

## Effects of Treatments and Fermentation Time on Phenolic Compounds, Glycoalkaloid Contents, and Antioxidant Capacity of Industrial Potato Waste

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### ABSTRACT

Potato processing plants generate waste in the form of peels, pulp, and rejects, which is estimated to be around 12–20 % of their total production volume. Potato peels, pulp, and unmarketable potatoes can be processed and incorporated into animal feed formulations. However, there is a limited information on phenolic compounds from industrial potato waste (IPW) subjected to short-term solid-state fermentation. Bioactive compounds could be improved via solid-state fermentation. *Lactiplantibacillus plantarum* (MW296876), *Saccharomyces cerevisiae* (MW296931), and *Aspergillus oryzae* (MW297015) were purposely selected to ferment IPW at 0, 24, 48, and 72 hr in a two-factor factorial design (treatment × fermentation time). The fermented products were analysed for phytochemical compounds such as total phenolic content (TPC), total flavonoid content (TFC), glycoalkaloid (GLA) content, and antioxidant capacity. The results revealed that the bioactive compounds, except phytic acid, had a significant interaction between treatment and fermentation time. Alpha solanine significantly ( $p < 0.05$ ) decreased while  $\alpha$  chaconine

increased ( $p < 0.05$ ) with fermentation time across all the treatments except in the control and *L. plantarum* treatment groups. IPW inoculated with *L. plantarum* significantly influenced the solubility of GLA compared to other treatment groups. Antioxidant capacity increased ( $p < 0.05$ ) across the fermentation time; at 48 hr of fermentation, *L. plantarum* had the highest ( $p < 0.05$ )

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antioxidant capacity than *S. cerevisiae* and *A. oryzae*. Among the three inocula, *L. plantarum* (MW296876) consistently increased TPC, antioxidant activity, and solubility of both GLA and tannin.

*Keywords:* Antioxidants, fermentation, glycoalkaloids, phytochemical compounds, potato waste

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## INTRODUCTION

Potato (*Solanum tuberosum* L.) contains vitamins, minerals, proteins, dietary fibre, and phytochemical compounds such as phenolic acids, flavonoids, and carotenoids (Cebulak et al., 2022). Phytochemicals are the largest group of phenolic compounds that account for most antioxidant activity in plants or plant products. Phytochemicals in potatoes are bioactive compounds that have wide effects on animal well-being (Hellmann et al., 2021).

The concentration of phenolic components varied in different scientific reports; chlorogenic acid has always been reported as the most abundant (Valiñas et al., 2017). It was reported that chlorogenic acid represents 90% of the total phenolic compounds in potato peels (Hellmann et al., 2021; Naveed et al., 2018). Other important phenolic acids include caffeic, ferulic, gallic, and syringic acids. The variation in concentration of phenolic acids is related to genotypes, extraction, and analytical methods (Akyol et al., 2016). It was reported that phenolic acids and flavonoids are the most abundant phenolic compounds, which are present in both free and bound forms (Ru et al., 2019) and are located in the peels and

adjoining tissues (Akyol et al., 2016; Yılmaz et al., 2017).

The presence of flavonoids influences the flavour and colour of fruits (Akyol et al., 2016). Some important flavonoids in potatoes include catechin, quercetin, anthocyanins, and kaempferol (Kim et al., 2019). In addition, some researchers observed the presence of rutin (Rodríguez-Martínez et al., 2021). Depending on the skin colour, the concentration of flavonoid, on a fresh-weight basis, ranges between 30 to 60 mg/100 g (Akyol et al., 2016). The higher content of flavonoids in red and purple potatoes is due to the higher content of anthocyanins (Frond et al., 2019).

In the past decades, phenolic compounds have been considered antinutrients; however, in recent times, they have been believed to be micronutrients with a high antioxidant capacity (Peluso, 2019). Antioxidants have wide bioactive properties, including prevention of oxidation in food materials, antimicrobial activities, and neutralising free radicals produced within the body (Hur et al., 2014).

Over 80 different steroidal glycoalkaloids (SGLAs) have been identified in potatoes, but  $\alpha$ -solanine and  $\alpha$ -chaconine are by far the most predominant (Kondamudi et al., 2017). However, concentrations of glycoalkaloids are higher in the potato peel than in the flesh (Ok & Şanlı, 2022). It was reported that GLA contents in potatoes are influenced by variety, postharvest storage conditions, and the type of fertiliser applied to soil (Ok & Şanlı, 2022). In general, GLA is heat stable and resistant to degradation via exposure to

boiling water, baking, frying, and microwave irradiation (Omayio et al., 2016). It was reported that the toxicity of  $\alpha$ -chaconine is higher than  $\alpha$ -solanine. However, both have a synergistic effect when present in the same tissue; the severity of their toxicity depends on their concentrations and ratio, which ranges from 1:2 to 1:7. It was observed that after peeling, slicing and washing out in water,  $\alpha$ -solanine content decreased more than  $\alpha$ -chaconine (Omayio et al., 2016).

Despite the fact that potatoes and their by-products contain GLA, previous studies show that it is being used in animal diets in the forms of fresh, dried, steamed, and ensiled (Ncobela et al., 2017). Similarly, it was reported that potatoes or their by-products could replace 10 to 40% of corn in beef cattle diets without any deleterious effects on animal growth, health, or meat quality (Paradhipta et al., 2020). Although GLAs are widely considered toxic substances, some recent studies have reported that they contain various health-promoting properties, which include a strong antioxidant capacity and anti-cancer activities (Hellmann et al., 2021; Wu et al., 2018).

Depending on the processing method and variety of potatoes, potato waste can range between 15 to 40% of the original fresh weight (Sepelev & Galoburda, 2015). However, local production of potatoes stands at 215,632.40 t in 2022, with a potential yield of 8.3 t/ha (Department of Agriculture [DOA], 2022). According to the Food and Agriculture Organization of the United Nations (FAO) (2023), Malaysia imported 217,636.81 t of potatoes in 2021 (FAO,

2023). The combined volumes of potatoes (local and import) could generate 64,99.38 to 173,307.68 t of waste (peels/skin/low grades and rejected potatoes) from processing plants, households, and restaurants. The higher composition of peels in the IPW virtually increased its phenolic contents than the tuber fraction. Nevertheless, few studies have been conducted on IPW as a potential source of bioactive compounds for ruminant animals, especially when subjected to microbial inoculation and short-term solid-state fermentation.

The objective of this study was to determine phenolic compounds, glycoalkaloid compounds, and antioxidant capacity of industrial potato waste fermented with *L. plantarum* (MW296876), *S. cerevisiae* (MW296931), and *A. oryzae* (MW297015). Although IPW could be stored fresh for a couple of months under controlled temperatures with good ventilation, such a process is economically impractical. Alternatively, IPW could be preserved longer by ensiling alone or in a mixture of straws or grasses. On the other hand, IPW could be processed by short-term fermentation via a solid-state fermentation method, which lasts for a couple of days (< 7 days). The major limitation of the longer ensiling method is the duration of time ( $\geq 21$  days) before the substrate reaches a stable anaerobic condition. A short-term fermentation provides an opportunity to quickly improve the nutrient and phenolic content of feedstuffs as well as reduce antinutrient factors (ANF) within a few days (24-96 hr). Afterwards, the feedstuff

could be dried to ensure a longer shelf-life before feeding or incorporating it into a ruminant diet.

## MATERIALS AND METHODS

### Fermentation of Substrate

About 100 kg of IPW was collected from French Fries (Malaysia) Sdn. Bhd., a local potato processing company in Malaysia. The IPW was then oven-dried at 65°C, cooled at room temperature (~ 28°C), milled, sieved to pass 1.0 mm and preserved in a cold room (4°C) until required for solid-state fermentation. A short-term IPW (substrate) fermentation was carried out using a method described by Aruna et al. (2017). Hence, about 100 g dry matter (DM) of the sample (substrate) was placed in a 250 ml Erlenmeyer flask. The moisture content was then adjusted to 60% by adding 140 ml of water, and then the flask was covered with a thin layer of paraffin. Thus, eighty samples were prepared and divided into four treatment groups. The first treatment group (control) was not inoculated with any microbe. In contrast, the remaining three treatment groups were inoculated with *L. plantarum* (MW296876) at  $1 \times 10^5$ /g (Abdul Rahman et al., 2017), *S. cerevisiae* (MW296931) at  $1 \times 10^5$ /g (Abdul Rahman et al., 2017), and *A. oryzae* (MW297015) at about 1 cm<sup>2</sup> of corresponding fungus per 50 g substrate (Ramin et al., 2011). Isolates of the three inoculants were initially obtained from the Malaysian Agricultural Research and Development Institute (MARDI). After the inoculation, five replicates from each treatment were randomly subjected

to fermentation times of 0, 24, 48, and 72 hr. The incubation temperature was maintained at 35°C inside the incubator (LM-450D, BIOBASE, China). Termination of fermentation was carried out by increasing the incubation temperature to 65°C until a constant weight of substrate was observed. The inoculated substrates were then carefully removed from the Erlenmeyer flask and preserved in a refrigerator until chemical analyses were required.

### Chemical Assays

Bioactive compounds were extracted from IPW using a method described by Ji et al. (2012). Following the procedure described by Ji et al. (2012), TPC was determined by the Folin-ciocalteu method, antioxidant activity was assayed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, and glycoalkaloid (GLA) content was also determined via high-performance liquid chromatography (HPLC). A simple ratio between solanine and chaconine was calculated by dividing chaconine concentration by solanine content. Furthermore, TFC was determined using the aluminium chloride (AlCl<sub>3</sub>) method described by Sulaiman and Balachandran (2012). Phytic acid content (mg/g) was determined by the iron (III) chloride (FeCl<sub>3</sub>) method described by Adeyemo and Orilude (2013). Tannin and carotenoid contents were analysed using the procedure described by Ru et al. (2019).

The extracts of fermented samples (Ji et al., 2012) were proceeded for GLA assay via HPLC (Ultimate 3000, Thermo

Fisher Scientific, USA) with ultraviolet to visible (UV-Vis) detection at 200 nm wavelength. Standard solutions containing both  $\alpha$ -chaconine (PHL80075, Sigma-Aldrich, USA) and  $\alpha$ -solanine (PHL80074, Sigma-Aldrich, USA) were prepared from stock solutions. A five-point standard curve was made for each compound with a linear range between 1 to 100 ppm ( $\mu\text{g/ml}$ ). Glycoalkaloid compounds were separated by injecting 20  $\mu\text{l}$  of the prepared sample across Siliachrome column (4.6 mm  $\times$  250 mm  $\times$  5  $\mu\text{m}$ ) (Siliachrome Plus C18, Canada) after the column temperature was adjusted to 25°C. A mixture (v/v) of acetonitrile (30%, Sigma-Aldrich, USA) and 0.05 M monobasic ammonium phosphate buffer (70%, ACS reagent, Sigma-Aldrich, USA) with pH 6.5 was used as a mobile phase at a flow rate of 1.0 ml/min. The GLA results were reported as mg per gram of dry sample.

### Research Design and Statistical Analysis

IPW collected from processing plants was fermented with zero inoculants, *L. plantarum* (MW296876), *S. cerevisiae* (MW296931), and *A. oryzae* (MW297015) for 0, 24, 48, and 72 hr. The experiment's layout was a two-factor factorial design (treatment  $\times$  fermentation time), with fermentation time randomly allotted to the treatment in a completely randomised design (CRD).

Data obtained were subjected to analysis of variance (ANOVA) using a general linear model (GLM) of SAS software version 9.4 (SAS, 2011). The model of the analysis was as follows:

$$Y_{ijr} = \mu + t_i + d_j + (td)_{ij} + e_{ijr}$$

where,  $Y_{ijr}$  = dependent variable/observation;  $\mu$  = overall population mean;  $t_i$  = effect of treatment;  $d_j$  = effect of fermentation time;  $(td)_{ij}$  = effect of interaction between treatment and fermentation time;  $e_{ijr}$  = random error/residual

Means were separated using Duncan's multiple range tests (Duncan, 1955).

## RESULTS

### Total Phenolic, Flavonoid, and Carotenoid Contents

There was significant ( $p < 0.05$ ) interaction between treatment and time of fermentation across all the treatments (Table 1). The TPC of fermented IPW shows an increased value with increasing fermentation time across the treatments. Both fermented IPW with *L. plantarum* and *A. oryzae* recorded the highest TPC at 72 hr of fermentation, while *S. cerevisiae* recorded the highest TPC at 48 hr. At 72 hr of fermentation, fermented IPW with *L. plantarum* had higher ( $p < 0.05$ ) TPC than another treatment group.

There was a significant ( $p < 0.05$ ) interaction between treatment and time of fermentation for fermented IPW with the three different inoculants. However, no significant ( $p > 0.05$ ) difference was observed in total flavonoid contents across the treatments. Fermented IPW with *S. cerevisiae* and *A. oryzae* inevitably showed a significant ( $p < 0.05$ ) difference across the fermentation time. In these two treatments, it was observed that total flavonoid content significantly ( $p < 0.05$ ) increased with the

Table 1  
*Total phenolic, flavonoid, and carotenoid contents of fermented industrial potato waste with Lactiplantibacillus plantarum, Saccharomyces cerevisiae, and Aspergillus oryzae inoculants at different time*

Parameters	Time (hr)	Treatments				p-value	
		Control	<i>Lactiplantibacillus plantarum</i>	<i>Saccharomyces cerevisiae</i>	<i>Aspergillus oryzae</i>	Treatment	Treatment*Time
Total phenolic content (mg/g)	0	48.51±0.12 <sup>d</sup>	48.23±0.06 <sup>d</sup>	48.32±0.17 <sup>d</sup>	48.32±0.19 <sup>c</sup>	0.5811	<.0001
	24	73.02±0.36 <sup>ab</sup>	77.01±0.36 <sup>ca</sup>	33.90±0.28 <sup>cd</sup>	47.43±0.44 <sup>cc</sup>	<.0001	<.0001
	48	90.16±0.33 <sup>ba</sup>	82.74±0.48 <sup>bc</sup>	86.50±1.05 <sup>ab</sup>	59.78±0.57 <sup>bd</sup>	<.0001	<.0001
	72	98.47±0.38 <sup>ab</sup>	151.53±0.29 <sup>ba</sup>	81.71±0.54 <sup>bc</sup>	67.90±0.55 <sup>ad</sup>	<.0001	<.0001
	p-value	<.0001	<.0001	<.0001	<.0001		
Total flavonoid content (mg QE/g)	0	14.25±0.66	11.87±2.20	9.71±0.46 <sup>b</sup>	12.56±2.20 <sup>b</sup>	0.2889	0.0387
	24	16.24±0.79	13.48±1.35	12.18±1.63 <sup>b</sup>	16.65±1.60 <sup>ab</sup>	0.1018	0.0387
	48	17.30±1.70	14.28±1.55	17.87±1.38 <sup>a</sup>	13.44±1.24 <sup>b</sup>	0.1286	0.0387
	72	14.99±2.89	17.23±1.20	19.16±0.79 <sup>a</sup>	19.42±0.99 <sup>a</sup>	0.2585	0.0387
	p-value	0.6294	0.1670	<.0001	0.0274		
Total carotenoid content (mg/kg DW)	0	96.04±1.74 <sup>a</sup>	96.04±1.74 <sup>a</sup>	94.59±1.28 <sup>a</sup>	97.01±1.67 <sup>a</sup>	0.7721	<.0001
	24	39.10±14.57 <sup>bab</sup>	65.15±6.53 <sup>ba</sup>	7.72±1.286 <sup>c</sup>	23.65±8.78 <sup>bbc</sup>	0.0118	<.0001
	48	8.20±1.74 <sup>cb</sup>	19.79±4.21 <sup>ca</sup>	10.62±0.96 <sup>bb</sup>	25.58±1.28 <sup>ba</sup>	0.0031	<.0001
	72	9.65±1.28 <sup>c</sup>	10.62±0.48 <sup>c</sup>	10.62±2.94 <sup>b</sup>	8.21±0.97 <sup>c</sup>	0.7206	<.0001
	p-value	<.0001	<.0001	<.0001	<.0001		

Note. A, B, C, D mean values with different superscripts within the same row were significantly different at  $p < 0.05$ ; a, b, c mean values with different superscripts within the same column were significantly different at  $p < 0.05$ ; DW = Dry weight



fermentation time; the highest values were recorded between 48 and 72 hr of fermentation.

The results on total carotenoid content (TCC) show a significant ( $p<0.05$ ) interaction between treatment and fermentation time. No difference ( $p>0.05$ ) in carotenoid content was observed between treatments after 48 hr of fermentation. It was observed that the carotenoid content of fermented IPW decreased significantly ( $p<0.05$ ) with fermentation time across all the treatments. At 72 hr of fermentation, the carotenoid content was similar ( $p>0.05$ ) across all the treatments.

### Tannin Content of Fermented

The tannin content presented in Table 2 shows a significant ( $p<0.05$ ) interaction between treatment and fermentation time. It was also observed that the tannin content of all fermented IPW increased ( $p<0.05$ ) over the fermentation time. At 24 hr of fermentation, fermented IPW with *S. cerevisiae* produced the highest ( $p<0.05$ ) tannin content than the other treatments and the control. However, at 48 hr, the tannin content of fermented IPW with *S. cerevisiae* was at par ( $p>0.05$ ) with *L. plantarum* and the control treatments, which were statistically higher ( $p<0.05$ ) than *A. oryzae*.

### Glycoalkaloid Content

The concentration of  $\alpha$ -solanine (Table 2) decreased ( $p<0.05$ ) linearly with fermentation time across all treatments except for the control. Fermented IPW with *L. plantarum* recorded a significant ( $p<0.05$ )

increase in  $\alpha$ -solanine content. Invariably, at 72 hr of fermentation, fermented IPW with *S. cerevisiae* and *A. oryzae* inoculants recorded significantly ( $p<0.05$ ) least  $\alpha$ -solanine content.

The chaconine content ( $p<0.05$ ) significantly increased with the fermentation time across all treatments. A concentration of 0.138 mg/g chaconine was recorded for unfermented IPW. At 72 hr of fermentation, fermented IPW with *L. plantarum* recorded ( $p<0.05$ ) higher concentration (0.479 mg/g) than all other treatments. However, 0.450 mg/g recorded for the control was significantly ( $p<0.05$ ) higher than 0.254 and 0.298 mg/g observed for fermented IPW with *S. cerevisiae* and *A. oryzae*, respectively.

Across all the treatments, except for fermented IPW with *A. oryzae*, which increased to 1:5 at 24 hr of fermentation, the ratios of  $\alpha$  solanine to  $\alpha$  chaconine (Table 3) varied from 1:2 at 0 hr to 1:3 at 24 and 48 hr. At 72 hr, the concentration of  $\alpha$ -chaconine in the control and fermented IPW with *L. plantarum* decreased to 1:1. However, fermented IPW with *S. cerevisiae* and *A. oryzae* recorded an increase in  $\alpha$  chaconine content with a ratio of 1:4.

### Phytic Acid Content

The result on phytic acid content (Table 4) shows no significant ( $p>0.05$ ) interaction between treatments and time of fermentation. No significant ( $p>0.05$ ) difference was observed across the treatments except at 48 hr, where the control recorded the highest concentration of phytic acid than

Table 2  
*Tannin, solanine, and chaconine contents of fermented industrial potato waste with Lactiplantibacillus plantarum, Saccharomyces cerevisiae, and Aspergillus oryzae inoculants at different time*

Parameters	Time (hr)	Treatments			p-value		
		Control	<i>Lactiplantibacillus plantarum</i>	<i>Saccharomyces cerevisiae</i>	<i>Aspergillus oryzae</i>	Treatment	Treatment*Time
Tannin (mg/g)	0	0.47±0.02	0.47±0.01 <sup>c</sup>	0.47±0.02 <sup>c</sup>	0.47±0.02 <sup>a</sup>	0.9996	<.0001
	24	0.53±0.02 <sup>c</sup>	0.75±0.02 <sup>bb</sup>	1.06±0.03 <sup>aA</sup>	0.15±0.01 <sup>cd</sup>	<.0001	<.0001
	48	0.47±0.03 <sup>A</sup>	0.49±0.02 <sup>eA</sup>	0.43±0.01 <sup>eA</sup>	0.27±0.00 <sup>bb</sup>	<.0001	<.0001
	72	0.47±0.02 <sup>C</sup>	1.20±0.01 <sup>aA</sup>	0.83±0.02 <sup>bb</sup>	0.41±0.04 <sup>aC</sup>	<.0001	<.0001
	<i>p</i> -value	0.1858	<.0001	<.0001	<.0001		
$\alpha$ -solanine (mg/g)	0	0.094 <sup>b</sup>	0.094 <sup>b</sup>	0.094 <sup>a</sup>	0.094 <sup>a</sup>	1.0000	<.0001
	24	0.080 <sup>dA</sup>	0.079 <sup>dA</sup>	0.062 <sup>dB</sup>	0.051 <sup>dC</sup>	<.0001	<.0001
	48	0.085 <sup>eA</sup>	0.072 <sup>cC</sup>	0.067 <sup>bd</sup>	0.079 <sup>bb</sup>	<.0001	<.0001
	72	0.373 <sup>ab</sup>	0.382 <sup>aA</sup>	0.065 <sup>cd</sup>	0.074 <sup>cC</sup>	<.0001	<.0001
	<i>p</i> -value	<.0001	<.0001	<.0001	<.0001		
$\alpha$ -chaconine (mg/g)	0	0.138 <sup>d</sup>	0.138 <sup>d</sup>	0.138 <sup>d</sup>	0.138 <sup>d</sup>	1.0000	<.0001
	24	0.263 <sup>eA</sup>	0.239 <sup>eB</sup>	0.159 <sup>cd</sup>	0.237 <sup>cC</sup>	<.0001	<.0001
	48	0.211 <sup>bd</sup>	0.242 <sup>bb</sup>	0.227 <sup>bc</sup>	0.249 <sup>bA</sup>	<.0001	<.0001
	72	0.451 <sup>ab</sup>	0.481 <sup>aA</sup>	0.255 <sup>ad</sup>	0.299 <sup>aC</sup>	<.0001	<.0001
	<i>p</i> -value	<.0001	<.0001	<.0001	<.0001		

Note. <sup>A,B,C,D</sup> mean values with different superscripts within the same row were significantly different at  $p < 0.05$ ; <sup>a,b,c,d</sup> mean values with different superscripts along the same column were different at  $p < 0.05$ ; NB = The SEM of  $\alpha$ -solanine and  $\alpha$ -chaconine in all the observations was  $\leq 0.00057735$



Table 3

*Solanine to chaconine ratio of fermented industrial potato waste with Lactiplantibacillus plantarum, Saccharomyces cerevisiae, and Aspergillus oryzae inoculants at different time*

Treatment	Fermentation time (hr)			
	0	24	48	72
Control	1:2	1:3	1:3	1:1
<i>Lactiplantibacillus plantarum</i>	1:2	1:3	1:3	1:1
<i>Saccharomyces cerevisiae</i>	1:2	1:3	1:3	1:4
<i>Aspergillus oryzae</i>	1:2	1:5	1:3	1:4

Table 4

*Phytic acid contents of fermented industrial potato waste with Lactiplantibacillus plantarum, Saccharomyces cerevisiae, and Aspergillus oryzae inoculants at different time*

Time (hr)	Treatment				p-value	
	Control	<i>Lactiplantibacillus plantarum</i>	<i>Saccharomyces cerevisiae</i>	<i>Aspergillus oryzae</i>	Treatment	Treatment*Time
0	1.95±0.11	2.05±0.11 <sup>a</sup>	2.06±0.12 <sup>a</sup>	2.14±0.12 <sup>a</sup>	0.7000	0.1415
24	1.85±0.10	1.56±0.09 <sup>b</sup>	1.75±0.10 <sup>ab</sup>	1.68±0.09 <sup>b</sup>	0.2679	0.1415
48	1.82±0.10 <sup>A</sup>	1.47±0.08 <sup>bb</sup>	1.65±0.09 <sup>bAB</sup>	1.48±0.08 <sup>bcB</sup>	0.0419	0.1415
72	1.75±0.10 <sup>A</sup>	1.39±0.08 <sup>bb</sup>	1.56±0.09 <sup>bAB</sup>	1.30±0.07 <sup>cB</sup>	0.0231	0.1415
p-value	0.6178	0.0036	0.0310	0.0012		

Note. <sup>A, B, C, D</sup> mean values with different superscripts within the same row were significantly different at  $p < 0.05$ ; <sup>a, b, c</sup> mean values with different superscripts along the same column were different at  $p < 0.05$

Table 5

*Antioxidant capacity of fermented industrial potato waste with Lactiplantibacillus plantarum, Saccharomyces cerevisiae, and Aspergillus oryzae inoculants at different time*

Fermentation time (hr)	Treatments				p-value	
	Control	<i>Lactiplantibacillus plantarum</i>	<i>Saccharomyces cerevisiae</i>	<i>Aspergillus oryzae</i>	Treatment	Treatment *Time
0	33.90±0.12 <sup>c</sup>	33.94±0.09 <sup>c</sup>	33.94±0.09 <sup>b</sup>	33.99±0.10 <sup>c</sup>	0.9406	<.0001
24	33.66±0.36 <sup>cC</sup>	42.07±0.11 <sup>aA</sup>	38.81±0.20 <sup>aB</sup>	42.28±0.41 <sup>aA</sup>	<.0001	<.0001
48	40.31±0.10 <sup>aB</sup>	41.39±0.086 <sup>bA</sup>	38.79±0.15 <sup>aC</sup>	40.04±0.14 <sup>bb</sup>	<.0001	<.0001
72	35.94±0.09 <sup>bA</sup>	31.51±0.08 <sup>dC</sup>	34.39±0.18 <sup>bb</sup>	27.02±0.05 <sup>dD</sup>	<.0001	<.0001
p-value	<.0001	<.0001	<.0001	<.0001		

Note. <sup>A, B, C, D</sup> mean values with different superscripts within the same row were significantly different at  $p < 0.05$ ; <sup>a, b, c, d</sup> mean values with different superscripts along the same column were different at  $p < 0.05$

the other three treatments that were similar. Invariably, it was observed that there was a significant ( $p < 0.05$ ) difference across the fermentation time except for the control. It was also observed that the phytic acid contents decreased linearly from 0 to 72 hr of fermentation time. At 72 hr fermentation time, *A. oryzae* recorded the least ( $p < 0.05$ ) phytic acid content.

### Antioxidant Capacity

The antioxidant capacity (Trolox equivalents mg/g) of fermented IPW increased significantly ( $p < 0.05$ ) across the time of fermentation until 48 hr (Table 5). Similarly, at 24 hr of fermentation, fermented IPW with *L. plantarum* and *A. oryzae* recorded a similar ( $p > 0.05$ ) concentration of the antioxidant, which was higher ( $p < 0.05$ ) than the concentration observed in fermented IPW with *S. cerevisiae*. But at 48 hr, fermented IPW with *L. plantarum* recorded the highest ( $p < 0.05$ ) concentration of antioxidants than both fermented IPW with *S. cerevisiae* and *A. oryzae* inoculants.

## DISCUSSION

Plants produced phenolic compounds during phenylpropanoid biosynthesis via shikimate pathways (Nkhata et al., 2018). It was observed that plants can convert synthesised phenolic components such as ferulic acid and caffeic acids into other related compounds like lignin, tannins, and flavonoids. Notwithstanding, common phenolic acids in potato peels are chlorogenic acid, chlorogenic acid isomer, and caffeic acid (Friedman et al.,

2017). The findings of this study show that the fermentation of IPW corroborated an earlier report that the fermentation process could result in an increase or decrease in phytochemical contents (Nkhata et al., 2018). The concentration of TPC recorded in the present work was within the range of earlier reports on different potato varieties (Ru et al., 2019). However, our result was higher than the values recorded in twenty potato clones (Ji et al., 2012) but lower than the concentrations recorded for both peeled and unpeeled potatoes (Ah-Hen et al., 2012). Variation in TPC has been attributed to many factors, including variety, skin colour, and tissue (tuber, flesh, and peel). Similarly, the increase in TPC observed in the present study could be related to the fact that during fermentation, phenolic compounds that are bound to proteins and minerals are released (Nkhata et al., 2018).

Flavonoids are a common group of phenolic compounds that influence fruits and vegetables' flavour and colour. Our study only analysed total flavonoid content, which should be composed of six significant subclasses: flavones, flavanols, flavanones, flavan-3-ols, anthocyanidins, and isoflavones. The present work recorded increased total flavonoid content over fermentation time, especially in treatments inoculated with *S. cerevisiae* and *A. oryzae*. It was also observed that inoculating industrial potato waste with *L. plantarum*, *S. cerevisiae*, and *A. oryzae* had no significant influence on total flavonoid content. The total flavonoid concentration recorded in our study was higher than the values

reported for organic and non-organic potato peel powders (Friedman et al., 2017). The variations observed from different reports on TFC could be related to various potato processing methods and analytical processes.

Carotenoids are important antioxidants that promote health, such as enhancing the immune system. It was discovered in the present work that the fermentation time and type of inoculation influenced the degradation of carotenoids. The work also revealed that *S. cerevisiae* and *A. oryzae* recorded higher degradation of total carotenoid content than the control and *L. plantarum* treatment groups. Similarly, it was observed that a high rate of degradation of total carotenoid content (mg/kg) occurred within 24 hr of fermentation. The degradation of total carotenoid content observed in our work corroborated an earlier finding that depending on the fermentation time, fermentation of high-carotenoid maize resulted in a significant loss of carotene (Ortiz et al., 2018). The values recorded in this study for total carotenoid content were higher but consistent with the fact that potato skin contains higher carotenoid content than flesh (Valcarcel et al., 2015). Among the various mechanisms proposed to explain the degradation of carotenoid content during fermentation is the disruption of the food matrix by endogenous enzymes and microorganisms, leading to the concentration of calcium, which might also enhance the saponification of free fatty acids (Nkhata et al., 2018). The most abundant carotenoids are  $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin,

lutein, zeaxanthin, and lycopene. In recent studies, fermentation either increased or decreased carotene content. The increase in carotenes during fermentation could be related to structural changes induced by fermentation, which increases the ability to extract carotenoids (Kiczorowski et al., 2022). However, loss of carotene during fermentation could also be related to the type of inoculum, time of fermentation, and oxidation (Zong et al., 2023). Therefore, differences in carotenoid content in different forms and morphological parts in potatoes could be associated with factors affecting carotenoid levels, such as genotype, climate, growing conditions, storage, and cooking processes (Lachman & Kotikova, 2016).

The effect of fermentation on tannin content (mg/g) was not consistent over time. Our result did not show a linear reduction of tannin due to fermentation time; however, a recent report on the fermentation of green coffee beans observed a significant reduction in tannin concentration (Haile & Kang, 2019). Reduction in tannin content during fermentation was previously recorded in cassava leaves (Hawashi et al., 2019). The tannin content recorded in this study was not higher than the values recorded for two potato cultivars (Taie et al., 2015). Our results showed that inoculation of IPW with the experimental microorganisms influenced tannin extraction from substrates rather than breaking down tannin into simple carbohydrate compounds. The absence of a reduction in tannin content in our results could be related to the fact that the present work recorded an increase in total flavonoid

content. Incidentally, condensed tannins contain flavonoids (flavan 3-ol or flavan 3, 4-diol) without a sugar core. In contrast, hydrolysable tannins comprise gallic acids with a sugar (mainly glucose) core (Das et al., 2020). Hence, an increase in flavonoid content due to microbial fermentation is likely to affect a reduction in tannin content during fermentation.

Our results on the phytic acid content of fermented IPW were not affected by inoculation but significantly influenced by the fermentation time. Phytic acid is a storage form of phosphorous in seeds and tubers; however, phytic acid is converted to phytate when it is bound to several mineral elements such as Ca, Fe, Zn, Mo, and Mg (Cominelli et al., 2020). The reduction of phytic acid during fermentation has been well-established (Kareem et al., 2017). Reduction of phytic acid could be related to the activities of phytase secreted by fermenting microorganisms (Nkhata et al., 2018). The values observed for phytic acid contents in fermented IPW were within a lower range of 3-23 mg/100 g fresh weight; a range of 3.39-61.34 mg/100 g fresh weight was reported for Indian potato cultivars (Joshi et al., 2021).

The results of glycoalkaloid content in fermented industrial potato waste showed that the fermentation time and inoculation of IPW influenced the concentration of GLA. It was also observed that the ratio between  $\alpha$ -solanine and  $\alpha$ -chaconine was affected by the fermentation time and inoculation. The range of glycoalkaloid concentrations recorded in the present study was lower by

far compared to values observed in peels of some selected potato cultivars (Taie et al., 2015), but within a range of values reported for twenty potato clones (Ji et al., 2012), and mashed Idaho variety of potato (Kondamudi et al., 2017).

The concentration of  $\alpha$ -solanine was consistently lower than  $\alpha$ -chaconine. Thus, the ratios of  $\alpha$ -solanine to  $\alpha$ -chaconine conformed with the report of a 1:2 to 1:7 range of ratios between the two glycoalkaloids (Omayio et al., 2016). Calculation of ratios between  $\alpha$ -solanine and  $\alpha$ -chaconine is important because it was observed that  $\alpha$ -chaconine has about five times more bioactive function than  $\alpha$ -solanine (Friedman et al., 2017). Variations in GLA contents in different works could be due to differences in potato variety, storage and processing methods (Kondamudi et al., 2017). Recent work reported an increase in glycoalkaloid content due to a decrease in the moisture content of potato vine silage (Juanjuan et al., 2019). However, the report on potato vine silage also observed a reduction of glycoalkaloid content from fresh potato vine. In contrast, our findings show that  $\alpha$ -chaconine content increased with fermentation time. This study recorded a sharp increase in both  $\alpha$ -solanine and  $\alpha$ -chaconine in the control and treatment groups fermented with *L. plantarum* at 72 hr of fermentation. An increase in GLA content was likely caused by the ability to extract GLA from solvent (methanol) and its solubility. It is thought that alcoholic solvents can break down food matrix and efficiently penetrate cell membranes, thus

permitting the extraction of a high amount of organic extracts (Nazarni et al., 2016).

Microbial enzymes such as  $\beta$ -glucosidase and lipase produced during fermentation can hydrolyse glucosides and break down plant cell walls to release organic compounds. In particular, *L. plantarum* has a strong glucosidase activity (Nkhata et al., 2018). Hence, apart from the effect of solvent and solubility of GLA, the sharp rise in glycoalkaloid concentration in the treatment group inoculated with *L. plantarum* could be ascribed to the ability of the lactic acid bacteria therein to produce  $\beta$ -glucosidase which can break down food matrix to release more GLA (Juanjuan et al., 2019).

The present study demonstrated that the antioxidant capacity of IPW fermented with *L. plantarum*, *S. cerevisiae*, and *A. oryzae* increased with the fermentation time. It was discovered that IPW inoculated with *L. plantarum* recorded the highest antioxidant capacity. Recently, potatoes have received special attention because of their high antioxidant activity (Ah-Hen et al., 2012). The antioxidant capacity (Trolox equivalents mg/g) observed in the present study was higher than values recorded for twenty potato extracts (peels, tuber, and granules). However, it corroborated that potato peels possess higher DPPH radical scavenging activity than any other potato part or form (Ji et al., 2012). It has been reported that the skin colour of potatoes influenced its antioxidant capacity owing to its higher level of phenolic content (Ji et al., 2012). In addition, our result confirmed that fermentation improves antioxidant

activity by increasing the release of phenolic compounds, which act as reducing agents or hydrogen donors (Hur et al., 2014). However, the antioxidant activity could not be predicted based on total phenolic content alone because other compounds in the extract may have contributed to the overall antioxidant capacity. Besides, other factors such as species of microorganisms, pH, temperature, solvent, fermentation time, the water content of the substrate types, and aerobic or anaerobic conditions may influence antioxidant capacity (Hur et al., 2014). Therefore, the higher antioxidant capacity (Trolox equivalents mg/g) recorded in IPW inoculated with *L. plantarum* at just 24 hr of fermentation agreed with an observation that substrate fermented with lactic acid bacteria produced higher antioxidant activity than substrate fermented with *S. cerevisiae* (Hur et al., 2014; Zhao & Shah, 2014). The superiority of *L. plantarum* over the two inocula used in the study was probably due to its ability to produce  $\beta$ -glucosidase that has high enzymatic hydrolysis over polymerised phenolic compounds during fermentation.

## CONCLUSION

A short-term fermentation of industrial potato waste with *L. plantarum* (MW296876), *S. cerevisiae* (MW296931), and *A. oryzae* (MW297015) shows changes in bioactive compounds during fermentation. Apart from the effect of fermentation time, the inocula used increased antioxidant activity and concentrations of phenolic and glycoalkaloid compounds. The concentrations of

glycoalkaloids ( $\alpha$ -solanine and  $\alpha$ -chaconine) and their ratios were increased by both the fermentation time and treatments (inoculation with microorganisms). Both fermentation time and treatments were responsible for the increase in the antioxidant capacity of industrial potato waste. Although the three microorganisms used in the study increased the concentration of different bioactive compounds, glycoalkaloids, and antioxidant activity, *L. plantarum* was therefore recommended because it was consistent in increasing total phenolic compounds, antioxidant capacity, and extraction yield of tannin,  $\alpha$ -solanine, and  $\alpha$ -chaconine. A future study on the nutritional implication of incorporating fermented industrial potato waste into a ruminant diet is recommended.

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