South Asian Journal of Research in Microbiology

7(3): 1-14, 2020; Article no.SAJRM.59567
ISSN: 2582-1989

Effects of Fermentation on the Antioxidant and Antinutritional Compositions of Green Pea

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Authors' contributions

This work was carried out in collaboration between both authors. Author AEO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors OAO and AEO managed the analyses of the study. Author AEO managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/SAJRM/2020/v7i330171
Editor(s):
(1) Dr. Ana Claudia Coelho, University of Tras-os-Montes and Alto Douro, Portugal.
Reviewers:
(1) I. Gusti Nyoman Gde Bidura, Udayana University, Indonesia.
(2) Abdouli Hedi, Carthage University, Tunisia.
Complete Peer review History: http://www.sdiarticle4.com/review-history/59567

Original Research Article

Received 27 May 2020
Accepted 01 August 2020
Published 11 August 2020

ABSTRACT

Green peas are known to contain anti-nutritional factors like enzymes inhibitors, phytates, oxalates, saponins and polyphenolic compounds, all of which limit their utilization hence, the study evaluate the effect of fermentation on the antioxidant and antinutrients content of green pea. Fermentation of green pea was done using both submerged and solid state fermentation for 7days. Isolation and identification of microorganism from the fermented sample was done on daily basis using standard microbiological and molecular techniques. The type of organism isolated from the submerged fermentation of Green pea included the bacteria (*Bacillus subtilis, Lactobacillus Plantarum, Micrococcus roseus, Lactobacillus lactis, and Lactobacillus fermentum*) and the fungi *Rhizopus oryzae, Penicillium chrysogenum* and *Rhizopus stolonifer*. While the type of organism isolated from the solid state fermentation of Green pea included some bacteria (*Bacillus subtilis, Lactobacillus Plantarum and Lactobacillus lactis*) and fungi (*Penicillium notatum, Rhizopus oryzae, Penicillium chrysogenum, Candida albicans, Alternaria alternate* and *Rhizopus stolonifer*). Fermentation reduced the antinutritional content of the fermented sample with submerged fermentation resulting in the highest reduction from 32.18 mg/g, 4.14 mg/g, 1.62 mg/g, 51.08 mg/g and 36.37 mg/g in the raw sample to 26.27 mg/g, 0.48 mg/g, 0.27 mg/g, 7.82 mg/g and 24.07 mg/g in submerged

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fermented green pea for saponin, tannin, oxalate, phytate and alkaloid respectively. However, Fermentation significantly $p \leq 0.05$ increased the phenol, flavonoid and FRAP content of the fermented green pea with the solid state fermentation resulting in the highest increase from 3.50, 0.03 and 1.41 in the raw sample to 9.32, 0.12 and 9.66 in the solid state fermented green pea for phenol, flavonoid and FRAP content respectively. This study revealed that fermentation had significant effect on the antioxidant and antinutritional compositions of Green pea thereby reducing the antinutrient composition of Green pea in which will improve the nutrient value of Green pea.

Keywords: Enzymes inhibitors; phytates; oxalates; saponins; polyphenolic compounds; submerged fermentation; solid state fermentation.

1. INTRODUCTION

Green pea (*Pisum sativum L*) is one of the most important pulses. It is an important dietary source of protein, fibre and carbohydrate in the humid tropical zones of Africa, Asia, India and Europe [1]. It is a good human food. It is a small, smooth, waxy and spherical in shape and the seeds of pea are like those of other legumes. It accumulates proteins in its cotyledons during development. These proteins are mainly globulins which are soluble in aqueous salt solutions at neutral pH. In keeping with their physiological storage Role, they are multimeric proteins readily rendered insoluble for deposition, and have high proportions of amide amino acids [2]. The Fibre from the pea has been reported to help in digestion while the vitamin and mineral contents in pea helps in the prevention of cancer and deficiency-related diseases [2].

However, Green pea has been proven to have high anti-nutrient properties [3]. Anti-nutrient compounds, when consumed may interfere with the absorption of beneficial and essential organic nutrients and inorganic minerals. Hence, reducing the antinutritional content of Green pea is essential. Several food processing methods such as germination, soaking, cooking and fermentation are known to reduce anti-nutritional factors in which fermentation has been reported to be most efficient [4].

Fermentation is the chemical breakdown of substance by bacteria, yeast or other microorganisms into alcohol, carbon dioxide or organic acids. Fermentation in food processing serves five main purposes which are to enrich the diet through development of a diversity of flavours, aromas, and textures in food substrates; to preserve substantial amounts of food through lactic acid, alcohol, acetic acid, and alkaline fermentations; to reduce cooking time and the associated use of fuel; to eliminate anti-nutrients and to retain food substrates with protein, enzymes, essential amino acids, vitamins and other nutrients that are usually destroyed by food processing since fermentation doesn't involve the use of heat [5]. The aim of this study was to access the effect of fermentation on the antioxidant and antinutritional compositions of green pea.

2. MATERIALS AND METHODS

2.1 Sample Collection

Mature Green peas were bought from shasha market in akure, ondo state Nigeria. The apparently healthy Green pea were transported to the laboratory in clean and sterile black opaque polythene bags sealed at the tip and kept at refrigerated temperature ($4^{o}C$) in the laboratory.

2.2 Preparation and Fermentation of Samples

The peas were sorted manually and were removed from their pods and sorted again to remove the immature seeds and spoilt seeds. 250 grams of the sorted peas was weighed using a digital weighing balance into 500 ml of sterile distilled water for submerged fermentation.

The same quantity was weighed for solid state fermentation. Fermentation was carried out in a transparent sterile container for 7 days at room temperature. The peas were physically evaluated for changes, chemical analysis such as pH, Temperature, Titratable acidity, proximate analysis, minerals and anti-nutritional factors analysis were also carried out for both submerged and solid state fermentation. Daily changes in the microbial population (cfu/ml) of the total viable count, fungi, and lactic acid bacteria were determined. The fermenting samples were collected at 0, 24, 48, 72, 96, 120, 144 and 168 hours interval respectively.

2.3 Microbiological Analysis of the Samples

Bacteria and fungi were evaluated using nutrient agar (NA) and potato dextrose agar (PDA) respectively while De Man Rogosa sharpe agar was used to isolate lactic acid bacteria. The bacterial culture was incubated at 37°C for 18 to 24 hours, fungal plates were inverted and incubated at 24°C for 48 to 72 hours. De Man Rogosa sharpe agar plates were incubated at 32°C for 18 to 24 hours anaerobically. The organisms were characterized based on biochemical and morphological observations according to the methods of [6].

2.4 Molecular Identification of Bacteria Isolate

Extraction of DNA using CTAB method was done according to [7], PCR analysis was run with a universal primer for fungi called 1TS1 and ITS4 and bacteria which was run with a universal primer called 16S rRNA. The amplicon was further purified before the sequencing using 2M Sodium Acetate wash techniques.

2.5 Determination of pH and TTA

The pH of all fermenting samples was determined at 24 hours interval using a pocket size pH meter. A 1 g sample was dissolved in 10 ml of distilled water and filtered. The pH meter was calibrated with buffer solutions of pH 4, 7 and 9, this was followed by dipping the electrode of the pH meter into the sample solution and the observed pH was read and recorded in triplicates. The total titratable acidity of the fermenting samples was determined at 24 hours interval. A 2 g macerated sample was weighed into a beaker. 20 ml of distilled water was added to it, it was mixed and filtered. 10 ml of the filtrate was measured into a beaker and 2 drops of phenolphthalein indicator was added into it. This was titrated with 0.1 M sodium hydroxide (NaOH) solution and the titre value was read. Total titratable acidity was expressed as percent (%) lactic acid. The acidity was calculated as: TTA= Titre value × 9 mg/100. The pH and TTA of the samples were carried out according to the method described by [8].

2.6 Antinutrient Determination

Tannin and Phytate was determining according to the method of [9]. Oxalate was determined using the method of [10]. The spectrophotometric method of [11] was used for Saponin determination.

2.7 Determination of Ferric Reducing Property

The ferric reducing property of the fermented sample was determined by the method of [12], 0.25 ml of the fermented broth culture was mixed with 0.25 ml of 200 mM of Sodium phosphate buffer pH 6.6 and 0.25 ml of 1% KFC. The mixture was incubated at 50^{o}C for 20min, thereafter 0.25 ml of 10% TCA was also added and centrifuge at 2000 rpm for 10min, 1ml of the supernatant was mixed with 1ml of distilled water and 0.1% of $FeCl_3$ and the absorbance was measure at 700 nm.

2.8 Determination of DPPH Scavenging Ability

The free radical scavenging ability of the fermented sample against DPPH (1, 1- diphenyl-2-picryhydrazyl) was done using the method of [13]. 1 ml of the fermented broth culture was mixed with 1ml of the 0.4 mM methanolic solution of the DPPH the mixture was left in the dark for 30 min before measuring the absorbance at 516 nm.

3. RESULTS

3.1 Types of Microorganisms Isolated during Submerged and Solid State Fermentation of Green Pea

The organisms isolated from raw green pea included *Bacillus subtilis* and *Micrococcus roseus* for bacteria and *Rhizopus oryzae, Candida albicans and Penicillium chrysogenum for fungi.* The bacteria *Bacillus subtilis, Lactobacillus* spp*, Micrococcus roseus, Lactococcus* spp*, and Staphylococcus aureus* and the fungi *Rhizopus oryzae, Penicillium chrysogenum* and *Rhizopus stolonifer* were associated with the submerged fermentation of green pea (Table 2). The bacteria *Bacillus subtilis, Lactobacillus* spp, *Lactococcus* spp, *Acinetobacter* spp and *Staphylococcus aureus* and the fungi *Penicillium notatum, Rhizopus oryzae, Aspergillus* spp*, Candida albicans, Saccharomyces cerevisiae* and *Rhizopus stolonifer* were associated from the solid state fermentation of green pea (Table 3).

Table 1. Colonial, morphological and biochemical characterization of bacterial isolates during solid and submerged fermentation

Isolate No	Colony Morphology	Gram's Reac-tion	Catal ase	Coa gula se	Moti lity	Man nitol	Gluc ose	Fruct ose	Malt ose	Lact ose	Su cro se	Citra te	Indole	Spore Forming	Methyl Red Test	Starch hydrolys is	Ure ase test	Probable Identity
1	Cream, circular, opaque, flat, rough	**+**	**+**	**NA**	+	+	AG	AG	AG	AG	AG	**+**	**-**	**+**	**-**	+	-	*Bacillus subtilis*
2	Circular, opaque, convex, cream, smooth colonies	**+**	**-**	**-**	-		A	AG	AG	A	AG	**-**	**-**		**+**	-	+	*Lactobacillus* spp
3	Cream, circular, smooth, entire	**+**	**+**	**NA**	-	-	AG	A	-	-	AG	**-**	**-**			-		*Lactococcus* spp
4	Cream, circular, raised and smooth	**-**	**+**	**-**	+	-	A	A	-	-	A		**-**	**NA**		+		*Acinetobacter* spp
5	Circular, translucent, convex, creamy, smooth colonies	**+**	**-**	**-**	-		AG	A	AG	AG	AG	**-**	**-**			-		*Staphylococcus aureus*

Keys: (+) = positive, (AG) = Acid and Gas, (-) = negative, (A) = Acid, (NA) = not applicable

Table 2. Types of microorganisms isolated from submerged fermentation at different fermentation duration

	Submerged fermentation	
Duration (days)	**Bacteria**	**Fungi**
1	*Bacillus subtilis, Acinetobacter* spp ,	*Rhizopus oryzae, Penicillium chrysogenum, Rhizopus stolonifer*
2	*Bacillus subtilis, Acinetobacter* spp ,	*Rhizopus oryzae, Penicillium chrysogenum, Rhizopus stolonifer, Aspergillus* spp
3	*Bacillus subtilis ,Lactococcus* spp*, Lactobacillus* spp	*Rhizopus oryzae, Penicillium chrysogenum, Rhizopus stolonifer, Aspergillus* spp
4	*Bacillus subtilis, Lactobacillus* spp *Lactococcus* spp*,*	*Rhizopus oryzae, Penicillium chrysogenum, Rhizopus stolonifer, Aspergillus* spp
5	*Bacillus subtilis, Lactobacillus* spp *Lactococcus* spp*,*	*Rhizopus oryzae, Penicillium chrysogenum, Rhizopus stolonifer, Aspergillus* spp
6	*Bacillus subtilis, Lactobacillus* spp *Lactococcus* spp*,*	*Rhizopus oryzae, Penicillium chrysogenum, Rhizopus stolonifer, Aspergillus* spp
7	*Lactobacillus* spp *Lactococcus* spp*,*	*Rhizopus oryzae, Penicillium chrysogenum, Rhizopus stolonifer, Aspergillus* spp

Table 3. Types of microorganisms isolated from Solid state fermentation at different fermentation duration

	Solid state fermentation	
Duration (Days)	**Bacteria**	**Fungi**
1	*Bacillus subtilis, Acinetobacter* spp,	*Rhizopus stolonifer, Rhizopus oryzae, Candida albican, Penicillium notatum, Alternaria alternate*
2	*Bacillus subtilis, Acinetobacter* spp,	*Rhizopus stolonifer, Rhizopus oryzae, Candida albican, Alternaria alternate*, *Penicillium notatum*
3	*Bacillus subtilis, Acinetobacter* spp, *Lactococcus* spp,	*Rhizopus stolonifer, Rhizopus oryzae, Candida albican, Alternaria alternate*, *Penicillium notatum, Penicillium chrysogenum, Saccharomyces cerevisiae*
4	*Bacillus subtilis*	*Rhizopus oryzae Penicillium chrysogenum, Penicillium notatum, Saccharomyces cerevisiae*
5	*Bacillus subtilis*	*Rhizopus oryzae Penicillium chrysogenum, Penicillium notatum, Saccharomyces cerevisiae*
6	*Bacillus subtilis*	*Rhizopus oryzae Penicillium chrysogenum, Penicillium notatum, Saccharomyces cerevisiae*
7	*Bacillus subtilis*	*Rhizopus oryzae Penicillium chrysogenum, Penicillium notatum, Saccharomyces cerevisiae*

Table 4. Morphological characteristics of fungal isolates during both solid and submerged fermentation

Isolate No	Cultural and Microscopy description	Probable Identity
1	Yellowish green to dark green hyphae. Conidiophores arising from the mycelium singly or less often in synnemata, branched near the apex, penicillate, ending in a group of phialides	*Penicillium notatum*
2	Colonies are very fast growing with some tendency to collapse, white cottony at first becoming brownish grey to blackish-grey, sporangiophores are non-septate, arising from stolons opposite rhizoids usually in groups of 3 or more. Sporangia are globose with a flattened base, greyish black, powdery in appearance, Columellae and apophysis together are globose. Sporangiospores are angular, subglobose to ellipsoidal, with ridges on the surface.	*Rhizopus oryzae*
3	Black mycelium , Conidiophore hyaline, slender with spairing upper part, branched conidia and septate hyphae	*Aspergillus* spp
4	White greenish growth with Conidia have a globose shape with rough surface wall	*Saccharomyces cerevisiae*
5	Hyphae broad, not or scarcely septate; rhizoids and stolons present; sporangiophores brown, solitary or in tufts on the stolons, diverging from the point at which the rhizoids form; sporangia rather round; apophysis absent or scarcely apparent; sporangiophores ovoid.	*Rhizopus stolonifer*

3.2 Frequency Distribution of Bacterial and Fungal Isolates in the Fermented Samples

The distribution of bacterial and fungal isolates in submerged and solid state fermentation are shown in Tables 5 and 6. The result revealed that *Bacillus subtilis* and *Rhizopus oryzae* were the most predominant bacteria and fungi, respectively.

3.3 Microbial Load of Microorganisms Isolated during Fermentation

The total bacterial (cfu/ml) and fungal count (sfu/ml) of the fermented samples increased during the first day in both submerged and solid state fermentation followed by subsequent reduction. The bacteria load is higher in submerged fermentation than solid state fermentation while the fungal count is higher in the solid state fermentation than submerged fermentation. The total lactic acid bacteria count decreased drastically during the first two day of the submerged fermentation after which there was a constant growth throughout the remaining fermentation duration. However, the total lactic acid count of the solid state fermentation further decreased drastically throughout the fermentation period. The details of the microbial load of microorganisms isolated from the fermented samples can be seen in Figs. 2, 3 and 4.

Table 5. Frequency distribution of bacteria in both solid and submerged fermentation

Isolates	Submerged Fermentation	Occurrence (%) in Submerged Fermentation	Solid State Fermentation	Occurrence (%) in Solid state Fermentation
Bacillus subtilis	+	60	+	65
Lactobacillus spp	+	10	-	0
Lactococcus spp	+	20	+	5
Acinetobacter spp	+	5	+	30
Staphylococcus aureus	+	5	-	0

Keys: + = Present, - = Absent

Table 6. Frequency distribution of fungi isolate in both solid and submerged fermentation

Isolates	Submerged Fermentation	Occurrence (%) in Submerged Fermentation	Solid State Fermentation	Occurrence (%) in Solid state Fermentation
Rhizopus oryzae	+	40	+	50
Penicillium notatum	-	0	+	15
Saccharomyces cerevisiae	-	0	+	15
Rhizopus stolonifer	_	0	+	10
Aspergillus spp	+	30	+	10

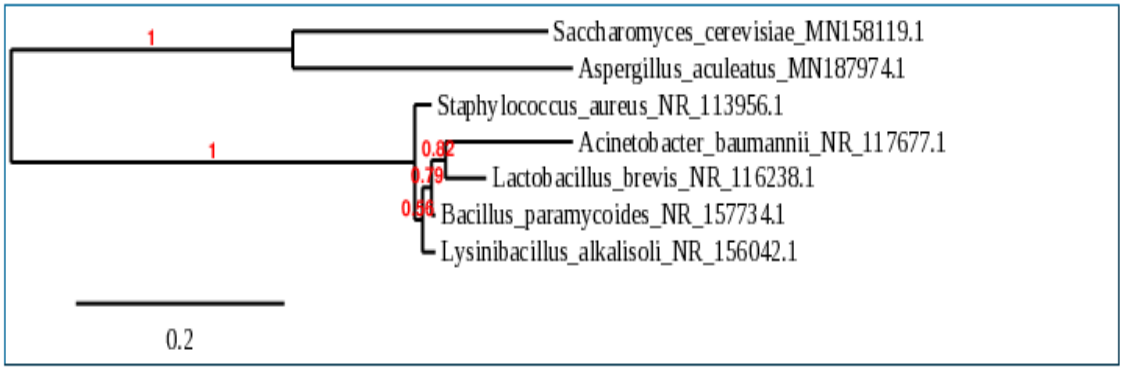

Fig. 1. Phylogenetic tree

Table 7. Molecular identification of isolated bacteria and yeast

Cultural and biochemical identification	Gene sequence identification	Max Identity	Accession number
Bacillus subtilis	*Bacillus paramycoides*	100	NR_157734.1
Lactobacillus spp	*Lactobacillus brevis*	99	NR_116238.1
Lactococcus spp	*Lysinibacillus alkalisoli*	99	NR_156042.1
Acinetobacter spp	*Acinetobacter baumannii*	100	NR_117677.1
Staphylococcus aureus	*Staphylococcus aureus*	99	NR_113956.1
Saccharomyces cerevisiae	*Saccharomyces cerevisiae*	95	MN158119.1

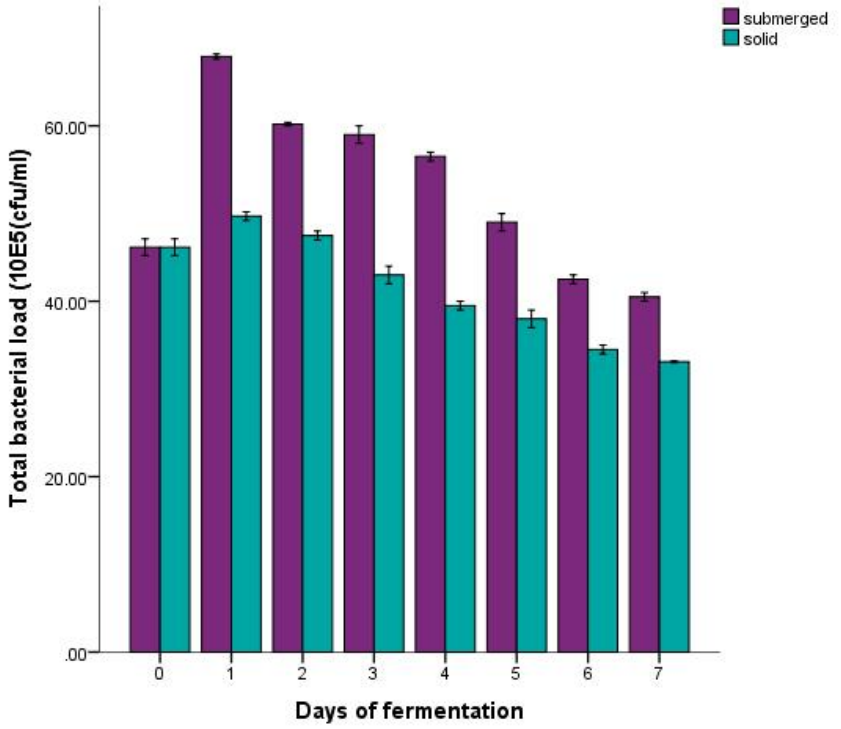

Fig. 2. Bacterial load of isolated organisms from solid and submerged fermentation

3.4 Molecular Identification of Bacterial Isolate

Molecular identification of the bacterial isolates is shown in Table 7. The sequence obtained was analysed with BLAST in National Centre for Biotechnology Information (NCBI) database. Based on the 16SrRNA sequences, the bacteria *Bacillus subtilis, Lactobacillus* spp, *Lactococcus* spp, *Acinetobacter* spp and *Staphylococcus aureus* were confirmed to be *Bacillus paramycoides, Lactobacillus brevis, Lysinibacillus alkalisoli, Acinetobacter baumannii and Staphylococcus aureus* while the fungi isolate *Saccharomyces cerevisiae* and *Aspergillus spp* were confirmed to be *Saccharomyces cerevisiae* and *Aspergillus aculeatus.* The Phylogenetic tree of the organisms isolated is shown in Fig. 1 for bacteria and Fig. 2 for fungi.

3.5 pH and Titratable Acidity of Fermented Broth Cultures

The pH of the submerged fermentation was found to decrease on daily basis while the total titratable acidity showed a drastic increase from day 1 to day 5 (Fig. 5). The pH of the solid state fermentation increased slightly with a slight increase in total titratable acidity (Fig. 6).

Table 8. Antioxidant activity of fermented samples

Techniques/ Free radicals	DPPH (%)	Fe^{2+} (%)
Solid state fermentation	48.14	4.62
Submerged fermentation	32.16	1.00

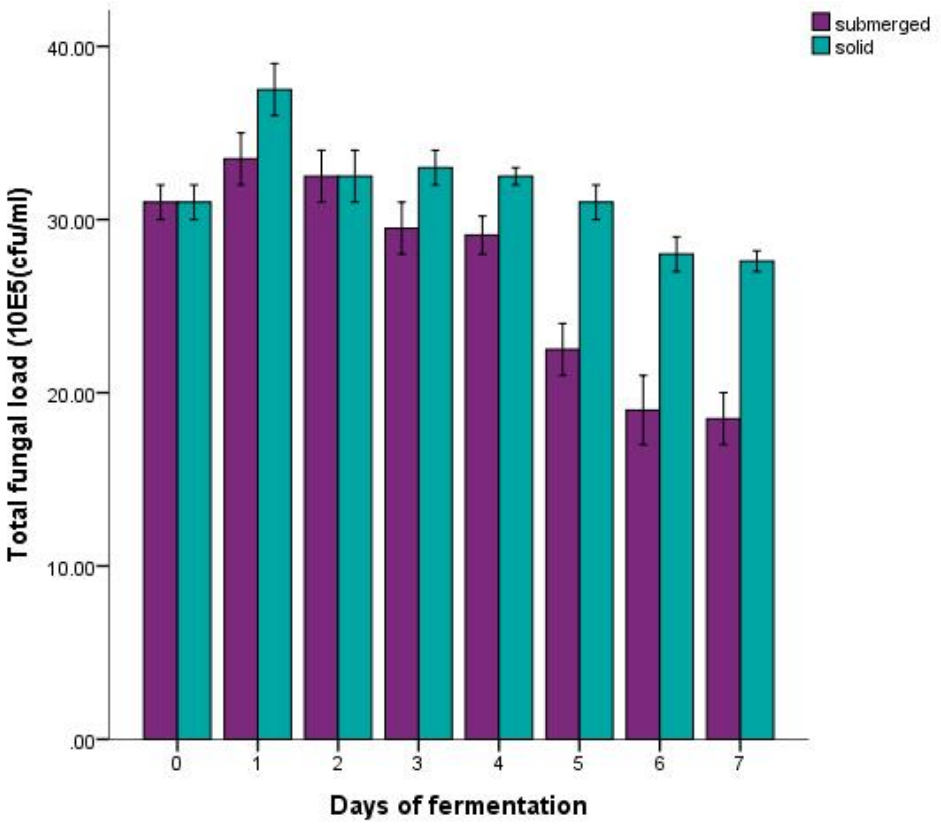

Fig. 3. Fungal load of isolated organisms from solid and submerged fermentation

3.6 Antioxidant Properties of Fermented Green Pea

Table 8 shows the antioxidant activity of fermented samples. The solid state fermented Green pea had the best antioxidant activity against DPPH and Fe^{2+} with scavenging inhibition activity of 48.14% and 4.62% respectively.

3.7 Effect of Fermentations on the Anti-nutrient Composition of Fermented Samples

There was significant reduction $P<0.05$ in the saponin, tannin, oxalate, phytate and alkaloid contents of the fermented samples. Submerged fermentation resulted in the highest reduction from 32.18 mg/g, 4.14 mg/g, 1.62 mg/g, 51.08 mg/g, 36.37 mg/g to 26.27 mg/g, 0.48 mg/g, 0.27 mg/g, 7.82 mg/g and 24.07 mg/ for saponin, tannin, oxalate, phytate and Alkaloid, respectively (Table 9). Fermentation increased the phenol, flavonoid and FRAP contents of the sample. The solid state fermentation resulted in the highest increase from 3.50, 0.03 and 1.41 to 9.32, 0.12 and 9.66 in the for phenol, flavonoid and FRAP contents respectively (Table 9).

4. DISCUSSION

In this study microorganisms associated with the fermentation of green pea were isolated and identified. *B. subtillis* was the most predominant

microorganism present in both solid state and submerged fermentations. *B. subtillis* has proteolytic ability and also possess the capacity to break down oils [14]. It has been associated with fermenting locust bean for iru production [15] and for fermenting soy bean for natto production [16] and this also agrees with the findings of [17] that *Bacillus* species constitute over 95% of the total microbial population density in ugba fermentation. *Lactobacillus lactis* was predominant towards the latter stage of the submerged fermentation probably because of the reduced pH which favours its growth and the ability of *Lactobacillus lactis* to produce lactic acid during fermentation. This might account for the isolation of some organisms during the first and second day of the fermentation which later disappeared towards the end of the fermentation. This agrees with the observation of [14], who reported that *Lactobacillus* produces acid medium during fermentation to inhibit the growth of other microbes that cannot grow in acidic medium. *Rhizopus oryzae* was the predominant fungi present during the submerged and solid state fermentations which could be due to the ability of the organism to produce acidic protease which contribute to its ability to adapt to acidic conditions and its capacity to utilize the high protein content of Green pea.

Molecular techniques are rapid, less laborious, more sensitive, specific and efficient compared to the conventional method [18]. Such technique revealed a difference in cultural identification of some organisms. A similar observation was also reported by [18], who reported differences in conventional method and molecular method of bacteria identification. However, the results of this study demonstrated clearly the interest and feasibility to introduce the 16S rRNA gene sequencing method in identification of bacteria. Combination of conventional techniques and molecular approach will improve bacteriological investigation and authentication, allowing specific and efficient identification of microorganisms as against cultural method that is probable.

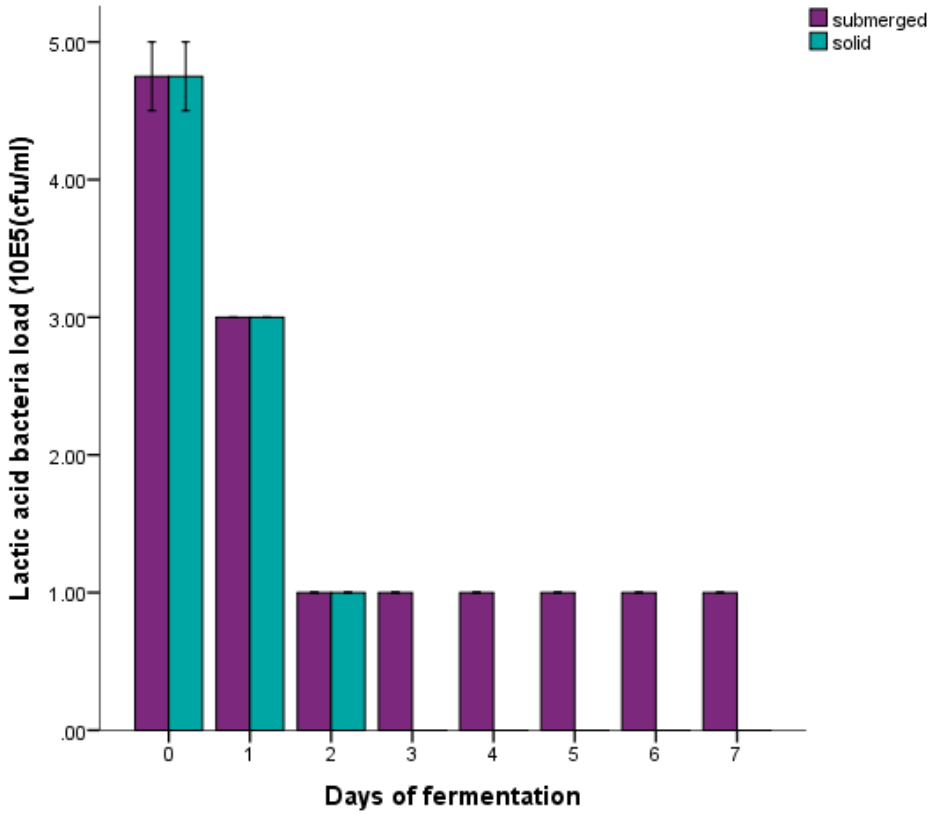

Fig. 4. LAB load of isolated organisms from solid and submerged fermentation

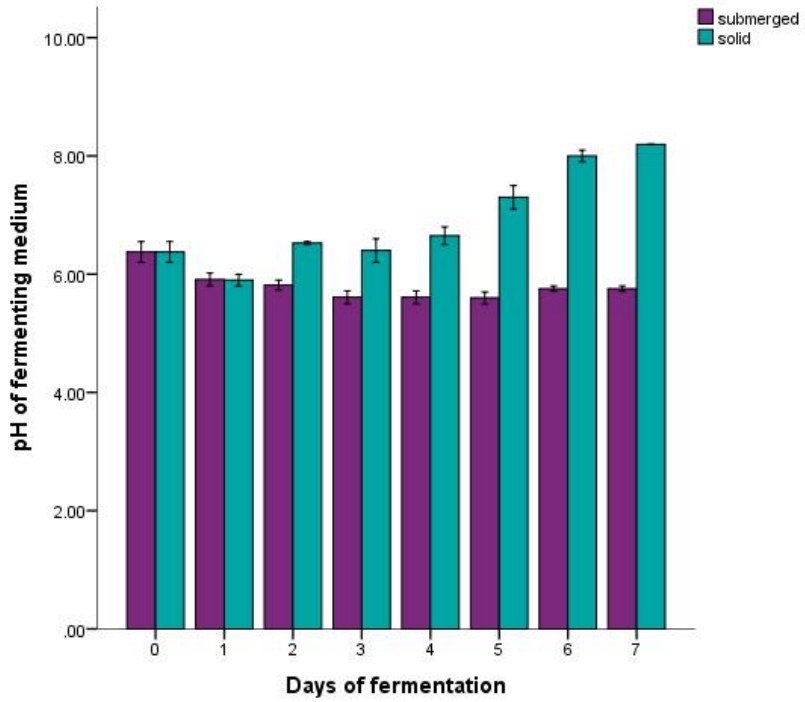

Fig. 5. Effects of fermentation on the pH of fermented samples

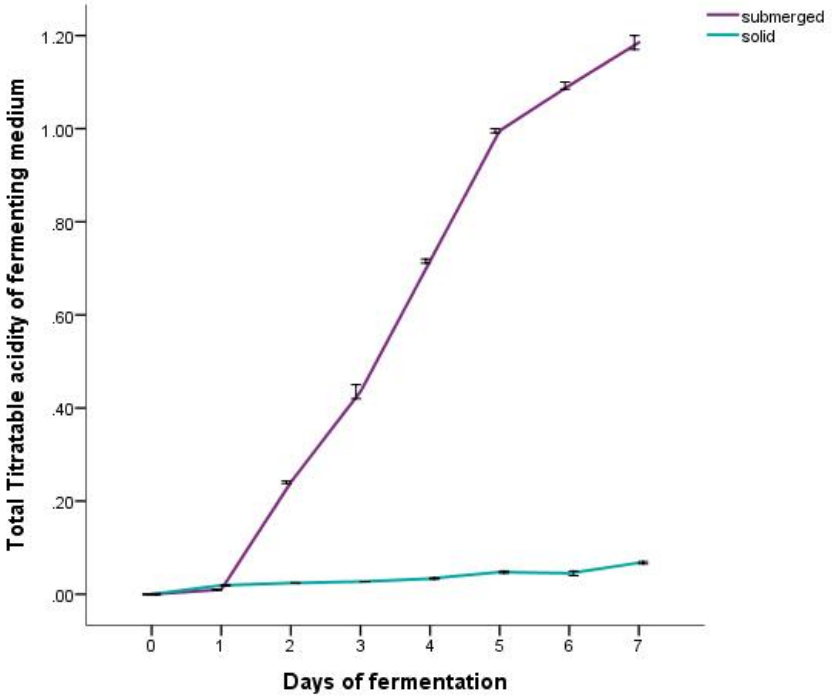

Fig. 6. Effects of fermentation on the total Titratable acidity of fermented samples

Table 9. Effects of fermentation on the Anti-nutrients content of Green pea

Samples	Anti-nutrient (mg/g)							
	Phenol	**Flavonoid**	**FRAP**	**Saponin**	**Tannin**	**Oxalate**	**Phytate**	**Alkaloid**
Raw	3.50±0.06^{a}	0.03±0.00^{a}	1.41±0.03^{a}	32.18±0.36^{b}	4.14±0.01^{c}	1.62±0.09^{c}	51.08±0.00^{c}	36.37±0.62^{b}
Submerged	4.83±0.09^{b}	0.04±0.00^{a}	2.34±0.53^{a}	26.27±0.45^{a}	0.48±0.03^{a}	0.27±0.00^{a}	7.82±0.41^{a}	24.07±0.22^{a}
Solid	9.32±0.09^{c}	0.12±0.03^{b}	9.66±0.64^{b}	23.72±1.36^{a}	0.99±0.02^{b}	0.85±0.04^{b}	40.37±0.82^{b}	32.37±2.12^{b}

Data are presented as Mean ±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05)

The microbial load was observed throughout the seven days of fermentation. The total bacterial (cfu/ml) and fungal count (sfu/ml) of the fermented samples increased on the first day and second day in both submerged and solid state fermentations before decreasing afterward. The increase in count showed that the microorganisms have adapted physiologically to the culture conditions and were in the exponential phase. This agrees with the view of [19] who reported that in a batch culture, after physiological adaptation of microorganisms to culture condition follows an exponential phase. While the subsequent reduction in the bacteria counts observed after the third day of fermentation could be as a result of the accumulation of toxic wastes material, depletion of nutrients and overpopulation of the organism thereby resulting in the ultimate death [20]. The bacteria load is higher in submerged fermentation than solid state fermentation while the fungal count is higher in the solid state fermentation than submerged fermentation. This could be as a result of the fact that submerged fermentation is characterized by very high water activity (the relative humidity of the gaseous phase in equilibrium with the moist solid is significantly) which is not suitable for the growth of fungi as compared to bacteria [21]. There was a drastic decrease of the total lactic acid bacteria during the first two days of the submerged fermentation after which there was a constant growth throughout the remaining fermentation duration. The increase toward the later end of the fermentation could be as a result of the ability of lactic acid bacteria to produce lactic acid, bacteriocins, hydrogen peroxide (H_2O_2) and diacetyl which equipped them with better chance to survive than other organisms [22,23,24]. The antimicrobial effect of lactic acid may be due to its undissociated form capable of penetrating the membrane and liberate hydrogen ion in the neutral cytoplasm thus leading to inhibition of vital cell functions of other organisms [24]. However the further drastic decrease of the total lactic acid count of the solid state fermentation throughout the fermentation period could be due to the alkaline milieu and the low water activity which might not be suitable for the growth of lactic acid bacteria.

The pH of the submerged fermentation was found to decrease on daily basis while the total titratable acidity increased drastically from day 1 to day 5. This may be due to the production of organic acids such as the lactic acid in the samples and this is in agreement with the findings of [7], who reported a decrease in the pH and increase in titratable acidity when checking for the effect of fermented palm wine on some diarrhoeagenic bacteria.

However, the pH of the solid state fermentation increased slightly with a slight increase in total titratable acidity and could be due to the fact that the fermentation is an alkaline fermentation. The temperature of both the solid state and the submerged fermentations increased slightly throughout the seven days of fermentation and could be due to the various metabolic activities of the organisms during the fermentation period.

Anti-nutrients are generally known to reduce nutrients utilization and or food intake [25]. Fermentation reduced the saponin, tannin, oxalate, phytate and alkaloid contents. Submerged fermentation resulted in the highest reduction. This could be due to the fact that some anti-nutrients like tannins are water soluble and also to the ability of some microorganisms to produce enzyme capable of breaking down some of these anti-nutrients e.g. phytases which has the ability of breakdown phytate content [26]. A similar observation was reported by [25] where fermentation resulted in a significant reduction of anti-nutrients. Also, the various arrays of microorganisms which were higher in submerged fermentation as compared to solid state fermentation could explain the highest anti-nutrients reduction recorded in submerged fermentation. Fermentation increased the phenol, flavonoid and FRAP contents of the fermented green pea. The solid state fermentation resulted in the highest increase and according to [27] and [21], It usually results in a higher yield of metabolite as compared to submerged fermentation. This could be the reason why solid state fermented Green pea had a high antioxidant activity against DPPH and Fe^{2+} since phenol, FRAP and flavonoid contents are responsible for the antioxidant activity.

5. CONCLUSION

In this study, it was discovered that both the solid and submerged fermentation significantly reduces the antinutrient composition of the green pea. Although the submerged fermentation was more efficient than the solid fermentations in reducing the anti-nutrient contents of the Green pea. Hence, both methods both methods can be employed to improve the nutritional quality of green pea as human food and medicine. Fermentation also helped to increase the phenol

and FRAP contents of Green pea. The high phenol and FRAP contents could be responsible for the high scavenging activity of fermented green pea against ferric oxide and DPPH observed in this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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The peer review history for this paper can be accessed here:
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