



Changes in Proximate and Antinutrient Contents of Irish Potato Peels Fermented with *Penicillium chrysogenum* and *Bacillus subtilis*

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Authors' contributions

This work was carried out in collaboration among all authors. Author OAO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KA and AOH managed the analyses of the study. Author AOH managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The study was carried out to investigate the effect of fermentation on Irish potato peels using pure strains of *Penicillium chrysogenum* and *Bacillus subtilis*. This was done at Department of Microbiology and Chemistry Department, Federal University of Technology Akure, Ondo State between March 2019 and October 2019. The temperature, pH, and total titratable acidity (TTA) of the fermenting substrates were determined at 24hours interval during fermentation. The proximate composition, anti-nutrient composition, and amylase activity were determined after fermentation using standard methods. There was a decrease in pH with an increase in total titratable acidity in all the samples. The result of the proximate analysis revealed that there was an increase in protein content in the fermented sample of Irish potato peels with *P. chrysogenum* having the highest protein content (20.96±0.14%) followed by the sample fermented with *B. subtilis* (18.23±0.17%) compared to the unfermented samples (14.11±0.11%) respectively. There was an increase in crude fibre and ash contents and a decrease in carbohydrate, fat and energy contents of the fermented samples. The effect of fermentation on the anti-nutritional content showed that there

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was decrease in the content of phytates, phytic acid, oxalate, tannin, phenols, and glycosides. Generally, maximum amylase activity was achieved at 48 hours for sample fermented with *B. subtilis* (132.86 mg/ml) and at 72 hours for sample fermented with *P. chrysogenum* (148.57 mg/ml). The fermented peels could therefore be of use in the production of amylase which has a wide range of applications in industry.

Keywords: Fermentation; starter culture; Irish potato peels; proximate; anti-nutrient; amylase activity.

1. INTRODUCTION

Roots and tubers are important sources of carbohydrates as energy source and are used as staple foods in tropical and subtropical countries [1]. These products have nutritionally beneficial components, such as a resistant starch and mucilage. Resistant starch has been with a slow digestion in the lower parts of the human gastrointestinal tract which results in the slow liberation and absorption of glucose and aids in the reduction of the risk of obesity, diabetes and other related diseases [1]. Whereas mucilage extracted from various tubers and roots has been reported to possess angiotensin conferring enzyme inhibitory and anti-oxidative activities [2, 3]. Also tubers and roots do not contain any gluten, which is an important factor when considering carbohydrate source. With these benefits in mind, examination of the physicochemical properties of a representative tubers and roots crop (Irish potato) was undertaken. The food industry utilizes some tubers and roots crops for their flour and starch products. Such an examination may demonstrate further potential uses within the food industry for the replacement of more traditional forms of carbohydrates or to produce entirely new food products.

Irish potato (*Solanum tuberosum* L.) belongs to the family of *Solanaceae*, it is the world's fourth largest food crop after wheat, rice and maize. The potato (*Solanum tuberosum*) originates from South America, most likely from the central Andes in Peru. The potato was domesticated and has been grown by indigenous farming communities for over 4,000 years. Introduced into Europe in the sixteenth century, the crop subsequently was distributed throughout the world, including Asia. The potato is a major staple fulfilling human nutritional requirements. In many countries potato serves as their staple food because of its excellent nutritional content [4]. There are two common varieties of potatoes; the Irish Potatoes (*Solanum tuberosum*) and the sweet potatoes (*Ipomeabatatas*). *Solanum tuberosum* is commonly called Irish potato in

Nigeria possibly because it was introduced into Nigeria by the Irish and sweet potatoes (*Ipomoea batatas*) are among the tuber crops grown in Nigeria. Irish potato and sweet potato are high starch yielding food crops and have the advantage of being harvested twice a year compared with cassava [5]. Irish potato was introduced into Nigeria early in the 20th Century by European miners in Jos-Plateau. Jos-Plateau has high altitude and thus, cool climate, which is favorable for the development of the crop. Jos South Local Government Area (LGA) accounts for 25% of the total Irish potato produced in Nigeria [6].

Generally, the aims of food technology are to exploit natural food resources as efficiently and profitably as possible [4,7,8]. Fermentation has been defined as a dynamic process during which several catabolic and anabolic reactions proceed simultaneously depending on several conditions including substrate micro flora and environmental factors, which usually result in the breakdown of carbohydrates and other nutrients to give products such as alcohols, acids, amino acids, other metabolites and antibiotics including small amount of energy by microbial enzymes such as bacteria, yeast and filamentous fungi such as *Acetobacter* spp., *Saccharomyces* spp., *Mucor* spp., *Lactobacillus* spp., among others [9,10]. Basically, there are two forms of fermentation; the lactic acid and alcoholic fermentations. In lactic acid fermentation, end products are determined by the conversion of pyruvic acid to lactic acid under anaerobic condition (two molecules of NAD⁺ reduced to NADH). However, in alcoholic fermentation, pyruvic acid is converted to ethanol with carbondioxide released and NADH recycled into NAD⁺ [7]. Fermentation process can generally be classified as aerobic or anaerobic [8]. In many developing countries, food substances ranging from cereals, legumes, root and tubers, etc. are fermented into various consumable products with improved nutritional qualities, shelf life and reduced anti-nutritional factors (phytates and oxalates) [4].

Nutritional value of potatoes is determined by the content of nutrients such as protein, starch, fat,

minerals (potassium, magnesium, sodium, phosphorus, zinc, calcium, iron) [11,12]. Potatoes have been found to be highly nutritious [13]. These nutrients have beneficial influence on human, as they protect against cardiovascular disease, and cancer, as well as reduce blood cholesterol level [14,15,16]. Also dietary fibre, high water content, low total lipid content, and energy (359 kJ) are important nutritional composition in Irish potato and its peels [17]. The fibre is made of cellulose, hemicelluloses, pectins and lignin (constituting so called raw fibre) [18]. Although dietary fibre is not regarded as a bio-active component, it is essential for proper human nutrition. It facilitates digestion by stimulating peristalsis, forms a growth medium for intestinal microflora, and exerts hypoglycemic and hypocholesterolaemic effect [12]. Therefore, it is important to maintain the current, high status of potatoes in our daily diet.

Since Irish potatoes play various roles around the world, the concept of nutritional quality and its contribution must transform to meet specific roles in human diet. Many of the nutrients in potatoes are found in their skin, so more benefits have been attributed to eating them whole as opposed to peeled [17]. The potato peels are rich in phytonutrients, carbohydrates, high in starch (8-28%) but with only about 1-4% protein. Potato starch is a large-grained starch containing 25% amylose and 73% amylopectin and high phosphate content [19].

Amylases constitute a class of industrial enzyme, which alone form approximately 25% of the enzyme market with application in sugar, textile, paper, brewing, distilling industries and pharmaceuticals [20,21]. The production of amylase from the bio-deterioration of selected agro-industrial wastes such as potato peels, cassava peels, using some fungal isolates such as *Mucor mucedo*, *Rhizopus stolonifer*, *Penicillium chrysogenum*, *Saccharomyces cerevisiae* and *Aspergillus* spp., and bacterial isolates such as *Bacillus subtilis*, *Bacillus licheniformis* has also been reported by Akinyosoye et al. [22] and Oboh [23]. Their study revealed that the isolates demonstrated high amylolytic activity on the above listed agro-industrial wastes. Agro-industrial residues are generally considered the best substrates for fermentation process [24]. The three major amylolytic enzymes in Irish potato roots and peels are α -amylase, β -amylase and starch phosphorylase [25]. The properties of amylase in yeast, bacteria, and moulds have been

documented and their evidences reported [26]. The aim of this research is to study the changes in amylase activity, physicochemical properties and proximate composition of fermented Irish potato with starter culture.

2. MATERIALS AND METHODS

2.1 Source of Sample

Fresh Irish potatoes were obtained from Oja-Oba market, Akure, Ondo State, Nigeria. The potatoes were peeled and used for the analysis.

2.2 Preparation and Inoculation of Substrate

The Irish potatoes were washed with sterile distilled water and peeled. Three hundred grams (300 g) of the peels were weighed and poured into a sterile container; 600mls of sterile distilled water was added to moisten it after sterilization in an autoclave at 121°C for 15 minutes. The samples were inoculated with pure cultures of *Bacillus subtilis* (15 ml) and *Penicillium chrysogenum* (15 ml) isolated from the traditional fermentation of Irish potato peels. The first bowl was inoculated with 10 ml culture of *B. subtilis* the second was inoculated with 10 ml culture of *P. chrysogenum*. They were allowed to ferment for 72 hours at room temperature. The fermentation process was terminated after 72 hours of fermentation by drying [27,28].

2.4 Physicochemical Analysis

2.4.1 pH and titratable acidity

The pH and titratable acidity of the fermenting samples was determined using the method described by AOAC [29]. Samples were taken every 24 hours during fermentation period according to the procedure described by Ojokoh et al. [28]. The pH was determined using pH meter (Hanna multi-parameter -H1-9828) equipped with glass electrode. The titratable acidity was determined by titrating 20 ml of the thoroughly mixed sample against 0.1 M NaOH using phenolphthalein as indicator. Values obtained were expressed as percent lactic acid. All analyses were carried out in triplicate.

2.5 Proximate Analysis

The moisture, ash, fat, crude fibre, protein, and carbohydrate content of the sample was analyzed according AOAC [29] after 72 hours of

fermentation. The test was carried out on fermented and unfermented potato peels. Moisture content was determined by drying to constant weight at 105°C in an oven, ash by ignition at 55°C in a muffle furnace, fat content by soxhlet extraction with hexane, nitrogen by micro-Kjedahl and the percentage nitrogen was converted to crude protein by multiplying by 6.25, crude fibre by acid/alkali digestion methods and carbohydrate determined by difference.

2.6 Determination of Anti-Nutrient Composition

The anti-nutrient contents of both fermented and unfermented peels were estimated. Phytate was determined by the method of Ola and Oboh [30]. The tannin content was determined using the [31] method. The oxalate content was determined using [29] method.

2.6.1 Phytate

Phytate was extracted by adding 0.1 g of the sample into 100 ml 0.2 M HCl and shaken for 1 hour before centrifuging at 5000 rpm for 15 minutes. A 0.5 ml of the supernatant was pipetted into a test tube fitted with ground glass stopper before adding 1 ml acidic ammonium iron (3) sulphate dodecahydrate (0.2 g $\text{NH}_4\text{Fe}(\text{SO}_4)_2$).

2.6.2 Tannin

A 200 mg of finely ground sample was weighed into a 50 ml sample bottle. Ten (10) ml of 70% aqueous acetone was added and properly covered. The bottles were put in an ice bath shaker for 2 hours at 30°C. Each solution was then centrifuged and the supernatant stored in ice. A 0.2 ml of each solution was pipetted into test tubes and 0.8 ml of distilled water was added. Standard tannic acid solutions were prepared from a 0.5 mg/ml stock and the solution made up to 1 ml with distilled water. A 0.5 ml of folin reagent was added to both sample and standard followed by 2.5 ml of 20% Na_2CO_3 . The solutions were then vortexed and allowed to incubate for 40 minutes at room temperature after which absorbance was read at 725 nm against a reagent blank concentration of the samples from a standard tannic acid curve.

2.6.3 Oxalate

A 1.0 g of the sample was weighed into 100 ml conical flask. 75 ml of 1.5 N H_2SO_4 was added and the solution was carefully stirred intermittently

with a magnetic stirrer for about 1 hour and then filtered using what man No.1 filter paper. 25 ml of the sample filtrate (extract) was collected and titrated hot (80 – 90°C) against 0.1 NKMnO_4 solution to the point when a faint pink colour appeared that persisted for at least 30 seconds.

2.7 Determination of Amylase Activity

The amylase activity of the samples was determined by 3, 5 – dinitro salicylic acid (DNSA) method using starch as substrate and 0.1 M pH 6.2 citrate-phosphate as buffer. The samples were taken every 24 hours during fermentation process. Blank was also prepared without the enzyme (extract). One unit of amylase enzyme activity was defined as the amount of starch hydrolyzed during 10 minutes incubation at 25°C for 1 ml of solution of extract [32].

2.8 Statistical Analysis

The data obtained were subjected to one-way analysis of variance (ANOVA) while differences in mean were determined using Duncan's New Multiple Range Test (DMRT). All data analyses were done with SPSS 23.0 version.

3. RESULTS

The isolates that were obtained were further screened and identified as *B. subtilis* and *P. chrysogenum*. The pH and temperature of the fermented substrate shows an initial increase and further decrease as the fermentation time increased. The pH of the sample fermented with *B. subtilis* ranged from 6.24 to 5.25 while that of sample fermented with *P. chrysogenum* ranged from 6.05 to 5.52. The total titratable acidity of the sample fermented with *B. subtilis* increased from 0.017 to 0.080 while that of sample fermented with *P. chrysogenum* increased from 0.010 to 0.062. The temperature of sample fermented with *B. subtilis* ranged from 32.8 to 29.7°C while sample fermented with *P. chrysogenum* ranged from 33.0 to 31.8°C as shown in Tables 1, 2 and 3.

The effect of fermentation on the proximate composition of Irish potato peels shows that there was a decrease in the carbohydrate (41.49 ± 0.13 to 11.64 ± 0.09 and 20.44 ± 0.04), fat (9.248 ± 0.06 to 3.446 ± 0.02 and 2.993 ± 0.02) and energy (1287.376 ± 0.40 to 700.420 ± 0.12 and 768.131 ± 0.07) contents i.e. fermented Irish potato peels with *B. subtilis* and *P. chrysogenum* compared with the unfermented Irish potato

peels while there was an increased in the protein (14.11 ± 0.11 to 22.06 ± 0.09 and 18.23 ± 0.17), ash (6.398 ± 0.28 to 7.95 ± 0.20 and 8.71 ± 0.10) and crude fiber (16.27 ± 0.04 to 27.84 ± 0.40 and 37.72 ± 0.17) contents of the fermented Irish potato peels respectively as shown in Table 4.

The anti-nutrients analyzed indicate that phytate content of the fermented Irish potato peels with *B. subtilis* decreased from 10.71 ± 0.04 to 5.56 ± 0.07 and the fermented Irish potato peels with *P. chrysogenum* decreased from 10.71 ± 0.04 to 5.77 ± 0.01 . Oxalate content of the fermented Irish potato peels with *B. subtilis* shows a great decrease from 24.76 ± 0.02 to 13.59 ± 0.04 and the fermented Irish potato peels with *P.*

chrysogenum decreased from 24.76 ± 0.02 to 19.36 ± 0.19 . A slight decrease was observed in the tannin content of fermented Irish potato peels with *B. subtilis* from 1.60 ± 0.02 to 0.33 ± 0.02 and fermented Irish potato peels with *P. chrysogenum* decreases from 1.6018 ± 0.015 to 1.49 ± 0.02 respectively as shown in Table 5.

The concentration of amylase activity increases for both Irish potato peels fermented with *B. subtilis* and *P. chrysogenum* throughout the fermentation period. Fermented Irish potato peels with *B. subtilis* increases from 11.43 to 117.14 and fermented Irish potato peels with *P. chrysogenum* increases from 8.57 to 148.57 as shown in Table 6.

Table 1. Change in pH during the controlled fermentation of Irish potato peels

| Time (hr) | Fermented Irish potato peels using <i>Bacillus subtilis</i> | Fermented Irish potato peels using <i>Penicillium chrysogenum</i> |
|-----------|---|---|
| 0 | 7.09 | 6.24 |
| 24 | 6.17 | 5.98 |
| 48 | 5.54 | 5.62 |
| 72 | 5.25 | 5.52 |

Table 2. Changes in total titratable acidity during the controlled fermentation of Irish potato

| Time (hr) | Fermented Irish potato peels using <i>Bacillus subtilis</i> (mEq/L) | Fermented Irish potato peels using <i>Penicillium chrysogenum</i> (mEq/L) |
|-----------|---|---|
| 0 | 0.017 | 0.010 |
| 24 | 0.054 | 0.028 |
| 48 | 0.077 | 0.053 |
| 72 | 0.080 | 0.062 |

Table 3. Changes in temperature during the controlled fermentation of Irish potato peels

| Time (hr) | Fermented Irish potato peels using <i>Bacillus subtilis</i> (°C) | Fermented Irish potato peels using <i>Penicillium chrysogenum</i> (°C) |
|-----------|--|--|
| 0 | 32.8 | 33.0 |
| 24 | 32.4 | 32.8 |
| 48 | 32.0 | 32.3 |
| 72 | 29.7 | 31.8 |

Table 4. Effect of fermentation on the proximate composition of Irish potato peels

| Proximate composition | Unfermented Irish potato peels | Fermented Irish potato peels using <i>Bacillus subtilis</i> | Fermented Irish potato peels using <i>Penicillium chrysogenum</i> |
|-----------------------|--------------------------------|---|---|
| Ash content | 6.398 ± 0.284 | 7.953 ± 0.201 | 8.712 ± 0.101 |
| Moisture content | 12.480 ± 0.066 | 27.060 ± 0.130 | 11.908 ± 0.081 |
| Fat content | 9.248 ± 0.055 | 3.446 ± 0.016 | 2.993 ± 0.023 |
| Crude fibre content | 16.274 ± 0.037 | 27.840 ± 0.402 | 37.717 ± 0.169 |
| Protein content | 14.113 ± 0.112 | 22.064 ± 0.129 | 18.229 ± 0.166 |
| Carbohydrate content | 41.487 ± 0.125 | 11.637 ± 0.099 | 20.441 ± 0.041 |
| Energy value (KJ/g) | 1287.376 ± 0.402 | 700.420 ± 0.119 | 768.131 ± 0.074 |

Error bars: +/- 2 SE

Table 5. Effect of fermentation on the antinutrient composition of Irish potato peels

| Antinutrient composition | Fermented Irish potato peels using <i>Bacillus subtilis</i> | Fermented Irish potato peels using <i>Penicillium chrysogenum</i> | Unfermented Irish potato peels |
|--------------------------|---|---|--------------------------------|
| Phytates (mg/g) | 5.562±0.070 | 5.768±0.013 | 10.712±0.036 |
| Phytic acid (mg/g) | 1.567±0.011 | 1.607±0.102 | 3.013±0.016 |
| Oxalates (mg/g) | 13.596±0.040 | 19.359±0.190 | 24.761±0.023 |
| Tannins (mg/g) | 0.328±0.015 | 1.496±0.016 | 1.6018±0.015 |

Error bars: +/- 2 SE

Table 6. Effect of fermentation on the concentration of amylase produced by Irish potato peels

| Time (hr) | Fermented Irish potato peels using <i>Bacillus subtilis</i> (mg/ml) | Fermented Irish potato peels using <i>Penicillium chrysogenum</i> (mg/ml) |
|-----------|---|---|
| 0 | 11.43 | 8.57 |
| 24 | 97.00 | 40.00 |
| 48 | 132.86 | 130.00 |
| 72 | 117.14 | 148.57 |

4. DISCUSSION

Fermentation lowered the pH of Irish potato peels fermented with *B. subtilis* from 6.24 to 5.25 and fermented Irish potato peels with *P. chrysogenum* 6.05 to 5.52 this indicate a greater mobility and activity of fermentation microorganisms as they feed on the carbohydrates of potato peels with subsequent release of organic acids. The acidification increased with increasing period of fermentation due to increased production of titratable acidity [22,23,27].

The proximate composition (%) of the Irish potato peels after fermentation varied. The fermented samples with *P. chrysogenum* showed the highest protein content (20.963±0.14) followed by the sample fermented with *B. subtilis* (18.229±0.17) compared with the unfermented sample (14.113±0.112). The increase in protein may be due to the activities of the microbial strains which might have secreted some extracellular enzymes (protein) [28]. Also, fungal fermentation has been reported by Oladebeye et al. [5] to increase protein content of starch substances. Decrease in fat content was observed for the fermented samples from 9.248±0.055 to 2.993±0.02 for *B. subtilis* and 3.395±0.05 for *P. chrysogenum* samples. Decrease in the percentage of carbohydrates from 41.487±0.13 to 20.441±0.04 and 21.625±0.23 and energy value from 1287.376±0.40 to 768.131±0.07 and 850.070±0.09 is an advantage because low carbohydrate content food is medically

recommended for diabetic (a major health problem in Africa) patients [12,15]. Increase in the percentage of ash content, moisture content, crude fibre content could be as a result of production of enzymes during growth which is essential for human nutrition as reported by [18]. The decrease and increase in the nutritional contents of Irish potato peels can also be linked to their utilization by microbes and production of metabolites by microorganisms during the fermentation process.

Anti-nutrients are compounds which affect the nutritive values of food products such as phytates, phytic acid, oxalates, and tannins. Before fermentation, the composition of anti-nutrient content was high in the sample but after fermentation, a decrease was observed. Phytates decrease from 10.712±0.04 to 5.768±0.01 in Irish potato peels fermented with *B. subtilis* and 5.665±0.01 for fermented Irish potato peels with *P. chrysogenum*. Phytate like oxalate are known to limit the availability of calcium, magnesium, iron and phosphorous and formation of insoluble compounds or salt with the minerals. Also, it has been reported Brown [19] and Lachman et al. [33] that phytate in foods have beneficial effects to the body as it contains antioxidants, a type of phytochemical that helps to eliminate free radicals from the body system and at the same time prevents and heal the body system from dangerous diseases.

Amylase produced during the fermentation of the samples increases in its concentration as the fermentation time increased. Maximum

concentration of alpha amylase was observed to reach its peak at 48 hours for fermented Irish potato peels with *B. subtilis* (132.86 mg/ml) and started to drop at increase in fermentation time. Sample fermented with *P. chrysogenum* was 148.57 mg/ml at 72 hours. The initial increase in the concentration of enzyme activity with increasing fermentation days agrees with the theory of microbial growth phase. This signifies that bacteria and fungi used are capable of breaking down soluble starch to simple sugar [22].

5. CONCLUSION

This study reveals that the fermentation of food wastes using starter cultures show improved characteristics such as improved nutritional quality, amylase activity and reduced anti-nutrients. The results obtained in this study have shown that fermentation of Irish potato peels with *B. subtilis* and *P. chrysogenum* can greatly enrich its protein content. In view of this significant protein yield increase, as well as decrease in anti-nutrient content, this by-product could be a good supplement in compounding animal feed provided that it is acceptable and highly digestible.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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