drug and /or new nutraceutical products discoveries and design. In order to progress the possible use of PPW as promising alternative source for natural antimicrobial production, and increase confidence in compound identifications, further studies on the investigation, purification and isolation of new natural products such as bioactive compounds an effective antimicrobial agent and evaluate their bioactivity, toxicity and clinical studies.

Acknowledgements

The authors acknowledge (i) the financial support from the SunGrant Initiative Western Region award # U0994G-D, (ii) the University of Idaho - Equipment and Infrastructure Support (EIS) Awards Program from the Office of Research and Economic Development (ORED) RISE Funding Programs for the support of the purchase of the GCMS, and (iii) Dr. Erik Coats for providing the MMC seed.

Conflict of Interest

The authors declare no financial interest or any conflict of interests.

Bibliography

1. Javed Ahsan*., et al. "*Potato Peel Waste-Its Nutraceutical, Industrial and Biotechnological Applacations". *AIMS Agriculture and Food* 4 3 (2019): 807-823.

2. Gebrechristos Haftom Yemane and Weihua Chen. "Utilization of Potato Peel as Eco-Friendly Products: A Review". *Food Science and Nutrition* 6 6 (2018): 1352-1356.

3. NPC. National Potato Council. " 2019 Potato Statistical Yearbook". (2020).

4. Pelletier RC*., et al. "*B624: A Comparison of Several Peeling Methods as Applied to Maine Potatoes for Processing". *Maine Agricultural Experiment Station Bulletin* 624 (1964): 1-20.

5. Liang Shaobo and Armando G McDonald. "Chemical and Thermal Characterization of Potato Peel Waste and Its Fermentation Residue as Potential Resources for Biofuel and Bioproducts Production". *Journal of Agricultural and Food Chemistry* 62 33 (2014): 8421-8429.

6. Scharf Regiane*., et al. "*Valorisation of Potato (Solanum Tuberosum) Peel Waste: Extraction of Fibre, Monosaccharides and Uronic Acids". *Waste and Biomass Valorization* (2018): 1-6.

7. Sadh Pardeep Kumar., *et al.* "Agro-Industrial Wastes and Their Utilization Using Solid State Fermentation: A Review". *Bioresources and Bioprocessing* 5 1 (2018): 1.

8. Zacharof MP and RW Lovitt. "Bacteriocins Produced by Lactic Acid Bacteria a Review Article". *Apcbee Procedia* 2 (2012): 50-56.

9. Balciunas Eduardo Marcos*., et al. "*Novel Biotechnological Applications of Bacteriocins: A Review". *Food Control* 32 1 (2013): 134-142.

10. Abdulbaset M., *et al.* "Fermentation of Apple Pomace Using Mixed Microbial Culture to Organic Acids". *EC Microbiology* 15 (2019): 870-884.

11. Liang S., *et al*. "Lactic Acid Production with Undefined Mixed Culture Fermentation of Potato Peel Waste". *Waste Management* 34 11 (2014): 2022-2027.

12. Liang S.., *et al. "*Comparative Analysis of Microbial Community of Novel Lactic Acid Fermentation Inoculated with Different Undefined Mixed Cultures". *Bioresource Technology* 179 (2015): 268-274.

13. Ma Jie*., et al. "*Assessment of Cross-Protection to Heterologous Strains of Flavobacterium Psychrophilum Following Vaccination with a Live-Attenuated Coldwater Disease Immersion Vaccine". *Journal of Fish Diseases* 42 1 (2019): 75-84.

14. Martinez-Fernandez Jose S*., et al. "*Recovering Valuable Bioactive Compounds from Potato Peels with Sequential Hydrothermal Extraction". *Waste and Biomass Valorization* (2020): 1-17.

15. Patelski Piotr*., et al. "*Conversion of Potato Industry Waste into Fodder Yeast Biomass". *Processes* 8 4 (2020): 453.

16. Burlingame Barbara., *et al.* "Nutrients, Bioactive Non-Nutrients and Anti-Nutrients in Potatoes". *Journal of Food Composition and Analysis* 22 6 (2009): 494-502.

17. Ahmad Sohail., *et al.* "In Vitro Antimicrobial Bioassays, Dpph Radical Scavenging Activity, and Ftir Spectroscopy Analysis of Heliotropium Bacciferum". *BioMed Research International* 2016 (2016).

18. Liou Je-Wen*., et al. "*The Antimicrobial Activity of Gramicidin a Is Associated with Hydroxyl Radical Formation". *PloS one* 10.1 (2015).

19. Nandhini S Usha., *et al.* "Gas Chromatography-Mass Spectrometry Analysis of Bioactive Constituents from the Marine Streptomyces". *Asian Journal of Pharmaceutical and Clinical Research* 8.2 (2015): 244-246.

20. El-Azazy Marwa*., et al. "*Potato Peels as an Adsorbent for Heavy Metals from Aqueous Solutions: Eco-Structuring of a Green Adsorbent Operating Plackett–Burman Design". *Journal of Chemistry* 2 (2019): 1-14.

21. Kamal Sabreen A., *et al.* "Antibacterial Activity of Secondary Metabolites Isolated from Alternaria Alternata". *African Journal of Biotechnology* 14.43 (2015): 2972-2994.

22. Chen Xiangrong*., et al. "*Antimicrobial Potential of Myristic Acid against Listeria Monocytogenes in Milk". *The Journal of Antibiotics* 72.5 (2019): 298-305.

23. Zhou Jiaju., *et al.* "Isolated Compounds (N-S)" In: Encyclopedia of Traditional Chinese Medicines - Molecular Structures, Pharmacological Activities, Natural Sources and Applications 4 (2011): 1-636.

24. Jing Jian., *et al*. "Diphenolic Acid Based Biphosphate on the Properties of Polylactic Acid: Synthesis, Fire Behavior and Flame Retardant Mechanism". *Polymer* 108 (2017): 29-37.

25. Van de Vyver Stijn*., et al. "*Mechanistic Insights into the Kinetic and Regiochemical Control of the Thiol-Promoted Catalytic Synthesis of Diphenolic Acid". *ACS Catalysis* 2.12 (2012): 2700-2704.

26. Shen Yue*., et al. "*Catalytic Synthesis of Diphenolic Acid from Levulinic Acid over Bronsted Acidic Ionic Liquids". *BioResources* 9.2 (2014): 3264-3275.

27. Thompson Richard C., *et al.* "Plastics, the Environment and Human Health: Current Consensus and Future Trends". *Philosophical Transactions of the Royal Society B: Biological Sciences* 364.1526 (2009): 2153-2166.

28. Adeyemo OM., *et al.* "Effect of Production Parameters and Inhibitory Activity of Antimicrobial Compounds Produced by Co-Cultured Strains of Streptomyces Xinghaiensis-Oy62 and S. Rimosus-Og95". *Journal of King Saud University-Science* 32.1 (2020): 294-301.

29. Osuntokun Oludare. "Bio-Guided Isolation, Chemical Purification, Identification, Antimicrobial and Synergistic Efficacy of Extracted Essential Oils from Stem Bark Extract of Spondias Mombin (Linn)". *Journal of Molecular Biology* 4 (2019): 135-143.

30. Ortiz Aurelio and Estibaliz Sansinenea. "Di-2-Ethylhexylphthalate May Be a Natural Product, Rather Than a Pollutant". *Journal of Chemistry* (2018).

31. Gong Rui., *et al.* "Effect of Dehp on Scfa Production by Anaerobic Fermentation of Waste Activated Sludge". *Advances in Polymer Technology* (2020).

32. Sharma Deeksha., *et al*. "Evaluation of Bioactive Secondary Metabolites from Endophytic Fungus Pestalotiopsis Neglecta Bab-5510 Isolated from Leaves of Cupressus Torulosa D. Don". *3 Biotech* 6.2 (2016): 210-224.

33. Aparna Vasudevan., *et al.* "Anti-Inflammatory Property of N-Hexadecanoic Acid: Structural Evidence and Kinetic Assessment". *Chemical Biology and Drug Design* 80.3 (2012): 434-439.

34. Paumgartner Gustav and Ulrich Beuers. "Ursodeoxycholic Acid in Cholestatic Liver Disease: Mechanisms of Action and Therapeutic Use Revisited". *Hepatology* 36.3 (2002): 525-531.

35. Akpuaka A., *et al.* "Biological Activities of Characterized Isolates of N-Hexane Extract of Azadirachta Indica A. Juss (Neem) Leaves". *Nature and Science* 11.5 (2013): 141-147.

36. Sanjaya P. "Preliminary Phytochemical Analysis, Gc-Ms Studies and Antioxidant Activity of Majidea Zangueberica J. Kirk Leaf Extracts". *Journal of Medicinal Plants* 7.2 (2019): 186-195.

37. Saranraj P., *et al*. "Lactic Acid Bacteria and Its Antimicrobial Properties: A Review". *International Journal of Pharmaceutical and Biological Archive* 4.6 (2013): 1124-1133.

38. Kocaçalışkan Ismail., *et al*. "Antimicrobial Activity of Catechol and Pyrogallol as Allelochemicals". *Zeitschrift für Naturforschung C* 61 9.10 (2006): 639-642.

39. Je In-Gyu., *et al.* "Tyrosol Suppresses Allergic Inflammation by Inhibiting the Activation of Phosphoinositide 3-Kinase in Mast Cells". *PLoS One* 10.6 (2015).

40. Hanif Muhammad Asif., *et al.* "Medicinal Plants of South Asia". *Novel Sources for Drug Discovery* (2019).

41. Krishnaveni Marimuthu., *et al.* "Gc-Ms Analysis of Phytochemicals, Fatty Acid Profile, Antimicrobial Activity of Gossypium Seeds". *International Journal of Pharmaceutical Sciences Review and Research* 27.1 (2014): 273-276.

42. Babu M., *et al.* "Chemical Constituents and Their Biological Activity of Ulva Lactuca Linn". International *Journal of Pharmaceutical Chemistry Analysis* 2.7 (2014): 595-600.

43. Hwang Byung Kook*., et al. "*Isolation and in Vivo and in Vitro Antifungal Activity of Phenylacetic Acid and Sodium Phenylacetate From-streptomyces Humidus". *Applied and Environmental Microbiology* 67.8 (2001): 3739-3745.

44. Mao Fei*., et al. "*Chemical Structure-Related Drug-Like Criteria of Global Approved Drugs". *Molecules* 21 (2016): 75.

45. Karimi Ehsan*., et al. "*Fatty Acid Composition, Antioxidant and Antibacterial Properties of the Microwave Aqueous Extract of Three Varieties of Labisia Pumila Benth". *Biological Research* 48.1 (2015): 9.

46. Girija Smiline*., et al. "*Chromatographic Characterization and Gc-Ms Evaluation of the Bioactive Constituents with Antimicrobial Potential from the Pigmented Ink of Loligo Duvauceli". *International Scholarly Research Notices* 2014 (2014).

47. Ahsan Taswar*., et al. "*Extraction and Identification of Bioactive Compounds (Eicosane and Dibutyl Phthalate) Produced by Streptomyces Strain Kx852460 for the Biological Control of Rhizoctonia Solani Ag-3 Strain Kx852461 to Control Target Spot Disease in Tobacco Leaf". *AMB Express* 7.1 (2017): 54.

48. Bedika Mallaiah., *et al.* "Isolation and Identification of Antifungal Compounds from Psedomonas Fluorescens Inhibiting the Growth of Fusarium Incarnatum (Desm.)Sacc. Incitant of Crossandra Wilt". 12 (2017).

49. Mallaiah B and M Muthamilan. "Isolation and Identification of Antifungal Compounds from Bacillus Subtilis Inhibiting the Growth of Fusarium Incarnatum (Desm.) Sacc. Incitant of Crossandra Wilt". *International Journal of Tropical Agriculture* 33.2 (2015): 1691-1696.

50. Ahamad M Sayed Iqbal and Shyam Kumar Vootla. "GC-MS Analysis of Anti-Microbial Compounds from Antheraea Mylitta Cocoons". *International Journal of Pharmaceutical Sciences and Research* 9.7 (2018): 2921-2926.

51. Parthipan B., *et al.* "GC-MS Analysis of Phytocomponents in Pleiospermium Alatum (Wall. Ex Wight and Arn.) Swingle, (Rutaceae)". *Journal of Pharmacognosy and Phytochemistry* 4 (2015): 216-222.

52. Subavathy P and RD Thilaga. "Gc-Ms Analysis of Bioactive Compounds from Whole Body Tissue Methanolic Extract of Cypraea Arabica (L. 1758)". *World Journal of Pharmaceutical Research* 5 (2016): 800-806.

53. Lawal Bashir*., et al. "*Gc-Ms Determination of Bioactive Constituents of Giant African Snail (Archachatina Maginata) Haemolymph". *IOSR Journal of Pharmacy and Biological Sciences* 10.2 (2015): 59-64.

54. Mishra Vineet Kumar*., et al. "*Determination and Production of Antimicrobial Compounds by Aspergillus Clavatonanicus Strain Mj31, an Endophytic Fungus from Mirabilis Jalapa L. Using Uplc-Esi-Ms/Ms and Td-Gc-Ms Analysis". *PloS one* 12.10 (2017): e0186234.

55. Phillips Sanitha*., et al. "*Preliminary Gc-Ms Analysis of an Ayurvedic Medicine "Kulathadi Kashayam". *Journal of Chemical and Pharmaceutical Research* 7.9 (2015): 393-400.

56. Hassan Sahar and Aida Shobier. "Gc/Ms Identification and Applications of Bioactive Seaweed Extracts from Mediterranean Coast of Egypt". *Egyptian Journal of Aquatic Biology and Fisheries* 22 (2018): 1-21.

57. Milne George WA. "Drugs: Synonyms and Properties: Synonyms and Properties". *Routledge* (2018).

58. Uddin Shaikh Jamal., *et al.* "Evaluation of Cytotoxic Activity of Patriscabratine, Tetracosane and Various Flavonoids Isolated from the Bangladeshi Medicinal Plant Acrostichum Aureum". *Pharmaceutical Biology* 50.10 (2012): 1276-1280.

59. Muthulakshmi, ARJM., *et al.* "GC-MS Analysis of Bioactive Components of Feronia Elephantum Correa (Rutaceae)". *Journal of Applied Pharmaceutical Science* 2.2 (2012): 69-74.

60. Hussein Ameera Omran*., et al. "*Phytochemical Screening of Methanolic Dried Galls Extract of Quercus Infectoria Using Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier Transform-Infrared (Ft-Ir)". *Journal of Pharmacognosy and Phytotherapy* 8.3 (2016): 49-59.

61. Fitzgerald Jay T., *et al*. "Engineered Biosynthesis of the Antiparasitic Agent Frenolicin B and Rationally Designed Analogs in a Heterologous Host". *The Journal of Antibiotics* 64.12 (2011): 759-762.

62. Kim Jiyoung*., et al. "*Lc-Ms/Ms Profiling-Based Secondary Metabolite Screening of Myxococcus Xanthus". *JMB Journal of Microbiolog and Biotechnology* 19.1 (2009): 51-54.

63. Gerth K*., et al. "*The Myxalamids, New Antibiotics from Myxococcus Xanthus (Myxobacterales)". *The Journal of Antibiotics* 36.9 (1983): 1150-1156.

Volume 17 Issue 7 July 2021