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INHIBITORY EVALUATION OF *Cola acuminata* AGAINST FOOD WASTE-DERIVED ALPHA-AMYLASE AND LIPASE

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Abstract

Food waste is an important environmental and resource-management issue, thus triggering a new interest in its valorisation into biotechnological products in the context of a circular bioeconomy. In the context of managing metabolic diseases, the inhibition of digestive enzymes, including α-amylase and lipase, has been well developed; however, concerns about synthetic inhibitors have led to the search for alternatives based on plant-derived compounds. Sweet potato peel (SPP) and tiger nut chaff (TNC) are abundant Nigerian food wastes rich in the nutrients needed for the target enzymes. SPP is rich in carbohydrate (primarily starch), and TNC is high in fibre and contains residual lipids, offering sustainable substrates for enzyme biotechnology and nutraceutical development through the transformation of agricultural waste into high-value bioproducts.

This study measured the proximate composition of sweet potato peel (SPP) and tiger-nut chaff (TNC), produced α-amylase and lipase from SPP and TNC, respectively, using solid-state fermentation (SSF) with *Aspergillus niger*, evaluated the inhibitory activity of *Cola acuminata* seed fractions against the enzymes, and characterised the n-hexane fraction by gas chromatography–mass spectrometry (GC–MS). Proximate analysis was conducted on SPP and TNC, which were used as substrates for α-amylase and lipase production via solid-state fermentation using *Aspergillus niger*. Enzyme activities were assessed after 3 and 7 days under different fermentation conditions. α-Amylase and lipase activities were determined using the DNSA and titration methods, respectively. Enzyme inhibition was evaluated using starch–iodine colourimetric and p-nitrophenyl butyrate assays, with acarbose and orlistat as reference inhibitors. SPP, with higher carbohydrate content (76.42%), yielded peak α-amylase activity (0.480U/mL) after 3 days of fermentation, while TNC, with higher fibre (25.00%) and lipid (8.20%) contents, yielded peak lipase activity (7.3U/mL) after 7 days when supplemented with olive oil/Tween-80 emulsion. The n-hexane extract of *C. acuminata* showed the highest inhibition of both enzymes (48% α-amylase; 49% lipase), although lower than acarbose (99%) and orlistat (76%). GC–MS analysis identified twenty-one compounds, with ethyl oleate (32.45%), 9,12-octadecadienoic acid ethyl ester (13.71%), and supraene (8.10%) as the major constituents. Overall, SPP and TNC are effective, inexpensive substrates for enzyme production, while *C. acuminata* demonstrates digestive enzyme inhibition of both alpha amylase and lipase, reinforcing its potential as a natural anti-obesity agent and supporting food-waste valorisation as a sustainable framework for enzyme and nutraceutical development.

Keywords: *Cola acuminata*, Food Waste, Alpha-amylase, Lipase, Solid-state fermentation.

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Introduction

Food waste (FW), which includes leftovers, partially consumed meals, and pre-cooked or processed food residues, is one of the world's serious environmental and resource management menaces. Globally, over a third of the food that is grown for people to eat, or 1.3 billion tonnes per annum, is lost or wasted (Barrera & Hertel, 2021). This happens at each level of the food chain, from pre-production and post-harvest handling, through distribution up to consumption, with serious consequences for food security and sustainability. There is a projection that food-waste-related emissions contribute approximately 6–8% of anthropogenic greenhouse-gas emissions while wasting water, soil nutrients and polluting the ecosystem (Amicarelli et al., 2021; Zhu et al., 2023). Conventional treatments such as landfill and incineration contribute to increased emissions and waste valuable reusable materials (Wu et al., 2022).

In this context, valorisation of food waste has emerged as a sustainable solution to manage and convert FW into a renewable resource for industrial and biotechnological applications (Ansari et al., 2024). Emerging valorisation methods such as microbial fermentation, enzymatic bioprocesses, anaerobic digestion, ultrasonic treatment, and pyrolysis allow for the extraction of high-value bio-products, including microbial enzymes, biofuels, nutraceuticals, and bioactive substances (Chilakamarry et al., 2022; Arya et al., 2022; Wu et al., 2022). These strategies form the foundation on which the framework of a circular bio-based economy as a means towards environmental preservation, resource optimisation and development of green economic avenues is based (Kaur et al., 2023).

Sweet-potato (*Ipomoea batatas*) peel (SPP) and tiger-nut (*Cyperus esculentus*) chaff (TNC) are two residues of food processing that are locally abundant in Nigeria and hold a lot of biotechnological potential but are currently massively underutilised. Sweet potato (*Ipomoea batatas*), which is the seventh most important food crop globally and a key staple in Nigeria, produces large quantities of peels in post-harvest operations such as sorting, cleaning, peeling, slicing, drying, and storage (Yigezu Wendimu, 2021; Vithu et al., 2019). These peels have a high carbohydrate content of starch, fermentable sugars, and dietary fibres, which provide a convenient low-cost carbon source and make them good substrates for enzyme synthesis, especially for α-amylase, in solid-state fermentation (SSF) systems. Their natural moisture levels also promote the growth of microorganisms and make fermentation efficient (Tlais et al., 2020).

Tiger nut (*Cyperus esculentus*) is an underutilised perennial tuber that is cultivated in Africa, Europe, and Asia, and is notably rich in dietary fibre, minerals, vitamins, and carbohydrates, and its oil composition is comparable to that of olive oil (Abdelkader et al., 2017; Ayeni, 2022; Oladele & Aina, 2007). During tiger nut milk production, a fibrous residue known as tiger nut chaff (TNC) is produced after soaking, grinding, and filtration. This residue is either usually discarded or burnt; however, the high fibre and lipid content of TNC provides a locally available good fermentation substrate to be used in the production of lipase and other biotechnological processes, converting waste into a renewable output (Oluseye et al., 2024; Rebezov et al., 2023).

Digestive enzymes such as α-amylase and lipase play essential roles in carbohydrate and lipid metabolism, respectively, and have broad industrial and biomedical relevance. α-Amylase catalyses the hydrolysis of α-1,4 glycosidic bonds in starch and glycogen and is widely used in food, brewing, baking, and biofuel industries (Farooq et al., 2021; Sheela et al., 2021). Lipase catalyses the breakdown of triglycerides into free fatty acids and glycerol and plays a central role in lipid catabolism and energy homeostasis. Dysregulation of lipase activity contributes to abnormal fat metabolism, which has been linked to obesity and cardiovascular diseases, while lipases are also extensively utilised in food, pharmaceutical, detergent, and biodiesel industries (Badraoui et al., 2020; Li & Zhang, 2019; Chandra et al., 2020; Costa et al., 2017).

Microbial fermentation, especially using *Aspergillus niger* on food-waste substrates, has been shown to offer a cheap and ecologically harmless method for enzyme production. Solid-state fermentation (SSF) is especially beneficial, offering high yields when optimised for substrate composition, pH, temperature, and moisture content while complying with the idea of circular bioeconomy (Putri et al., 2020; Makeri et al., 2021).

In the biomedical setting, inhibiting the activity of α-amylase and lipase is an established approach in the control of metabolic-related diseases, including type 2 diabetes mellitus and obesity. By slowing carbohydrate and lipid digestion, enzyme inhibitors reduce energy absorption and contribute to weight management (Rajan et al., 2020; Haguet et al., 2023). Although synthetic inhibitors such as acarbose and orlistat are effective, concerns regarding their toxicity have driven increasing interest in plant-based alternatives rich in bioactive phytochemicals, including flavonoids, alkaloids, and saponins, which possess enzyme-inhibitory and metabolic-regulating properties (Oluwagunwa et al., 2021; Anyanwu et al., 2021; Anyanwu et al., 2023).

Cola acuminata (kola nut) tree is a culturally and economically valued West African tree known for its pharmacological potential. Its fruit is rich in caffeine, theobromine, and theophylline, as well as phenolic and flavonoid compounds that play antioxidant, anti-inflammatory, antimicrobial, and antidiabetic roles (Ekalu et al., 2020; Chukwuma et al., 2023). Although little investigation has been conducted on its inhibition of α-amylase and lipase.

While sustainable biotechnological processes and natural therapeutics are in high demand, the present study aimed to evaluate the enzyme-inhibitory properties of *Cola acuminata* seed extracts on α-amylase and lipase synthesised from sweet-potato peel (SPP) and tiger-nut chaff (TNC), using *Aspergillus niger* under solid-state fermentation (SSF), thereby incorporating food-waste valorisation with enzyme biotechnology and plant-based anti-obesity studies.

Material and Methods

Collection and Identification of Plants

Fresh sweet potato (*Ipomoea batatas*) tubers, dried tiger nut (*Cyperus esculentus*) and seed of *Cola acuminata* were bought from Zuba Fruit Market, FCT Abuja, Nigeria. Identification and authentication of the samples were done by Mr Akeem A. Lateef in the Herbarium Department of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja. Voucher specimen numbers NIPRD/H/7478, NIPRD/H/7479 and NIPRD/H/7481 were assigned, and the authenticated samples were kept in the National Institute for Pharmaceutical Research and Development (NIPRD) herbarium.

Preparation of food waste

The fresh tubers of sweet potato (*Ipomoea batatas*) were washed and peeled, and the peels were utilised as a source of substrate. Brown dried tiger nut (*Cyperus esculentus*) tubers were processed by sorting to remove all broken pieces, rot, stones and other impurities, followed by washing and soaking for 3 hours before blending. The milk was filtered through a muslin cloth, and the chaff obtained after filtering was collected as substrate. Peels of both sweet potato and tiger nut chaff were air-dried independently for 14 days to lower their moisture level. The dried sweet potato peels were then crushed into powder and kept in Ziploc bags at room temperature; the tiger nut chaff was also stored similarly until further analysis.

Proximate composition for food waste substrate

The proximate composition of SPP and TNC substrates was estimated using the AOAC (2000) procedures, assessing the moisture, fat, protein, ash, fibre and carbohydrate content.

Culture of Aspergillus niger

A fungal strain of *A. niger* ATCC 16888 was sourced from the Microbiology and Biotechnology Department, National Institute for Pharmaceutical Research and Development (NIPRD), Idu-Abuja, Nigeria. The organism was cultured on Potato Dextrose Agar (PDA) slants at 30 °C for 5 days before being kept at 4°C (Mahmood et al., 2016).

Inoculum preparation

The inoculum was prepared by adding 60 mL of sterile distilled water to a sporulated 5-day-old slant. A dry cotton swab was used to remove the spores under sterile conditions, which was agitated for uniform suspension of the spores for substrate inoculation. The spore suspension was standardised to approximately 1.0×10^7 spores/mL, resulting in an estimated total spore load of 6.0×10^8 spores in the 60 mL inoculum.

Solid-state fermentation for α-amylase activity

A mineral salt solution was formulated using the procedure detailed by Mahmood et al. (2016), with minor changes. The solution contained KH_2PO_4 (10 g/L), $MgSO_4$ (2.0 g/L), NaCl (2.0 g/L), and $MnSO_4$ (0.5 g/L) in 250 mL of distilled water.

Table 1: Experimental Design for α-Amylase Production using Sweet potato peel (SPP)

Groups	Base	Microorganism inoculum	Incubation period	Incubation period
Flask 1 (Control)	20 g SPP + 10 mL mineral salt solution	Starch (0.25g) + Peptone (0.3g) + 5 mL *A. niger*	3 days	7 days
Flask 2 (Group 1)	20 g SPP + 10 mL mineral salt solution	5 mL *A. niger*	3 days	7 days
Flask 3 (Group 2)	10 g SPP + 10 mL mineral salt solution	5 mL *A. niger*	3 days	7 days
Flask 4 (Group 3)	20 g SPP + 10 mL mineral salt solution	10 mL *A. niger*	3 days	7 days

The substrate mixture was homogenised, sterilised in an autoclave at 121 °C for 15 minutes, cooled, inoculated, and incubated at 30 °C for 3 and 7 days, respectively.

Solid-state fermentation for lipase activity

A diluent solution was prepared using the procedure detailed by Mukhtar et al. (2015) with slight changes: $MgSO_4$ (0.5 g), KH_2PO_4 (1.0 g), $FeSO_4$ (0.01 g), KCl (0.05 g), glucose (0.8 g) and peptone 20g were dissolved in 250 ml of distilled water.

Table 2: Experimental Design for Lipase Production using Tiger nut chaff (TNC)

Groups	Base	Microorganism inoculum	Incubation period	Incubation period
Flask 1 (Control)	10 g of TNC + 10 mL diluent	2 mL of Tween 80: olive oil (1:1) + 1.0 mL *A. niger*	3 days	7 days
Flask 2 (Group 1)	15 g of TNC + 10 mL diluent	2 mL of Tween 80: olive oil (1:1) + 1.0 mL *A. niger*	3 days	7 days
Flask 3 (Group 2)	10 g of TNC + 10 mL diluent	1.0 mL *A. niger*	3 days	7 days
Flask 4 (Group 3)	15 g of TNC + 10 mL diluent	1.0 mL *A. niger*	3 days	7 days

The substrate mixture was homogenised, sterilised in an autoclave at 121 °C for 15 minutes, cooled, inoculated, and incubated at 30 °C for 3 and 7 days, respectively.

Alpha-Amylase Extraction

The extraction of enzymes followed the protocol of Mahmood et al. (2016), slightly modified. Fermentation was achieved after 3 and 7 days, respectively, and 75 mL of citrate buffer (pH 5.0) was added to both flasks, which were then shaken using a Stuart orbital shaker at a rate of 200 rpm over 60 minutes. The extract was filtered using muslin cloth and filter paper, followed by centrifugation at 4,000 rpm for 15 minutes. To determine α-amylase activity, the supernatant was taken as a crude enzyme extract.

Lipase Extraction

The extraction of enzymes followed the protocol of Mukhtar et al. (2015), slightly modified. Fermentation was achieved after 3 and 7 days, respectively, and 100 mL of phosphate buffer (pH 6.5) was added to both flasks, which were then shaken using a Stuart orbital shaker at a rate of

200 rpm over 60 minutes. The extract was filtered using muslin cloth and filter paper, followed by centrifugation at 4,000 rpm for 15 minutes. To determine lipase activity, the supernatant was taken as a crude enzyme extract.

Alpha-Amylase Activity

The activity of α-amylase was measured using the modified procedures of Okolo et al. (1995), Okonji et al. (2019) and Miller (1959). A mixture of 1 mL of crude enzyme extract and 1 mL of 2% soluble starch was incubated at 50°C for 10 minutes in the reaction mixture. To stop the reaction, 1 mL DNSA reagent was added and boiled for 5 minutes. Then the mixture was cooled and pipetted 200 µL onto a 96-well plate, and the absorbance at 492 nm was measured using a microplate reader. The enzyme amount was evaluated using one unit (IU) of α-amylase activity. The enzyme's activity was measured as one unit (IU) of α-amylase necessary to release one µg of glucose/min as measured using a glucose standard curve.

Lipase Activity

To assess the lipase activity, a titrimetric assay was performed following the procedure given by Mukhtar et al*.* (2015), with slight changes. The mixture reaction was composed of 2 mL of an olive oil emulsion, 1 mL of phosphate buffer (pH 7.0), and 1 mL of the enzyme extract and incubated for 15 minutes at 37 °C. Hydrolysis was terminated with the addition of 4 mL of ethanol and three drops of phenolphthalein indicator. The freed fatty acids were titrated using 0.01N NaOH until a light pink colour developed. The activity of lipases was determined as a record of the amount of fatty acid released, where one unit (U) is the ability of the enzyme to liberate one 1 µmol of fatty acid per minute under the experimental conditions.

Preparation of Cola acuminata Extract and Fractions

Fresh *C. acuminata* seeds were sliced, allowed to air-dry, ground, and then soaked for 3 days with frequent shaking in ethanol. The filtrate was filtered through muslin cloth and then filter paper and concentrated using a rotary evaporator at 70 °C (Anyanwu et al., 2020). The crude extract was partitioned in water and organic solvents, hexane and ethyl acetate, respectively, following Hostettmann et al. (1991) with minor changes in order to obtain n-hexane, ethyl acetate, and aqueous fractions. The hexane and ethyl acetate fractions were concentrated at 40 °C, and the aqueous fraction was concentrated at 70 °C, again using a rotary evaporator.

Alpha-Amylase Inhibition Assay (starch-iodine colour assay)

The α-amylase inhibitory assay was conducted in a 96-well microtiter plate using the procedure of Xiao et al. (2006), with minor changes. The reaction mixture included 25 µL of 0.02 M sodium phosphate buffer (pH 6.9, 6 mM NaCl), 20 µL of 2% soluble starch, and 20 µL of plant extract or acarbose (0-2000 µg/mL) as substrates. After 5 minutes of incubation at 37°C, 40 µL of crude α-amylase extract was added and incubated for another 8 minutes at the same temperature. The enzymatic process was then stopped with 20 µL of 1 M HCl, followed by 100 µL of Iodine reagent. Absorbance readings were taken at 620 nm; dark blue showed strong inhibition, brown partial inhibition, and yellow no inhibition. The positive control for the assay was acarbose, with blanks containing no enzyme or extract.

The percentage inhibition of α-amylase activity was calculated as follows:

$$\% \text{ Inhibition} = \left(\frac{Absorbance\ of\ Control - Absorbance\ of\ Sample}{\text{Absorbance of Control}}\right) \times 100$$

Lipase Inhibition Assay

The lipase inhibitory effect was measured using the modified method described by Kim et al. (2009). Each well contained a total volume of 200 µL of Tris buffer (100 mM TrisHCl, 5 mM $CaCl_2$, pH 7.0), 10 µL crude lipase, and plant extract or orlistat (0-2000 µg/mL). The substrate (10 mM p-NPB) 10 µL, was added after 5 minutes at 37°C, and 15 minutes at 37°C allowed additional incubation. At 405 nm, absorbance was measured using a microplate reader. The effect of the plant constituents on the inhibition of lipase activity was then calculated using the following equation: Lipase inhibition (%) = $100 - \left(\frac{B-b}{\text{A}-\text{a}}\right) \times 100\Big)$.

Where: A = activity without inhibitor**,** a = negative control without inhibitor**,** B = activity with inhibitor and b = negative control with inhibitor.

Gas Chromatography Mass Spectrometry (GC-MS) analysis of the n-hexane fraction of Cola acuminata

The n-hexane fraction of *C. acuminata* was analysed by GC-MS using a Shimadzu GC-MS-QP2010 Ultra system with an AOC-20i auto injector and an OPTIMA 5MS capillary column (60 m × 0.25 mm × 0.25 µm). Helium was employed as a carrier gas at a constant pressure. The oven was programmed from 60 °C to 280 °C at a rate, which was connected to a FID detector (split:10.1:1) and operated in split mode with an injection volume of 1 µL. Compounds were identified with a quadrupole mass spectrometer operating in EI mode at 70 eV and comparing their spectra against the NIST11 library.

2.16 Statistical Analysis

The data are expressed as the mean ± SEM of three replicates. The data were analysed using a one-way ANOVA in SPSS version 27. Duncan's multiple range test was used to compare mean differences at a P-value < 0.05. The graphs were created using GraphPad Prism (version 10).

Results

Proximate Composition of Sweet potato peel (SPP) and Tiger nut chaff (TNC)

The proximate composition of SPP and TNC is given in Table 1, confirming that there are apparent differences in nutritional contents between the two substrates. The moisture content (12.7%) of SPP was slightly higher than that of TNC; however, the observed difference was not statistically significant ($p > 0.05$). The ash (3.40%) and carbohydrate (76.42%) concentrations were significantly higher ($p \leq 0.05$) in SPP, indicating its higher energy and mineral potential. Conversely, TNC had a slightly higher crude protein content (5.26%) than SPP; the observed difference was not statistically significant ($p > 0.05$). However, TNC had significantly higher ($p \leq 0.05$) crude fibre (25.00%) and lipid (8.20%) content, suggesting that it has more nutritional value and can be converted into lipase. Therefore, SPP is more favourable in the production of α-amylase because of its high carbohydrate content. In contrast, TNC is more appropriate in the production of lipase because of its high fibre and fat content.

Table 3: Proximate Composition of Sweet potato peel (SPP) and Tiger nut chaff (TNC)

Parameters	Sweet potato peel (SPP)	Tiger nut chaff (TNC)
Moisture content (%)	12.7 ± 0.75[a]	10.25 ± 0.60[a]
Crude protein (%)	4.38 ± 0.23[a]	5.26 ± 0.30[a]
Crude fibre (%)	2.20 ± 0.12[b]	25.00 ± 1.44[a]
Crude lipid (%)	0.90 ± 0.06[b]	8.20 ± 0.50[a]
Ash (%)	3.40 ±0.17[a]	1.55 ± 0.12[b]
Carbohydrate (%)	76.42 ± 2.90[a]	49.74 ± 2.90[b]

The values presented are expressed as mean ± SEM. Mean values within the same row with different superscript letters are significantly different ($p \leq 0.05$) according to an independent-samples t-test.

Alpha-amylase activity

Figure 1 illustrates the α-amylase activity of *A. niger* cultured on SPP after 3 days of fermentation under different inoculum and supplementation conditions. The highest activity (0.480 U/mL) occurred in Group 3 (20 g SPP + 10 mL inoculum), followed by the control (0.420 U/mL), Group 1 (0.300 U/mL), and Group 2 (0.240 U/mL).

Figure 2 shows the α-amylase activity of *A. niger* cultured on SPP after 7 days of fermentation. Enzyme activity declined across all groups compared to day 3, with Group 3 (0.333 U/mL) and the control (0.320 U/mL) showing the highest values, though not statistically different, while Groups 1 and 2 recorded lower activities of 0.260 U/mL and 0.230 U/mL, respectively. Overall, α-amylase production was highest at day

3 and decreased by day 7, with reduced variation among the groups.

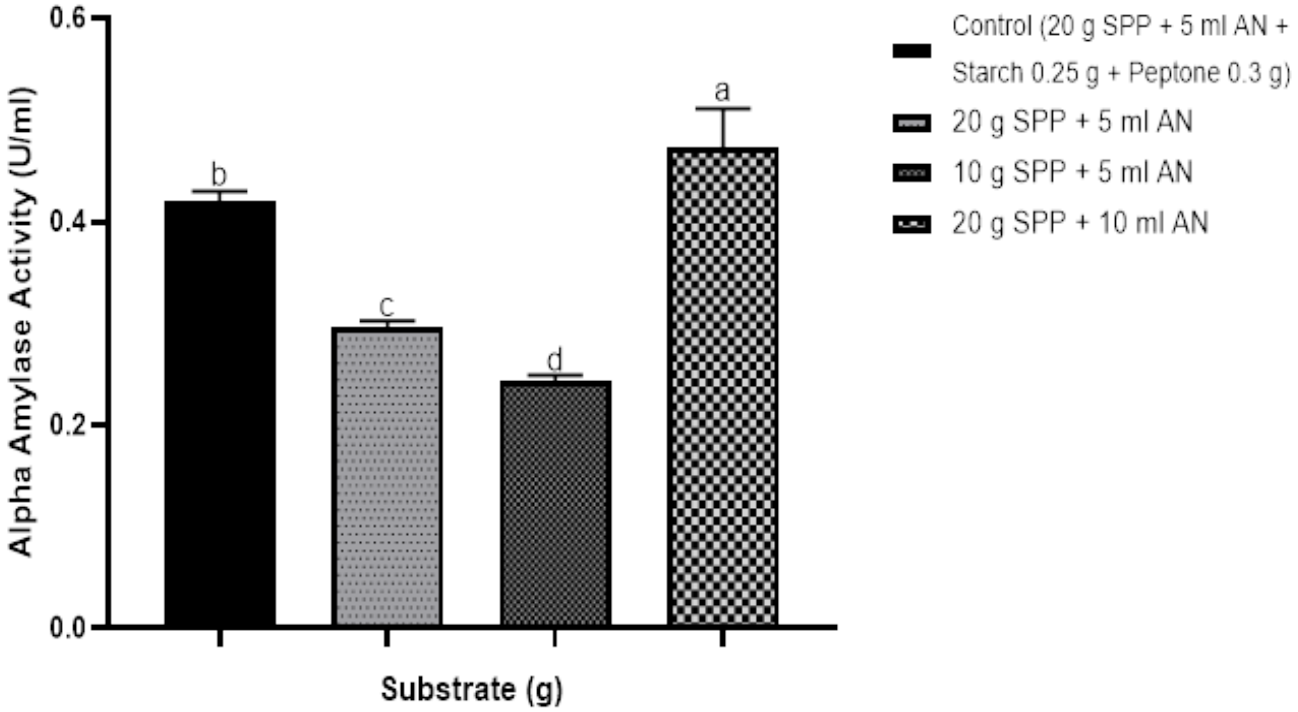


Figure 1: Alpha amylase activity obtained using SPP at 3 days of fermentation.
The data are shown as mean ± SEM. Statistically significant differences are shown by the unique letters above the bars ($p < 0.05$). SPP stands for sweet potato peel; AN represents *A. niger*; SSF is an abbreviation for solid-state fermentation. The experimental groups are Control, Group 1, Group 2, and Group 3.

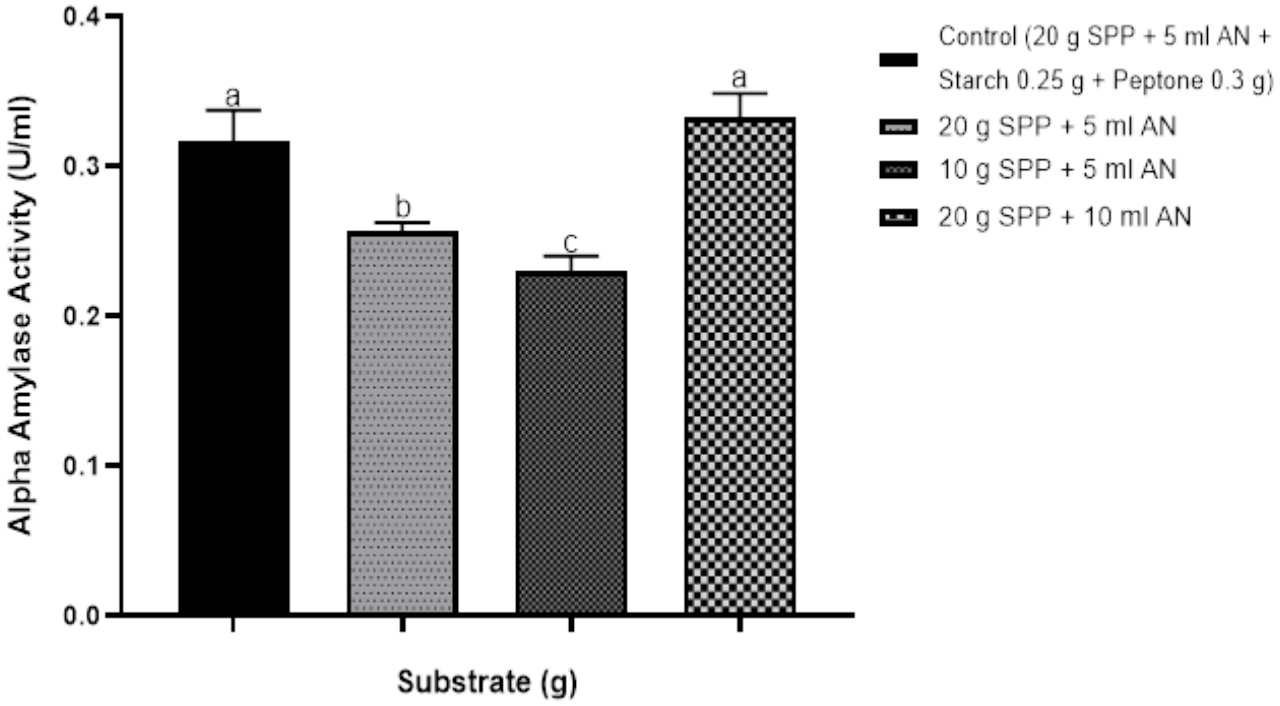


Figure 2: Alpha amylase activity obtained using SPP at 7 days of fermentation.
The data are shown as mean ± SEM. Statistically significant differences are shown by the unique letters above the bars ($p < 0.05$). SPP stands for sweet potato peel; AN represents *A. niger*; SSF is an abbreviation for solid-state fermentation. The experimental groups are Control, Group 1, Group 2, and Group 3.

Lipase activity

Figure 3 presents the lipase activity of *A. niger* cultured on TNC under different substrate loads and supplementation conditions. After 3 days of incubation, the highest activity was observed in Group 3 (15 g TNC without emulsion, 5.1 U/mL), followed closely by the control (10 g TNC + 1 mL inoculum + 2 mL emulsion, 4.8 U/mL), while Groups 1 and 2 showed lower activities of 3.3 U/mL and 3.9 U/mL, respectively. Overall, lipase production increased with higher substrate load, whereas emulsion addition did not significantly enhance enzyme activity at this stage.

Figure 4 presents the lipase activity of *A. niger* cultured on TNC after 7 days of fermentation. At this stage, Group 1 (15 g TNC with emulsion) showed the highest activity (7.3 U/mL), significantly higher ($p<0.05$) than the control (4.0 U/mL) and Group 3 (15 g TNC without emulsion, 4.3 U/mL), while Group 2 (10 g TNC without emulsion) exhibited the lowest activity (2.0 U/mL). These results indicate that the addition of emulsion enhanced lipase production during extended fermentation.

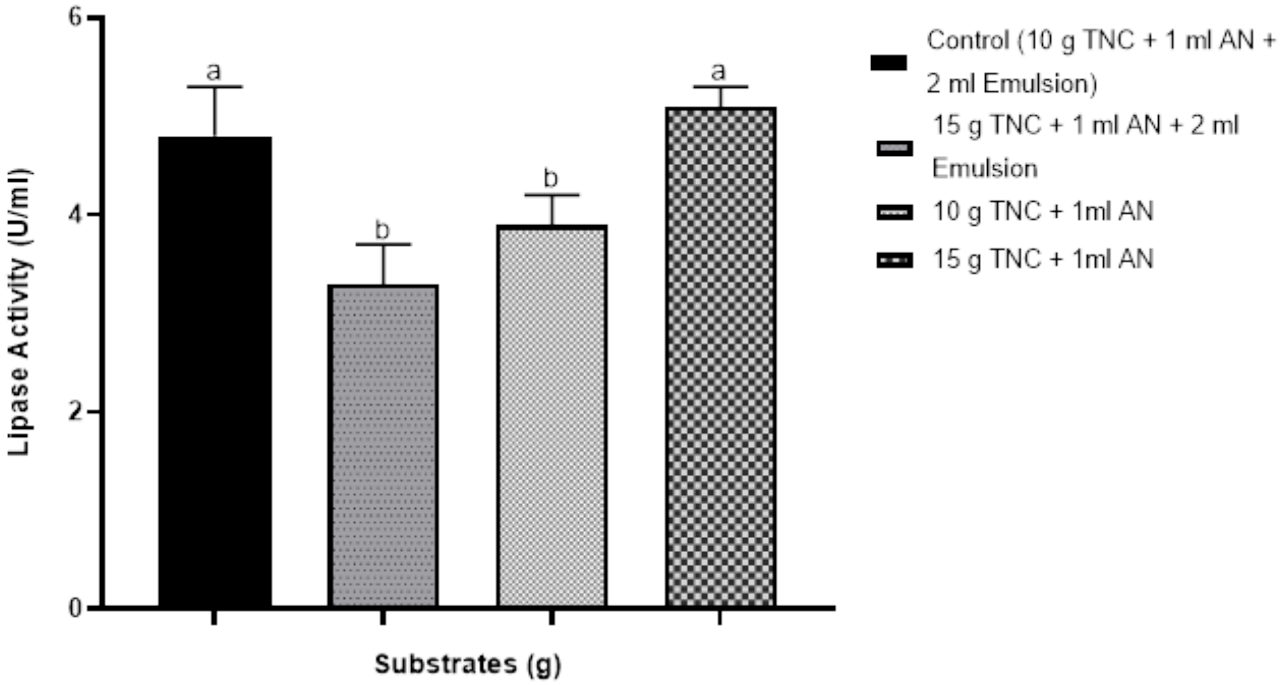


Figure 3: Lipase activity obtained using TNC at 3 days of fermentation.
The data are shown as mean ± SEM. Statistically significant differences are shown by the unique letters above the bars ($p < 0.05$). TNC stands for Tiger nut chaff; AN, *A. niger*; SSF, Solid-state fermentation; Emulsion denotes olive/tween 80 (1:1); Control; Group 1; Group 2; Group 3.

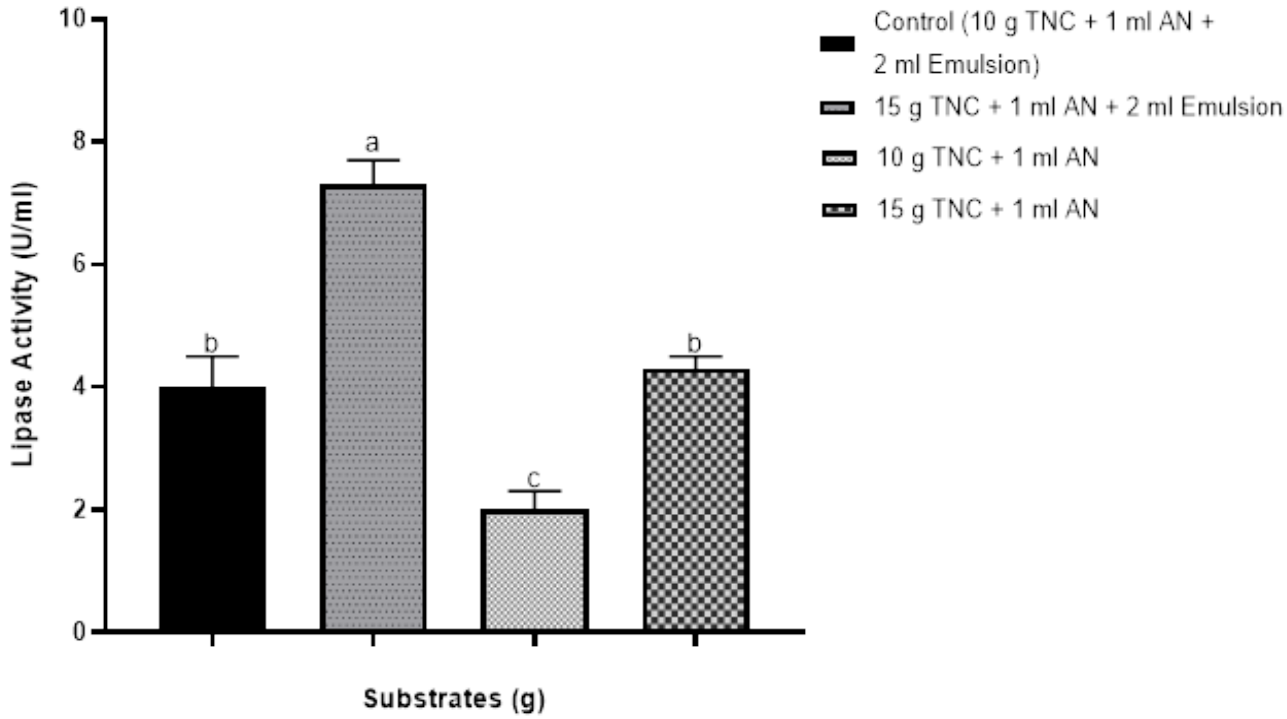


Figure 4: Lipase activity obtained using TNC at 7 days of fermentation.
The data are shown as mean ± SEM. Statistically significant differences are shown by the unique letters above the bars ($p < 0.05$). TNC stands for Tiger nut chaff; AN, *A. niger*; SSF, Solid-state fermentation; Emulsion denotes olive/tween 80 (1:1); Control; Group 1; Group 2; Group 3.

Inhibition of Crude α-Amylase and Lipase by n-hexane fraction of Cola acuminata

Figures 5 & 6 show the percentage inhibitory activities of the aqueous, n-hexane, and ethyl acetate fractions of *Cola acuminata* against crude α-amylase and lipase, compared with the standard inhibitors acarbose and orlistat. The n-hexane fraction showed the highest inhibition for both enzymes, 48% for α-amylase and 49% for lipase, while the aqueous (15% and 22%) and ethyl acetate (17% and 9%) fractions showed considerably lower activities. Although none of the fractions matched the potency of the standards (acarbose 99% or orlistat 76%), the n-hexane fraction showed notable lipase inhibition.

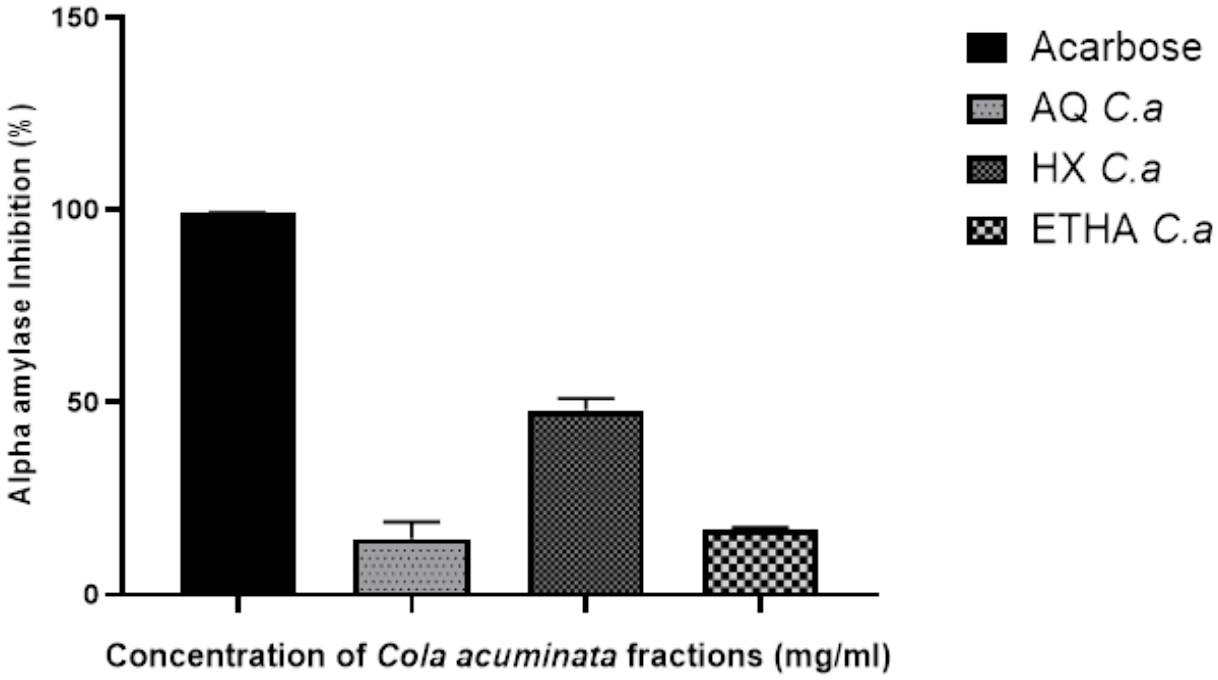


Figure 5: Percentage Inhibition of Crude α-Amylase by *C. acuminata* Fractions
Data are expressed as mean ± SEM. AQ, Aqueous; HX, Hexane; ETHA, Ethyl acetate; C.a, *C. acuminata*.

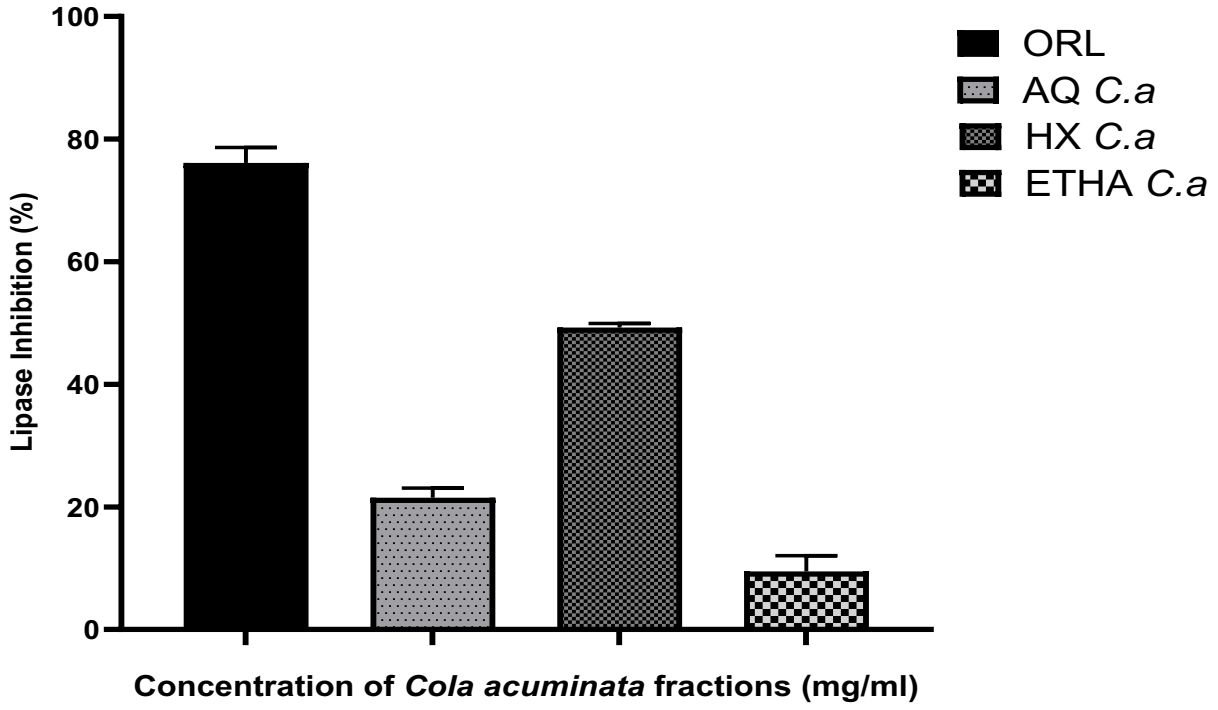


Figure 6: Percentage Inhibition of Crude Lipase by *C. acuminata* Fractions

Data are expressed as mean ± SEM. ORL, Orlistat; AQ, Aqueous; HX, Hexane; ETHA, Ethyl acetate; C.a, *C. acuminata*.

GC-MS analysis of the hexane fraction of C. acuminata

Table 2 and Figure 7 present the GC-MS chromatogram and result of the n-hexane fraction of *Cola acuminata*, revealing twenty-one (21) volatile compounds. The major constituents identified were Ethyl oleate (32.45%), 9,12-Octadecadienoic acid ethyl ester (13.71%), and Supraene (8.10%), while Heptadecanoic acid ethyl ester (0.61%) was the least abundant compound in the fraction.

Table 2: GC-MS analysis of the n-hexane fraction of *Cola acuminata*

Peak	Retention time	Area %	Compounds in n-hexane fractions	Molecular formula
1	10.318	0.91	1-Decyne	$C_{10}H_{18}$
2	13.184	3.06	Ethyl 9-hexadecenoate	$C_{18}H_{34}O_2$
3	14.147	6.35	Eicosanoic acid, ethyl ester	$C_{22}H_{42}O_2$
4	15.775	2.61	2-Dodecen-1-yl (-) succinic anhydride	$C_{16}H_{26}O_3$
5	17.449	1.66	1, 3-Dipalmitin trimethylsilyl ether	$C_{38}H_{76}O_5Si$
6	17.991	3.18	Fumaric acid, allyl octadecyl ester	$C_{25}H_{44}O_4$
7	19.524	1.17	Diisooctyl phthalate	$C_{24}H_{38}O_4$
8	20.137	3.07	Nonadecanoic acid, ethyl ester	$C_{21}H_{42}O_2$
9	20.385	1.45	Octadecanoic acid, 2-propenyl ester	$C_{21}H_{40}O_2$
10	21.654	4.36	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$
11	22.324	0.69	Nonadecanoic acid, ethyl ester	$C_{21}H_{42}O_2$
12	22.651	0.61	Heptadecanoic acid, ethyl ester	$C_{19}H_{38}O_2$
13	23.121	13.71	9, 12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$
14	23.498	32.45	Ethyl Oleate	$C_{20}H_{38}O_2$
15	23.686	3.30	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$

16	24.131	4.64	9, 12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$
17	24.180	2.72	Z, Z-4, 15-Octadecadien-1-ol acetate	$C_{20}H_{36}O_2$
18	24.601	1.46	Ethyl 9-hexadecenoate	$C_{18}H_{34}O_2$
19	24.783	3.83	Ethyl tetracosanoate	$C_{26}H_{52}O_2$
20	25.346	8.10	Supraene	$C_{30}H_{50}$
21	25.800	0.66	Eicosanoic acid, ethyl ester	$C_{22}H_{44}O_2$

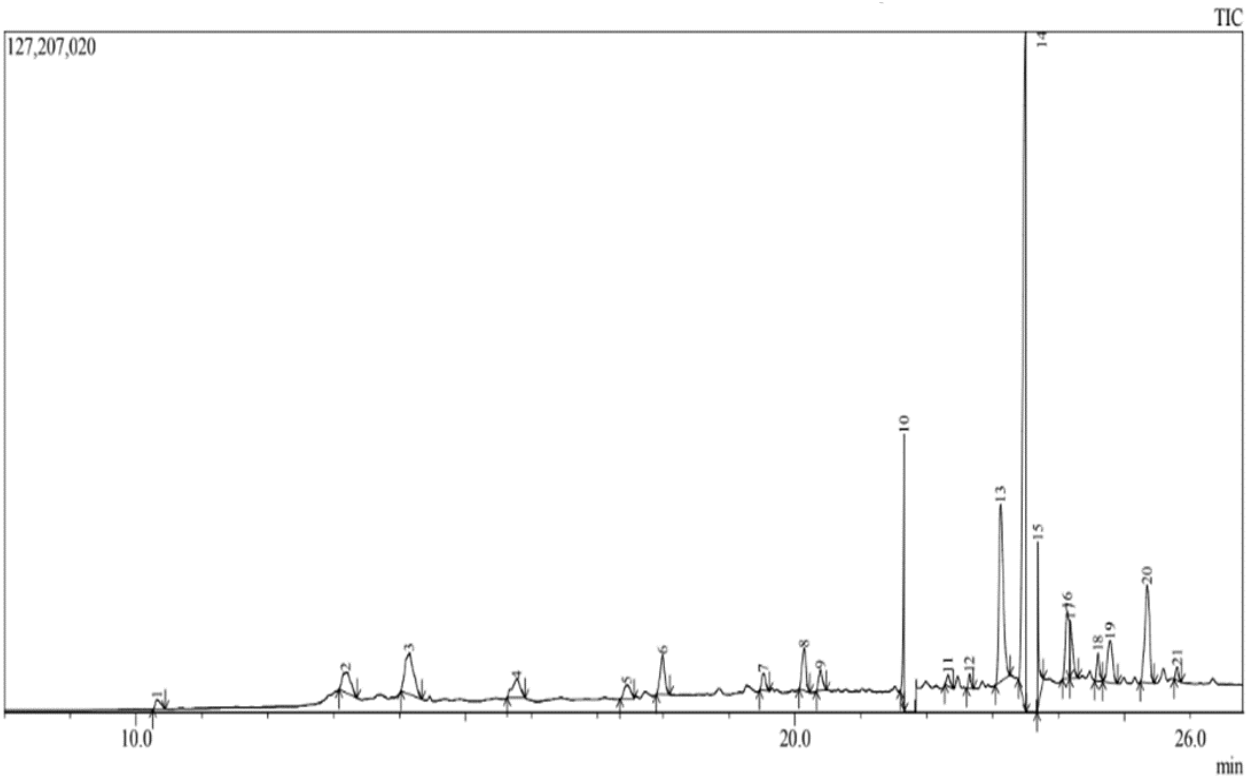


Figure 7: Chromatographic result of the hexane fraction of *Cola acuminata*

Discussions

The current study shows that food waste substrates, such as SPP and TNC, can be utilised as effective, low-cost substrates for microbial enzyme production via SSF with *A. niger*. The α-amylase activity observed at both 3 and 7 days further justified the use of SPP, given its high carbohydrate content (76.42%) and the strong inducers of amylase, starch and fermentable sugars (Pereira et al., 2017; Tlais et al., 2020). Maximum α-amylase production (0.480 U/mL) was attained on day 3 with 20 g SPP and 10 mL inoculum, while supplementation with starch and peptone further enhanced enzyme production (Li et al., 2012). However, the activity decreased after day 3 due to nutrient depletion and proteolytic degradation (Perwez & Al Asheh, 2025; da Silva-López et al., 2022), indicating that the fermentation time could be optimised. Essentially, TNC, a lipid-rich by-product of milk extraction, exhibited the highest lipase activity (7.3 U/mL) after 7 days when 15 g of substrate was combined with an olive oil/Tween-80 emulsion. This demonstrates that the quantity of substrate and the nature of the emulsifier actually influence the quantity of enzyme obtained

(Mukhtar et al., 2015; Costa et al., 2017). That the maximum activity was subsequent to the addition of the emulsifier may be due to the increased time in the induction phase, which is likely due to the increased accessibility of the lipids (Mahadik et al., 2002; Colla et al., 2015). Overall, this indicates that SPP and TNC are potentially promising, less damaging sources for producing α-amylase and lipase, which may assist in using waste in a circular bio-economy (Sharma et al., 2022; Sarker et al., 2024).

The screening of crude α-amylase and lipase from food-waste substrates revealed varied inhibitory activities from *Cola acuminata* seed fractions. The most potent fraction was the n-hexane extract, which inhibited 48% and 49% of α-amylase and lipase, respectively. This indicates that the non-polar, lipophilic compounds are primarily responsible for enzyme inhibition. This observation is consistent with the growing evidence that lipophilic phytochemicals exhibit stronger affinity for digestive enzymes due to their ability to interact with hydrophobic pockets within the enzyme active sites (Itoh et al., 2019; Anyanwu et al., 2021; Jawed et al., 2019; Haguet et al., 2023).

Although the inhibition of *C. acuminata* was lower than that of standard drugs acarbose (99%) and orlistat (76%), the moderate inhibition observed is pharmacologically relevant. Natural inhibitors are often less potent but safer, with fewer gastrointestinal side effects, making them suitable for long-term dietary management of obesity (Rajan et al., 2020). Similar moderate inhibition profiles have been reported for other Nigerian plant extracts such as *Piper guineense* and *Mucuna flagellipes*, which also demonstrate multi-target metabolic effects (Anyanwu et al., 2021).

The molecular mechanisms of lipase and α-amylase inhibition by lipophilic compounds are mostly controlled by hydrophobic forces, competition, and conformational equilibrium of catalytic sites.

Ethyl oleate, the dominant compound (32.45%) identified, is a long-chain fatty acid ester with a structure similar to natural lipase substrates (triglycerides). This structural similarity allows it to serve as a competitive or pseudo-substrate inhibitor, binding to the active site in the enzyme and blocking access to actual substrates. Studies have demonstrated that the catalytic triad (Ser–His–Asp) of the pancreatic lipase can be occupied by fatty acid esters, thus inhibiting hydrolysis (Rajan et al., 2020; Itoh et al., 2019). Also, the hydrophobic chains increase binding affinity due to van der Waals interactions in the lipid-binding domain of the enzyme.

In the case of α-amylase, lipophilic compounds can inhibit the enzyme through allosteric modulation, where the compounds bind to the enzyme not at its catalytic site but instead to change the enzyme conformation and decrease the proximity of substrates to the catalytic site (Haguet et al., 2023). The unsaturated fatty acid esters, such as 9,12-octadecadienoic acid ethyl ester (13.71%), can further promote the inhibition due to their structural flexibility and can thus better fit in the enzyme pockets. Additionally, molecular docking studies on similar compounds have revealed that lipophilic phytochemicals have high binding energies and stability in enzyme active sites compared to polar ones, which further supports the superiority of the hexane fraction (Anyanwu et al., 2023). Thus, the inhibitory effect of the *C. acuminata* could be due to the synergistic action of fatty acid esters through competitive and hydrophobic attachment.

While *C. acuminata* showed moderate enzyme inhibition, it is necessary to place the efficacy of *C. acuminata* relative to other Nigerian medicinal plants traditionally used for obesity and metabolic disorders. Studies on native plants such as *Ceratotheca sesamoides*, *Jatropha tanjorensis*, *Pterocarpus mildbraedii* and *Vernonia amygdalina* have been reported with an increased inhibitory efficiency, usually over 60-80% against lipase and α-amylase (Anyanwu et al., 2021; Oluwagunwa et al., 2021). Polyphenols and flavonoids present in these plants typically exhibit strong enzyme inhibition through hydrogen bonding and metal ion binding.

However, comparable findings from *C. nitida,* another Cola species, revealed the presence of palmitic, oleic, linoleic, and stearic acids in dichloromethane extracts, which have shown stronger antioxidant and antimicrobial properties (Adesanwo et al., 2017), corroborating the presence of related bioactive lipophilic esters in *C. acuminata*. While most previous studies on *C. acuminata* and related *Cola* species have focused on alkaloids and phenolics (Osaro et al., 2024), limited GC–MS data exist on their hexane fractions, and this shows that *C. acuminata* demonstrates a unique profile of fatty acid esters,

indicating species-specific phytochemical variability.

Conclusions

This study demonstrates that the carbohydrate (76.42%) and lipid (8.20%) content of sweet potato peel (SPP) and tiger nut chaff (TNC), respectively, makes them effective, low-cost food waste substrates for α-amylase and lipase production by *A. niger* under solid-state fermentation. Maximum enzyme yields were obtained with SPP (0.480 U/mL for α-amylase) and TNC (7.3 U/mL for lipase), confirming their suitability for microbial enzyme biosynthesis. The n-hexane fraction of *Cola acuminata* exhibited the most potent inhibitory activity against both enzymes, with 48% inhibition for α-amylase and 53% inhibition for lipase, attributed to its lipophilic compounds, including ethyl oleate, 9,12-octadecadienoic acid ethyl ester, and supraene. In particular, the GC-MS results shed new light on the composition of the n-hexane portion of *C. acuminata*, which has barely been documented. On the whole, the research highlights the two-fold importance of food-waste valorisation and *C. acuminata* bioactives in the creation of natural enzyme inhibitors, which may find application in obesity management.

References

Adesanwo, J. K., Ogundele, S. B., Akinpelu, D. A., & McDonald, A. G. (2017). Chemical analyses, antimicrobial and antioxidant activities of extracts from *Cola nitida* seed. *J. Explor. Res. Pharmacol*, *2*(3), 67-77. http://dx.doi.org/10.14218/JERP.2017.00015

Amicarelli, V., Lagioia, G., & Bux, C. (2021). Global warming potential of food waste through the life cycle assessment: An analytical review. Environ. Impact Assess. Rev, *91*, 106677. https://doi.org/10.1016/j.eiar.2021.106677.

Ansari, S. A., Kumar, T., Sawarkar, R., Gobade, M., Khan, D., & Singh, L. (2024). Valorisation of food waste: A comprehensive review of individual technologies for producing bio-based products. *J. Environ. Manage*, *364*, 121439. https://doi.org/10.1016/j.jenvman.2024.121439.

Anyanwu, G. O., Anzaku, D., Donwell, C. C., Usunobun, U., Adegbegi, A. J., Ofoha, P. C., & Rauf, K. (2021). Chemical composition and in vitro anti-obesity and in vivo anti-hyperlipidaemic effects of *Ceratotheca sesamoides*, *Jatropha tanjorensis*, *Mucuna flagellipes*, *Pterocarpus mildbraedii* and *Piper guineense*. *Phytomedicine Plus*, *1*(3), 100042. https://doi.org/10.1016/j.phyplu.2021.100042

Anyanwu, G. O., Ejike, U. D., Gyebi, G. A., Rauf, K., Iqbal, J., Zaib, S., ... & Batiha, G. E. S. (2023). Phytochemical analysis, in vitro and in silico effects from Alstonia boonei De Wild stem bark on selected digestive enzymes and adipogenesis in 3T3-L1 preadipocytes. *BMC Complement. Med. Ther*, *23*(1), 370. https://doi.org/10.1186/s12906-023-04202-6

Anyanwu, G. O., Kolb, A. F., & Bermano, G. (2020). Anti-obesity functional leads and targets for drug development. In *Phytochemicals as lead compounds for new drug discovery* (pp. 143-160). Elsevier.

Arya, P. S., Yagnik, S. M., Rajput, K. N., Panchal, R. R., & Raval, V. H. (2022). Valorisation of agro-food wastes: Ease of concomitant-enzymes production with application in food and biofuel industries. *Bioresour. Technol*, *361*, 127738. https://doi.org/10.1016/j.biortech.2022.127738

Association of Official Analytical Chemists. (2000). *Official methods of analysis of the Association of Official Analytical Chemists* (Vol. 11). The Association.

Ayeni, A. O. (2022). Hoop House and Field Evaluation of Tigernut (*Cyperus esculentus* L. var. sativus Boeck) Selections in New Jersey, USA. *Plants*, 11(7), 897. https://doi.org/10.3390/plants11070897

Badraoui, R., Ben-Nasr, H., Bardakçi, F., & Rebai, T. (2020). Pathophysiological impacts of exposure to an endocrine disruptor (tetradifon) on α–amylase and lipase activities associated with metabolic disorders. *Pestic. Biochem. Physiol*, *167*, 104606. https://doi.org/10.1016/j.pestbp.2020.104606.

Barrera, E. L., & Hertel, T. (2021). Global food waste across the income spectrum: Implications for food prices, production and resource

use. *Food Policy*, *98*, 101874. https://doi.org/10.1016/j.foodpol.2020.101874.

Chandra, P., Enespa, Singh, R., & Arora, P. K. (2020). Microbial lipases and their industrial applications: a comprehensive review. *Microb. Cell Fact*, *19*(1), 169. https://doi.org/10.1186/s12934-020-01428-8.

Chilakamarry, C. R., Sakinah, A. M., Zularisam, A. W., Sirohi, R., Khilji, I. A., Ahmad, N., & Pandey, A. (2022). Advances in solid-state fermentation for bioconversion of agricultural wastes to value-added products: Opportunities and challenges. *Bioresour. Technol*, *343*, 126065.

Chukwuma, I. F., Apeh, V. O., Nwora, F. N., Nkwocha, C. C., Mba, S. E., & Ossai, E. C. (2023). Phytochemical profiling and antioxidative potential of phenolic-rich extract of Cola acuminata nuts. *Biointerface Res Appl Chem*, *13*(1), 29-39. https://doi.org/10.33263/briac131.029.

Colla, L. M., Ficanha, A. M., Rizzardi, J., Bertolin, T. E., Reinehr, C. O., & Costa, J. A. V. (2015). Production and characterisation of lipases by two new isolates of Aspergillus through solid-state and submerged fermentation. *Biomed Res. Int*, *2015*(1), 725959. https://doi.org/10.1155/2015/725959

Costa, T., Hermann, K., García-Román, M., Valle, R., & Tavares, L. (2017). Lipase production by *A. niger* grown in different agro-industrial wastes by solid-state fermentation. *Braz. J. Chem. Eng*, 34, 419-427. https://doi.org/10.1590/0104-6632.20170342s20150477.

da Silva-López, R. E., de Araujo, T. A. A., Monteiro, H. J. J., Teixeira, É. M. G. F., Tupi, L., & da Silva Bon, E. P. (2022). Study of protease activity from *Aspergillus awamori* INCQS2B. 361U2/1 extracellular fraction and modification of culture medium composition to isolate a novel aspartic protease. *Braz. J. Microbiol*, *53*(3), 1599-1611. https://doi.org/10.1007/s42770-022-00750-0

Ekalu, A., & Habila, J. D. (2020). Phytochemistry, pharmacology and medicinal uses of Cola (Malvaceae) family: a review. *Med. Chem. Res*, *29*(12), 2089-2105. https://doi.org/10.1007/s00044-020-02637-x.

Farooq, M. A., Ali, S., Hassan, A., Tahir, H. M., Mumtaz, S., & Mumtaz, S. (2021). Biosynthesis and industrial applications of α-amylase: A review. *Arch. Microbiol*, *203*(4), 1281-1292. https://doi.org/10.1007/s00203-020-02128-y.

Haguet, Q., Le Joubioux, F., Chavanelle, V., Groult, H., Schoonjans, N., Langhi, C., Michaux, A., Otero, Y. F., Boisseau, N., Peltier, S. L., Sirvent, P., & Maugard, T. (2023). Inhibitory Potential of α-Amylase, α-Glucosidase, and Pancreatic Lipase by a Formulation of Five Plant Extracts: TOTUM-63. *Int. J. Mol. Sci*, *24*(4), 3652. https://doi.org/10.3390/ijms24043652.

Hostettmann, K., Hostettmann, M., & Marston, A. (1991). Saponins. *Terpenoids (Charlwood BV, Banthorpe DV, eds.), Methods Plant Biochem (Dey, PM and Harborne, JB, eds.)*, *7*, 435-471. https://doi.org/10.1016/j.biortech.2021.126065.

Itoh, K., Matsukawa, T., Murata, K., Nishitani, R., Yamagami, M., Tomohiro, N., Kajiyama, S. I., Fumuro, M., Iijima, M., Shigeoka, S., Endo, Y., & Matsuda, H. (2019). Pancreatic lipase inhibitory activity of *Citrus unshiu* leaf extract. *Nat. Prod. Commun*, 14(9). https://doi.org/10.1177/1934578X19873439

Jawed, A., Singh, G., Kohli, S., Sumera, A., Haque, S., Prasad, R., & Paul, D. (2019). Therapeutic role of lipases and lipase inhibitors derived from natural resources for remedies against metabolic disorders and lifestyle diseases. *S. Afr. J. Bot*, *120*, 25-32. https://doi.org/10.1016/j.sajb.2018.04.004

Kaur, M., Singh, A. K., & Singh, A. (2023). Bioconversion of food industry waste to value-added products: Current technological trends and prospects. *Food Biosci*, 55, 102935. https://doi.org/10.1016/j.fbio.2023.102935.

Kim, J., Jang, D. S., Kim, H., & Kim, J. S. (2009). Anti-lipase and lipolytic activities of ursolic acid isolated from the roots of Actinidia arguta. *Arch. Pharm. Res*, *32*(7), 983-987.

Li, F., & Zhang, H. (2019). Lysosomal acid lipase in lipid metabolism and beyond. *Arterioscler. Thromb. Vasc. Biol*, *39*(5), 850-856. https://doi.org/10.1161/atvbaha.119.312136.

Li, S., Yang, X., Yang, S., Zhu, M., & Wang, X. (2012). Technology prospecting on enzymes: application, marketing and engineering. *Comput. Struct. Biotechnol. J*, *2*(3), e201209017. https://doi.org/10.5936/csbj.201209017

Mahadik, N. D., Puntambekar, U. S., Bastawde, K. B., Khire, J. M., & Gokhale, D. V. (2002). Production of acidic lipase by *Aspergillus niger* in solid-state fermentation. *Process Biochem*, *38*(5), 715-721. https://doi.org/10.1016/S0032-9592(02)00194-2

Mahmood, S., Shahid, M. G., Nadeem, M., Irfan, M., & Syed, Q. (2016). Production and optimisation of α-amylase from *Aspergillus niger* using potato peel as substrate. *Pak. J. Biotechnol*, *13*(2), 101-109.

Makeri, M. S., Bala, M., Wante, S. P., Bitrus, K. V., & Aliyu, H. U. (2021). Activity of α-amylase produced by *Aspergillus niger* at different pH, temperature and incubation time using the solid-state fermentation process of corn and wheat wastes. *Asian J Biotechnol Biores Technol*, *7*, 1-11. https://doi.org/10.9734/ajb2t/2021/v7i230095.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for the determination of reducing sugar. *Anal. Chem*, *31*(3), 426-428.

Mukhtar, H., Hanif, M., Rehman, A., Nawaz, A., & Haq, I. U. (2015). Studies on the lipase production by *Aspergillus niger* through solid-state fermentation. *Pak. J. Bot*, *47*, 351-354.

Okolo, B. N., Ezeogu, L. I., & Mba, C. N. (1995). Production of raw starch-digesting amylase by *Aspergillus niger* grown on native starch sources. *J. Sci. Food Agric*, *69*(1), 109-115.

Okonji, R. E., Itakorode, B. O., Ovumedia, J. O., & Adedeji, O. S. (2019). Purification and biochemical characterisation of pectinase produced by *Aspergillus fumigatus* isolated from soil of decomposing plant materials. *J Appl Biol Biotechnol*, *7*(3), 1-8.

Oladele, A. K., & Aina, J. O. (2007). Chemical composition and functional properties of flour produced from two varieties of tigernut (Cyperus esculentus). *Afr. J. Biotechnol*, *6*(21).

Oluseye, O. A., Christianah, O. I., Barnabas, O. S., & Bola, O. O. (2024). Cultivation, Nutrition, and Market Trends of the Multi-Faceted Tubers: Tiger Nuts. *Int. J. Curr. Microbiol. App. Sci*, *13*(06), 216-227. https://doi.org/10.20546/ijcmas.2024.1306.023.

Oluwagunwa, O. A., Alashi, A. M., & Aluko, R. E. (2021). Inhibition of the in vitro Activities of α-Amylase and Pancreatic Lipase by Aqueous Extracts of Amaranthus viridis, Solanum macrocarpon and Telfairia occidentalis Leaves. *Front. Nutr*, *8*, 772903. https://doi.org/10.3389/fnut.2021.772903.

Osaro, I. E., Chinedu, I., & Ojochenemi E, Y. (2024). Proximate and Phytochemical Constituents of Ethanolic Extract of Seeds of *Cola Acuminata* and *Cola Nitida*. *Asian J. Sci. Technol. Eng. Art*, *2*(2), 129-144. https://doi.org/10.58578/ajstea.v2i2.2738

Pereira, C. R., Resende, J. T. V., Guerra, E. P., Lima, V. A., Martins, M. D., & Knob, A. (2017). Enzymatic conversion of sweet potato granular starch into fermentable sugars: feasibility of sweet potato peel as an alternative substrate for α-amylase production. *Biocatal. Agric. Biotechnol*, *11*, 231-238. https://doi.org/10.1016/j.bcab.2017.07.011

Perwez, M., & Al Asheh, S. (2025). Valorisation of agro-industrial waste through solid-state fermentation: Mini review. *Biotechnol. Rep*, *45*, e00873. https://doi.org/10.1016/j.btre.2024.e00873

Putri, D. N., Khootama, A., Perdani, M. S., Utami, T. S., & Hermansyah, H. (2020). Optimisation of *Aspergillus niger* lipase production by solid-state fermentation of agro-industrial waste. *Energy Reports*, *6*, 331-335. https://doi.org/10.1016/j.egyr.2019.08.064.

Rajan, L., Palaniswamy, D., & Mohankumar, S. K. (2020). Targeting obesity with plant-derived pancreatic lipase inhibitors: A comprehensive review. *Pharmacol. Res*, *155*, 104681. https://doi.org/10.1016/j.phrs.2020.104681.

Rebezov, M., Usman Khan, M., Bouyahya, A., Imran, M., Tufail, T., Loretts, O., Neverova, O., Artyukhova, S., Kuznetsova, E., Ermolaev, V., Balahbib, A., Thiruvengadam, M., Zengin, G., & Shariati, M. A. (2023). Nutritional and technical aspects of tiger nut and its micro-constituents: An overview. *Food Rev. Int*, *39*(6), 3262-3282. https://doi.org/10.1080/87559129.2021.2011910.

Sarker, A., Ahmmed, R., Ahsan, S. M., Rana, J., Ghosh, M. K., & Nandi, R. (2024). A comprehensive review of food waste valorisation for the sustainable management of global food waste. *Sustain. Food Technol*, *2*(1), 48-69.

Sharma, V., Tsai, M. L., Nargotra, P., Chen, C. W., Kuo, C. H., Sun, P. P., & Dong, C. D. (2022). Agro-industrial food waste as a low-cost substrate for sustainable production of industrial enzymes: a critical review. *Catalysts*, *12*(11), 1373. https://doi.org/10.3390/catal12111373

Sheela, J. M., Divya, K., & Premina, S. (2021). Amylase production by *Aspergillus Niger* and *Penicillium species* by solid-state and submerged cultivation using two food industrial wastes. *Nat. Environ. Pollut. Technol*, *20*(3), 1127-1135. https://doi.org/10.46488/NEPT.2021.v20i03.020

Skaf, L., Franzese, P. P., Capone, R., & Buonocore, E. (2021). Unfolding hidden environmental impacts of food waste: An assessment for fifteen countries of the world. *J. Clean. Prod*, *310*, 127523. https://doi.org/10.1016/j.jclepro.2021.127523.

Tlais, A. Z. A., Fiorino, G. M., Polo, A., Filannino, P., & Di Cagno, R. (2020). High-value compounds in fruit, vegetable and cereal by-products: an overview of potential sustainable reuse and exploitation. *Molecules*, 25(13), 2987. https://doi.org/10.3390/molecules25132987.

Vithu, P., Dash, S. K., & Kalpana Rayaguru, K. R. (2019). Post-harvest processing and utilisation of sweet potato: A review. *Food Rev. Int, (726–762).* https://doi.org/10.1080/87559129.2019.1600540

Wu, Y., Yao, S., Narale, B. A., Shanmugam, A., Mettu, S., & Ashokkumar, M. (2022). Ultrasonic Processing of Food Waste to Generate Value-Added Products. *Foods*, *11*(14), 2035. https://doi.org/10.3390/foods11142035

Xiao, Z., Storms, R., & Tsang, A. (2006). A quantitative starch-Iodine method for measuring alpha-amylase and glucoamylase activities. *Anal. Biochem*, *351*(1), 146-148.

Yigezu Wendimu, G. (2021). Biology, Taxonomy, and Management of the Root-Knot Nematode (Meloidogyne incognita) in Sweet Potato. *Adv. Agric*, 2021(1), 8820211. https://doi.org/10.1155/2021/8820211.

Zhu, J., Luo, Z., Sun, T., Li, W., Zhou, W., Wang, X., Fei, X., Tong, H., & Yin, K. (2023). Cradle-to-grave emissions from food loss and waste represent half of total greenhouse gas emissions from food systems. *Nat. Food*, *4*(3), 247-256. https://doi.org/10.1038/s43016-023-00710-3.