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Effect of Microbial Cell-Size on Solid State Fermentation of Cowpea (*Vigna unguiculata* L. Walp) and Groundnut (*Arachis hypogaea* L.) by *Rhizopus oligosporus*

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ABSTRACT: The solid state fermentation of *Vigna unguiculata* L. Walp (cowpea) and *Arachis hypogaea* L. (groundnut) was carried out using *Rhizopus oligosporus* at different inoculum size (1.89 x 10^2, 3.78 x 10^2, 5.67 x 10^2, 7.56 x 10^2, 9.45 x 10^2 and 11.34 x 10^2 CFU g^{-1}). Following fermentation for 72 hours, biochemical parameters were determined in fermented and unfermented samples. All inoculum sizes produced significantly more phenol and flavonoids in the fermented groundnut samples as compared to the control, with the 15% inoculum size providing the most phenols (255.0 mg GAE/g). The fermentation of cowpea showed a similar pattern. The recognized antioxidant molecules flavonoids and phenols have sufficient capacity to reduce the negative effects of free radicals. The fermented samples' 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical increased significantly as compared to the unfermented sample. Comparing the fermented samples to the unfermented sample (control) at various inoculum sizes, amylase activity rose dramatically. Solid-state fermentation (SSF) can be employed to enhance the nutritional characteristics of cowpea and groundnut as soluble protein, glucose, and reducing sugars were also significantly increased in fermented cowpea and groundnut (at the different inoculum sizes) compared to the control. From this study, it is obvious that the selected fungus isolate, *R. oligosporus* showed high enzymatic activities and increased value added product of cowpea at varying inoculum size. This is an important characteristic for possible biotechnological applications.

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Cowpea and groundnut are leguminous crops popularly grown in the tropic and subtropic regions of the world; the most common usage is as food for humans and a component of animal feeds. Legumes have significant advantages in sustainable cropping systems; their capacity to fix atmospheric nitrogen through symbiotic associations has great potential in the fight against global warming (Dequiedt and Moran, 2015). Groundnut (*Arachis hypogaea*), also known as peanut is a tropical legume valued by humans for its nutrition and taste, the production of oil and as feed for animal consumption. The health benefits associated with the consumption of groundnut have been widely reported; for example, Guasch-Ferré et al. (2017) reported based on three (3) large cohort studies with up to 32 years of follow-up, that nut

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consumption resulted in lowered risk of cardiovascular disease (CVD) and coronary heart disease (CHD). A risk reduction of at least 13% and 15% was reported for CVD and CHD respectively following consumption of one serving (28 g) of peanut two or more times a week (Guasch-Ferré et al., 2017). Given its documented robust nutritional composition; including proteins, calories, fatty acids, vitamins, minerals, antioxidants etc., increased consumption of groundnut and groundnut-based foods is highly recommended, especially among nutritionally vulnerable groups such as infants, young children, and women of reproductive age (Ojiewo et al., 2020). A second leguminous crop currently making waves in the combat against food and nutritional insecurity is cowpea (*Vigna unguiculata* L.Walp). Among the pulses, cowpea is described as the most valuable economically probably due to the fact that it is widely distributed and most consumed in many developing countries for its role in combating protein malnutrition (Jayathilake et al., 2018; Bessada et al., 2019). Aside from being a high-quality protein source, cowpea is also reported as rich in nutraceuticals, i.e. chemical compounds with medicinal or health benefits such as antioxidants, polyunsaturated fatty acids, polyphenols etc. Reported applications of the functional proteins of cowpea seeds range from as antimicrobial agents, additives for meat preservation/tenderization, to cowpea protein isolates (CPIs) which have varied industrial and medicinal usage (da Silva et al., 2021). As grain legumes, groundnut and cowpea have gained significant recognition in terms of their use in sustainable farming as well as nutritional and health benefits to humans and animals alike (Foyer et al., 2016). Nevertheless, there have been reports of stagnation and/or decline in the overall usage and production of grain legumes compared to cereal crops, in developing countries and other parts of the world. Some identifiable challenges include: low profitability to farmers due to high variability in yields, pre- and post-harvest contamination, low digestibility of carbohydrates and fibres, presence of anti-nutritive components and allergens, adoption of policies and market drivers that favour cereals production, availability of resistant seeds with high market acceptability, etc. (Magrini et al., 2016; Zander et al., 2016; Kebede, 2020; Aderibigbe et al., 2022). Microbial fermentation methods have been demonstrated to significantly enhance the nutritional quality, digestibility and bioactivity of food materials including cereals/grain legumes crops and various agricultural wastes (Aganbi et al., 2015; Anigboro et al., 2020; Egbune et al., 2023). Solid state fermentation (SSF) has proven to be more widely acceptable over submerged fermentation due to advantages such as: low investment costs, reduced risk of contamination and simplicity of the technology among others. Saharan et al. (2020) reported higher levels of phenolic and flavonoids contents, accompanied by increases in antioxidant activity and secretions of specific microbial enzymes following SSF of a group of pulses that included cowpea and pigeon pea. In a different study, SSF of soybean meal with *Bacillus subtilis* was shown to substantially improve not only the phytochemicals content and antioxidant activity but increased production of compounds that exhibited angiotensin I-converting enzyme (ACE I) inhibitory activity (De Villa et al., 2021). Chi and Cho (2016) observed a reduction to varying degrees, in the concentrations of protein- and carbohydrate-based anti-nutritional factors (ANFs), as well as allergens during SSF of soy bean meal (SBM) with *Saccharomyces cerevisiae*, *Bacillus amyloliquefaciens* and *Lactobacillus* spp. Reports like this and others by various workers have successfully confirmed fermentation-induced improvements in both nutritional and bioactive properties; increasing the usability of important pulses like soybean, lupin, cowpea, etc. In line with this, the overall outcomes of SSF could be greatly influenced by the choice of microbe used as starter, the fermentation time as well as environmental conditions like pH, temperature, etc. *R. oligosporus* is a GRAS (generally regarded as safe) fungi popular for its use in the production of the Indonesian *tempe*; a good number of studies have reported the suitability of this mould in SSF for the enhancement of antioxidant properties, protein digestibility and protein content among others, in different plant-based products (Ojo et al., 2022; Ndego et al., 2023; Egbune and Tonukari, 2023). To our knowledge, there are no existing studies that have explored the application of *Rhizopus oligosporus* in Solid State Fermentation (SSF) of cowpea (*Vigna unguiculata* L. Walp) and groundnut (*Arachis hypogaea* L.), nor have they investigated the potential advantages of adjusting the concentration of the starter organism on the fermentation-related properties of these functional foods. Hence, the main objective of this study is to assess the impact of microbial cell size on the SSF of cowpea and groundnut using *Rhizopus oligosporus*.

MATERIALS AND METHODS

Collection of samples and preparation: Groundnut (*Arachis hypogaea* L.) and cowpea (*Vigna unguiculata* L. Walp) were purchased from the main market in Abraka, Delta State. The samples were verified and authenticated by the Department of Botany, Delta State University and was given the voucher number (DELSUH: 20 and 117). The samples were air dried for four days, before grinding using an industrial blender (SM-1 Retsch GmbH 5667 HAAN, West Germany). Dry, grounded samples were stored in clean dry containers until required for analyses. Seven (7) grams of dry grounded groundnut and cowpea were added separately to sterile petri dishes (7 per sample type) and served as substrate for the fermentation.

Starter organism and substrate preparation for SSF: The Harmony Pathological Laboratory, based at Songhai in Amukpe, Sapele, Delta State, provided the *Rhizopus oligosporus* strains (produced by PT Aneka Fermentas Industry, Bandung, Indonesia). *R. oligosporus*, in dried preserved form was weighed (0.35, 0.70, 1.05, 1.40, 1.75 and 2.10 g), and added to 7 g of either dried grounded groundnut or cowpea in sterile petri dishes; the mixture was homogenized using 15 ml of 50 mM acetate buffer solution at pH 6 to obtain final inoculum concentrations of 5, 10, 15, 20, 25 and 30 % w/w. The corresponding number of cells in the mass of fungal stock used was: 1.89 x 10^2, 3.78 x 10^2, 5.67 x 10^2, 7.56 x 10^2, 9.45 x 10^2 and 11.34 x 10^2 CFU g^{-1}. The combinations were placed in covered petri dishes and let to ferment for 72 hours. Alongside the test samples, unfermented control samples (0%; containing dried, ground-up groundnut or cowpea, free of any molds, with buffer only) were made. The samples were given the following labels to indicate the final inoculum concentration: 0%, 5%, 10%, 15%, 20%, 25%, and 30%. Approximately 6 g of each type of sample—fermented and unfermented—was collected separately after fermentation. 40 ml of sterile distilled water was then added, and the mixture was homogenized with a mill and pestle. Test tubes containing 10 ml of each homogenate were spun at 3000 rpm for 10 min to collect the supernatant. To ascertain the impact of inoculum size on the parameters examined, various experiments used the supernatant as the crude extract.

Quantification of total phenols and flavonoids content: Singleton and Rossi (1965) approach was used to determine the phenolic content of fermented and unfermented samples. In a nutshell, 1 ml of Folin C reagent was mixed with 1 ml of supernatant. After 3 minutes, 1 ml of saturated Na_2CO_3 solution was added, followed by 10 ml of distilled water. Prior to measuring absorbance at 765 nm, reaction mixtures were stored in the dark for 90 minutes. Following that, total phenolic content was estimated as milligram gallic acid equivalent per gram of extract (mg GAE /g). For flavonoids quantification, the colorimetric method reported by Jia et al. (1999) with modifications was used. 250 µL of crude extract was mixed with 1.25 ml of distilled water and 75 µL of 5% NaNO2 solution. After 5 minutes, 150 µL of 10% $AlCl_3.H_2O$ was added, then 500 µL of 1 M NaOH, and finally 275 µL of distilled water after 6 minutes. The solution was thoroughly mixed, and the color intensity at 415 nm was measured. The total flavonoid concentration was determined and represented as milligram catechin equivalent per gram of extract (mg CE /g).

Determination of antioxidant activity based on the DPPH (2,2-diphenyl-1-picrylhydrazyl) method: The antioxidant activity of fermented and unfermented samples was measured using the DPPH test, as described by Hatano et al (1988). A 2.7 ml methanolic solution of DPPH radical (6 x 10^{-5} mol l^{-1}) was added to 0.3 ml of the extract. The mixture was briskly agitated and allowed to stand in the dark for 60 minutes until stable absorption values could be obtained. The absorbance at 517 nm was used to calculate the reduction of the DPPH radical.

$$\%\ \text{Inhibition of DPPH radical} \quad = \frac{(\text{A0} - \text{A1})}{\text{A0}} X\ 100$$

Where A0 is the absorbance of the control DPPH solution (without extract) and A1 is the absorbance in the presence of the extract.

Biochemical assays: α-amylase activity and total soluble proteins concentrations: The Nouadri, et al. (2010) method was used to measure the alpha α-amylase activity using the DNS (3,5-dinitrosalicylic acid) reagent. The principle is based on the conversion of starch to maltose by α-amylase. Maltose released is detected from the coloured pigment formed when the pale yellow alkaline 3,5-dinitrosalicylic acid is changed to orange-red colour. The intensity of the colour produced is proportional to the concentration of maltose present in the sample; the absorbance of the coloured pigment was measured at 540 nm. 0.2% (w/v) maltose solutions were prepared in the concentration range 0 - 2 mg ml^{-1}, and used as standard for calibration. The amount of enzyme that released 1 g of maltose (as reducing sugar equivalent) per ml per minute under the assay conditions was considered one unit (U) of amylase activity. Enzyme activity in samples was determined using the relationship shown below;

$$Enzyme\ Activity = \frac{A}{B\ x\ C}$$

Where A = microgram (µg) Maltose released; B = volume (in mL) of enzyme used; C = incubation time (0 mins.)

Total soluble protein was determined by the Biuret method described by Gornall, et al. (1949). Samples and protein standard (bovine serum albumin) in the concentration range 0.5 – 10 mg ml^{-1} was prepared in series of test tubes to a final volume of 0.5 ml. 2.5 ml of Biuret reagent was added to each sample and standard solutions, vortexed and allowed to react for 30 min. After incubation, the absorbance of samples and standards was read at 540 nm. A standard calibration curve was plotted and used to extrapolate the amount of proteins present in samples.

Miller's DNS colorimetric technique was used to perform the reducing sugars assay (1959). In a nutshell, 1.5 ml of DNS reagent was mixed with 1.5 ml of samples in slightly covered test tubes. The mixture was heated at 90 degrees Celsius for 15 minutes to generate the red-brown coloration. To stabilize the color, 0.5 ml of a 40% potassium sodium tartrate (Rochelle salt) solution was added. Absorbance was measured at 575 nm after cooling to room temperature in a cold water bath.

Glucose and reducing sugars concentrations: Glucose was measured using standard kits and the manufacturer's recommended procedure (Randox, UK). In brief, 20 µL of standard solution and 20 µL of each sample were pipetted into a series of test tubes, and each test tube received 2 ml of reconstituted glucose working reagent. The contents of the test tubes were combined and incubated at 25ºC for 25 minutes. At 500 nm, the absorbance of the standard and samples was measured. In all cases, distilled water was utilized as a blank, and sample preparations were done in triplicate. The glucose concentration (mg dl^{-1}) was estimated using the following formula.

$$Glucose\ Conc. = \frac{Abs_{sample}}{Abs_{standard}} x Std\ Conc.$$

Where Std con. = standard concentration is 120 m/mL

*Statistical analysis***:** Data obtained were subjected to statistical analysis using one-way ANOVA (analysis of variance) and Fischer’s test of least significance (LSD); values are presented as Mean ± Standard deviation. Results were considered significant at p-values less than 0.05, that is, at 95% confidence level ($p< 0.05$).

RESULTS AND DISCUSSION

The study evaluated the effect of incremental rise in inoculum amounts on fermentation-induced parameters of nutritional benefits using grounded groundnut and cowpea as substrates in solid state fermentation by *R. oligosporus*.

Determination of total phenols and flavonoids concentration in fermented and unfermented cowpea and groundnut: All inoculum sizes produced significantly more phenol and flavonoids in the fermented groundnut samples as compared to the control, with the 15% inoculum size providing the most phenols (255.0 mg GAE/g). The fermentation of cowpea showed a similar pattern, showing that the amount of phenol and flavonoids rose as a result of fermentation. This finding is consistent with a previous study's finding that SSF of pearl millet (*Pennisetum glaucum*) by *R. oligosporus* was significantly more effective than the unfermented sample at increasing total phenol and flavonoid levels (Egbune et al., 2021; Egoamaka et al., 2021; Ezedom et al., 2022). Phenolic compounds and flavonoids are secondary metabolites that are produced by plants and are known for their beneficial health effects, such as antioxidant, anti-inflammatory, and anti-carcinogenic activities (Abeysinghe et al., 2021; Rakha et al., 2022). The concentration of these compounds can be increased through fermentation, which is a process that involves the breakdown of complex organic compounds by microorganisms (Nozhevnikova et al., 2022).

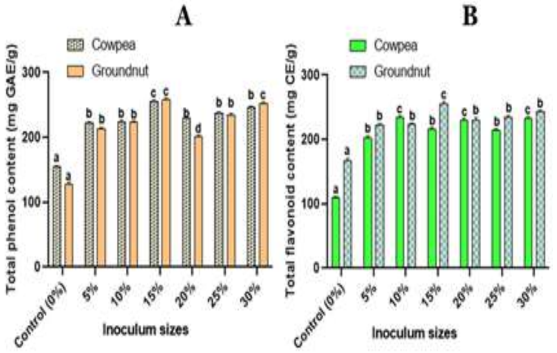

Fig 1. (**A**) Total phenolic and (**B**) Total flavonoids during solid state fermentation of cowpea and groundnut using different inoculum sizes of *Rhizopus oligosporus*. Significant differences from the control exist in superscripts with distinct letters ($p < 0.05$)

Fermentation of cowpea and groundnut has been shown to result in an increase in the concentration of total phenols and flavonoids. A study by Oyelere et al. (2010) investigated the effect of fermentation on the phenolic compounds in cowpea. The authors found that the concentration of total phenols was significantly higher in fermented cowpea compared to unfermented cowpea. They attributed this increase to the breakdown of complex phenolic compounds by the microorganisms involved in the fermentation process, which made the compounds more bioavailable. Similarly, a study by Zhou et al. (2021) examined the outcome of fermentation on the flavonoid content of groundnut. They found that the concentration of total flavonoids was significantly higher in fermented groundnut compared to unfermented groundnut. They suggested that the increased availability of flavonoids in fermented groundnut was due to the action of hydrolytic enzymes produced by the microorganisms during the fermentation process, which broke down the complex flavonoid compounds into simpler, more bioavailable compounds. The increase in total phenols and flavonoids concentration in fermented cowpea and groundnut is a result of the action of microbes in the course of the fermentation procedure (Saharan et al., 2020; Duhan et al., 2021). The microorganisms produce hydrolytic enzymes that break down complex phenolic and flavonoid compounds into simpler, more bioavailable compounds, resulting in an increase in the concentration of these beneficial compounds.

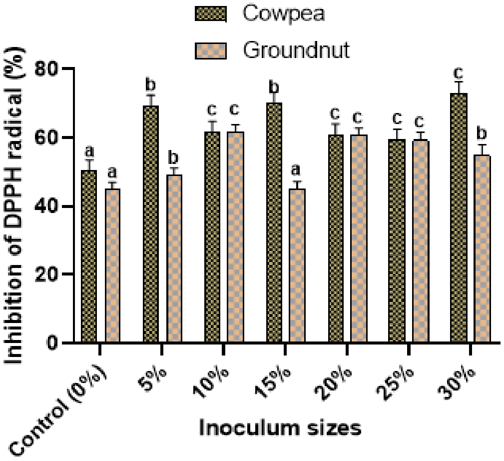

Fig 2. Percentage inhibition of DPPH during solid state fermentation of cowpea and groundnut using different inoculum sizes of *Rhizopus oligosporus.* Significant differences from the control exist in superscripts with distinct letters ($p < 0.05$)

Determination of free radical scavenging activity of DPPH in fermented and unfermented cowpea and groundnut: Free radicals are highly reactive compounds that can cause damage to cells and contribute to the development of various diseases, such as cancer, heart disease, and aging. Antioxidants, such as phenolic compounds and flavonoids, can scavenge free radicals and prevent oxidative damage. Fermentation of cowpea and groundnut has been shown to result in an increase in the free radical scavenging activity of these foods. A study by Avanza et al. (2021) investigated the effect of fermentation on the antioxidant properties of cowpea. The authors found that the fermented cowpea had a higher scavenging activity against the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical compared to the unfermented cowpea. They attributed this increase to the higher concentration of phenolic compounds in the fermented cowpea, which acted as antioxidants and scavenged free radicals. Similarly, a study by Zhou et al. (2021) investigated the effect of fermentation on the antioxidant activity of groundnut. The authors found that the fermented groundnut had a higher scavenging activity against the DPPH radical compared to the unfermented groundnut. They suggested that the increased antioxidant activity of fermented groundnut was due to the increased concentration of flavonoids, which are potent antioxidants and scavenge free radicals. The increase in the free radical scavenging activity of DPPH in fermented cowpea and groundnut compared to unfermented samples is a result of the increased concentration of antioxidants, such as phenolic compounds and flavonoids, in the fermented foods. The increased antioxidant activity of these fermented foods can have important implications for human health, as it may help to prevent oxidative damage and reduce the risk of various diseases. The recognized antioxidant molecules flavonoids and phenols have sufficient capacity to reduce the negative effects of free radicals (Orororo et al., 2018a,b; Orororo et al., 2022). Therefore, it is not surprising that the fermented samples' ability to block the DPPH radical increased significantly as compared to the unfermented sample (at the various inoculum sizes) (control). Egbune et al. (2021), (2022b) and Sadh et al. (2022) all found similar findings. A similar increase in DPPH radical scavenging capacity in black soybean by SSF with *Bacillus subtilis* was also found by Juan and Chou (2010). The research by Sadh et al. (2018), Saharan et al. (2020), and Duhan et al. (2020) also showed that *Aspergillus oryzae*'s SSF of *O. sativa* and *Lablab purpureus* boosted both the ABTS and DPPH scavenging characteristics. Similar to this, Sadh et al. (2018) discovered that peanut press cake's free radical scavenging abilities increased after Aspergillus awamori fermentation. All of these point to the presence of chemicals in the fermented samples that can donate hydrogen to free radicals (chemical entities with lone pair electrons), reducing them to their inert states and perhaps preventing free radical-induced cellular damage when ingested (Tonukari et al., 2016). Fermentation has been linked to an increase in phenolic compounds and antioxidant activity following the breakdown of the grain cell wall and subsequent enzyme activities that result in the release of bound phenolic molecules, which promote antioxidant capabilities (Salar et al., 2012; Adebo and Medina-Meza, 2020). Phenolic chemicals typically exist in grains and legumes in an esterified state that is connected to the cell wall matrix and, as a result, is not easily accessible. The release of the insoluble bound phenolic acids during fermentation is thought to boost the bioavailability of the antioxidants as well as their antioxidant activity (Moore et al., 2007). During fermentation, fermenting organisms metabolize and modify phenolic chemicals into additional conjugates, glucosides, and/or related forms. According to studies (Gänzle 2014; Katina et al., 2012), this metabolism of phenolic compounds during fermentation increases their bioavailability and results in the production of chemicals that affect flavor (Czerny and Schieberie, 2002; Rodriguez et al., 2009). Any type of oxidative/nitrosative stress or its effects can be reduced by antioxidants, which can be endogenous or exogenous substances (Kurutas, 2016). According to Slavin (2003), the primary defensive role of antioxidants in the body is their interaction with free radicals. Antioxidants work as free radical scavengers, singlet oxygen quenchers, and reducing agents by inhibiting prooxidant enzyme activity (Verma, 2008; Sevgi et al., 2015). The stimulation of detoxification mechanisms through phase II conjugation processes, which stop carcinogens from forming from their precursors as well as from reacting with vital cellular macromolecules, is one potential mechanism by which phenols and flavonoids give antioxidant protection (Slavin et al., 2000). Additionally, phenolic compounds have an impact on some cellular signaling processes, donate an electron to free radicals or transfer a hydrogen atom to them, activate endogenous antioxidant mechanisms, boosting the levels of antioxidant enzymes, and act as chelators for trace metals necessary for free radical defense (Juurlink et al., 2014).

Effect of inoculum size on fermentation-induced α-amylase activity and total soluble proteins concentrations: Comparing the fermented samples to the unfermented sample (control) at various inoculum sizes, amylase activity and soluble protein content rose dramatically. This discovery was made for both cowpea and groundnut, and it is compatible with the findings of Egbune et al. (2022a), who discovered an increase in amylase activity during solid state fermentation of cassava (*Manihot esculenta* Crantz) with the fungus *R. oligosporus*. This rise was attributable to microorganisms producing amylolytic enzymes during fermentation, which broke down complex starch molecules into simple sugars (Arya et al., 2022). Aganbi et al. (2020) demonstrated that solid state fermentation of yam (Dioscorea Spp) peels by *Rhizopus oligosporus* increased amylase activity, whereas Egbune et al. (2021) established that solid state fermentation of pearl millet (P. glaucum) by *R. oligosporus* increased amylase activity statistically ($p<0.05$) compared to that present in the unfermented. At various inoculum sizes, fermentation has been demonstrated to boost the activity of enzymes such amylase, cellulose, and -glucosidase (Sulyman et al., 2020). Sulyman et al. (2020) assert that the quantity of microorganisms added to the fermentation medium constitutes the size of the inoculum. When a single inoculum is used to manufacture enzymes during fermentation, it may take longer for enzymes to be secreted and a much smaller amount of enzymes will be produced than when the inoculum is doubled or even tripled. Abduh et al's 2020 solid-state fermentation of groundnut (*Arachis hypogaea*) shell to make cellulose using *Trichoderma sp*., tape yeast, and tempeh yeast revealed that mixed cultures exhibited cellulase activity that was greater than that produced by single cultures. However, a significant decrease in the generation of amylase activity was seen in this investigation at inoculum sizes above 20%. Amylase activity and production may have decreased when inoculum size increased due to cell clumping, which may have decreased the amount of macro- and micronutrients in the fermentation medium (Kunamneni et al. 2005; Sulyman et al., 2020).

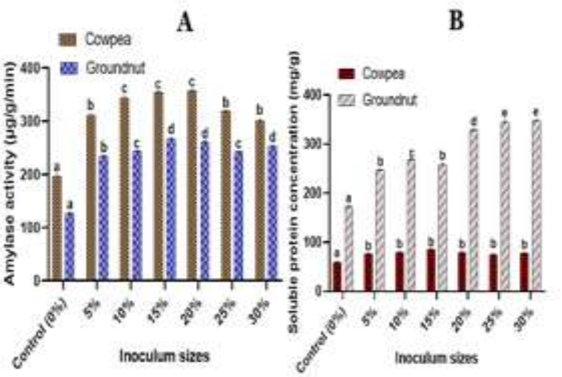

Fig 3. (**A**) Amylase activity and (**B**) Soluble protein concentration during solid state fermentation of cowpea and groundnut using different inoculum sizes of *Rhizopus oligosporus*. Significant differences from the control exist in superscripts with distinct letters ($p < 0.05$)

Similarly the increase in soluble protein concentration was due to the production of proteolytic enzymes by the microorganisms during fermentation, which broke down complex protein molecules into simpler, more digestible forms. A study by James et al. (2021) found that fermented groundnut had a higher concentration of total soluble proteins compared to unfermented groundnut. This increase was due to the production of proteolytic enzymes by the microorganisms during fermentation, which broke down complex protein molecules into simpler, more digestible forms. Anigboro et al (2020)'s findings that *R. oligosporus* increased the protein content of maize offal compared to control are also consistent with the findings of this investigation. Anigboro et al. (2022a), who examined the biochemical parameters of solid-state fermented cocoyam (*Colocasia esculenta*) using *R. oligosporus* at various inoculum sizes, likewise showed an increase in protein content as a result of SSF. The extracellular proteins and enzymes secreted by the microorganisms for their metabolic processes during fermentation were thought to be the cause of the elevated protein content

(Oseni and Ekperigin, 2007; Chomini et al., 2020; Egbune et al., 2021). In order to increase the protein content of cereals and legumes, fermentation is one of the finest food processing methods (Kumitch, 2019; Adhikari et al. 2013; Anigboro et al., 2022b).

Determination of glucose and reducing sugar concentration in fermented and unfermented cowpea and groundnut: The concentration of glucose and reducing sugar increased in all the inoculum sizes examined. The increase in glucose and reducing sugar concentration in fermented cowpea and groundnut using *Rhizopus oligosporus* compared to the unfermented samples has been widely studied and documented in the scientific literature. *Rhizopus oligosporus* is a type of filamentous fungus that is commonly used for the fermentation of legumes, including cowpea and groundnut. During fermentation, *Rhizopus oligosporus* secretes various enzymes, including amylases, which break down complex carbohydrates into simpler sugars like glucose. This result in an increase in the concentration of glucose and reducing sugars in the fermented cowpea and groundnut compared to the unfermented samples. For example, in one study, fermented cowpea using *Rhizopus oligosporus* was found to have higher concentrations of glucose and reducing sugars compared to unfermented cowpea (Ogunniyi et al., 2006). Similarly, another study found that fermentation of groundnut using *Rhizopus oligosporus* resulted in an increase in glucose and reducing sugar concentration compared to unfermented groundnut (Adekunle and Adebowale, 2013). The concentration of glucose and reducing sugars in fermented and unfermented cowpea and groundnut can vary depending on several factors, including the type of fermentation process used, the duration of fermentation, the type of microorganisms involved, and the conditions under which the fermentation took place (such as temperature, pH, and moisture content).

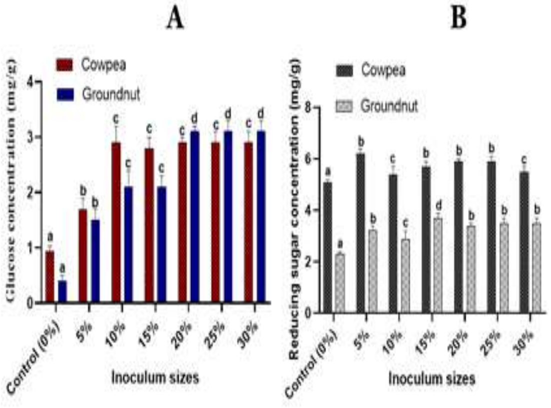

Fig 4. (**A**) Glucose and (B) Reducing sugar concentration during solid state fermentation of cowpea and groundnut using different inoculum sizes of *Rhizopus oligosporus*. Significant differences from the control exist in superscripts with distinct letters ($p < 0.05$)

In general, however, fermented cowpea and groundnut tend to have higher concentrations of glucose and reducing sugars compared to unfermented samples. This is because fermentation can break down complex carbohydrates, such as starch, into simpler sugars like glucose. This is accomplished through the action of enzymes produced by microorganisms during fermentation, which break down the complex carbohydrates into simpler sugars that can be more easily utilized by the body. Additionally, some fermentation processes can enhance the production of amylolytic enzymes, which further increases the breakdown of starch into glucose and other reducing sugars. This increased availability of glucose and reducing sugars in fermented cowpea and groundnut can make these foods a more valuable source of nutrition for human consumption.

Conclusion: From this study, it is obvious that the selected fungus isolate, *Rhizopus oligosporus* showed high enzymatic activities and increased value added product of cowpea and groundnut at varying inoculum size during fermentation. Therefore, fermenting cowpea and groundnut before using them as functional ingredients may be nutritionally interesting because doing so results in a product that is more stable, safer, and has the potential to be used in novel cuisines.

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