Green Synthesis for the Production of Glucose Syrup from Waste Cassava Starch Using Alpha-Amylase

*[1]Fatai A. Aderibigbe, [1]Esther O. Babatunde, [1]Sunday O. Ochapa and [2]Harvis B. Saka
[1]Department of Chemical Engineering, University of Ilorin. Ilorin, Kwara State
[2]Department of Chemical and Environmental Engineering, University of Arizona, Tucson, USA
aderibigbe.fa@unilorin.edu.ng | babatunde.eo@unilorin.edu.ng | ochapasunday1@gmail.com | sakaharvis@gmail.com

Received: 21-APRIL-2024; Reviewed: 19-JUNE-2024; Accepted: 20-JUNE-2024

https://dx.doi.org/10.4314/fuoyejet.v9i2.24

ORIGINAL RESEARCH

Abstract --- Research on production of glucose syrup from waste cassava starch has not been adequately covered. In this paper, we report on the process for breaking down starch through harnessing the catalytic properties of silver nanoparticles (AgNPs) in an enzymatic procedure that encompasses the breakdown of starch by alpha-amylase. The analysis carried out on the starch-synthesized AgNPs had its peak absorbance in the region between 400 nm and 500 nm, the X-ray diffraction (XRD) result also showed that the AgNPs were crystalline and this indicated that the nanoparticle formed could be used for hydrolysis. The presence of silver nanoparticles was observed to enhance the speed at which starch was enzymatically broken down as sample G1 (which was the sample with an equal amount of silver nanoparticle and alpha-amylase) has a concentration of 5.23 mg/mL (which implies a percentage conversion of approximately 52.3%). It is hypothesized that alpha-amylase became immobilized on the nanoparticle surface giving rise to a better glucose yield in this study.

Keywords: alpha-amylase, hydrolysis, starch, silver nanoparticles

1. INTRODUCTION

Silver nanoparticles (AgNPs) have found extensive applications in numerous consumer products. They have been employed for catalytic reactions, energy production, electronics, biotechnology, textiles, beauty products, and food packaging due to their remarkable optoelectronic properties (Aritonang et al., 2019). Also, they demonstrate significant promise for diverse medical applications, including wound recovery, diagnostic tools, biological sensing technology, and the transportation of pharmaceuticals. In the food industry, they also serve as effective antimicrobial agents, leveraging silver's well-known exceptional electrical conductivity (Ernest et al., 2012).

Silver nanoparticles (AgNPs) facilitate enhanced electron transfer in biosensors compared to gold nanoparticles. The interaction between metallic nanoparticles and biological molecules has attracted considerable interest in recent times, driving progress in diagnostics, sensor technology, and precise drug delivery.

*Corresponding Author

Section D- MATERIAL/CHEMICAL ENGINEERING & RELATED SCIENCE

Can be cited as:

F. A. Aderibigbe, E. O. Babatunde, S. O. Ochapa and H. B. Saka (2024). Green Synthesis for the Production of Glucose Syrup from Waste Cassava Starch Using Alpha-Amylase, FUOYE Journal of Engineering and Technology (FUOYE**JET), 9(2), 320-325.**
https://dx.doi.org/10.4314/fuoyejet.v9i2.24

Significantly, the immobilization of glucose oxidase on AgNPs led to a threefold improvement in sensitivity for glucose biosensors (Basiri et al., 2018).

Presently, organic synthesis of complex molecules incorporates nanoparticles for catalysis due to their exceptional catalytic properties and the selectivity of nanomaterials (Bhosale and Bhanage, 2015). The size and shape of the nanoparticles, the conditions of preparation, the addition of support materials, and the capping agent all play pivotal roles in catalysis.

The food industry extensively relies on starch as a primary raw material for producing maltodextrin, liquid dextrose, liquid glucose, high fructose corn syrups, and corn steep liquors. Enzymes, which are biological catalysts capable of accelerating reactions with or without cofactors, play a crucial role in these processes without altering their activity. An example of this is a-amylases, prominent digestive enzymes found in animal saliva and the pancreas, which break down complex starch into maltose and glucose by catalyzing the endo hydrolysis of α-(1--->4)-D-glucosidic linkages within starch (Matheson and McCleary, 1985).

Recent studies have explored green synthesis methods for producing glucose syrup from waste cassava starch utilizing alpha-amylase enzymes. Researchers have investigated the optimization of reaction conditions, such as pH and temperature, to enhance enzyme activity and maximize glucose yield (Selina *et al.*, 2023). Additionally, studies have focused on isolating and characterizing alpha-amylase-producing fungi from cassava flour, highlighting the potential of Aspergillus species for large-scale enzyme production (Munawar *et a.*, 2023). Furthermore, the extraction of starch from cassava agro-industrial wastes like cassava peel and bagasse has been studied, demonstrating the feasibility of utilizing these non-edible sources for starch extraction and potential applications in biodegradable food packaging. These findings collectively contribute to sustainable practices in the production of glucose syrup from waste cassava starch, showcasing the versatility and efficiency of alpha-amylase enzymes in converting cassava-derived materials into valuable products. In their study, (Zohrevandi et al., 2021; Kumar et al., 2008) utilized nickel nanoparticles as a catalyst to selectively promote the oxidative coupling of thiols, resulting in the production of disulfides. They achieved the catalytic conversion of n-dodecanethiol, n-butanethiol, and n-octanethiol into their respective disulfides by employing silver nanoparticles coated with polyvinylpyrrolidone (PVP). Additionally, they harnessed silica-supported AgNPs as robust and recyclable catalysts in Diels–Alder cycloadditions of 20-hydroxy chalcones and dienes, yielding substantial product yields and turnover rates (Madhavan & Okamoto; Chopra et al., 2016).

The current study is focused on producing glucose syrup from waste starch obtained from the 'garri' processing industry using alpha-amylase hydrolysis and silver nanoparticle synthesis. This research lays the groundwork for understanding the interactions between nanoparticles and biomolecules, potentially opening new avenues for applications in the food industry.

2. MATERIAL AND METHODS

2.1 CHEMICALS AND REAGENTS

Chemicals and reagents used for this work were alpha-amylase, soluble starch, 3, 5-dinitrosalicylic acid (DNSA), potassium sodium tartrate (Rochelle salt), sodium hydroxide, and silver nitrate and all other chemicals were analytical grade from Labio Scientific Centre, Lagos, Nigeria.

2.2 SILVER NANOPARTICLE SYNTHESIS

Simultaneously, soluble starch with concentrations varying between 10 mg/mL and 80 mg/mL was mixed with the solution, depending on the specific sample composition as presented in Table 1 and subjected to autoclaving at 121°C and 15 psi for a time of 4 and 6 min. The development of a brown-hued solution indicated the formation of AgNPs (Pani et al., 2016; Kumar, et al., 2022). After cooling, this solution was utilized for subsequent analyses.

2.3 UV - VIS SPECTROPHOTOMETRY

The AgNPs derived from starch synthesis underwent UV-visible analysis. At specific time intervals, the samples were examined using a UV-visible spectrophotometer (Vigneshwaran *et al.*, 2006; Nangare et al., 2021)). To confirm the existence of silver nanoparticles, spectral analysis was conducted across the wavelength span from 200 nm to 1000 nm.

2.4 TRANSMISSION ELECTRON MICROSCOPY (TEM)

The morphology and dimensions of the created AgNPs were examined using transmission electron microscopy (TEM) with a JEM-ARM200F-G TEM instrument. The TEM sample preparation procedure encompassed the following steps: initial specimen fixation to prevent further alterations or harm to the cell, followed by dehydration, infiltration, polishing, and cutting. The resulting sections, ranging in size from 30 nm to 60 nm for optimal resolution, were collected in a receptacle and then transferred to a copper grid for observation under the microscope.

2.5 X-RAY DIFFRACTION (XRD)

The morphology and dimensions of the AgNPs were examined using transmission electron microscopy (TEM) with a JEM-ARM200F-G TEM instrument. The TEM sample preparation procedure encompassed the following steps: initial specimen fixation to prevent further alterations or harm to the cell, followed by dehydration, infiltration, polishing, and cutting. The resulting sections, ranging in size from 30 nm to 60 nm for optimal resolution, were collected in a receptacle and then transferred to a copper grid for observation under the microscope. The specifications of the equipment used for this analysis are as follows: Diffractometer type: PW 1800, Model: Rigaku D/Max-IIIC; Tube anode: Cu; Generator tension [kV]: 40; Generator current [mA]: 20; Wavelength Cuka 1[A^{O}]: 1.667; Wavelength Cuka 2[A^{O}]: 1.659; Intensity ratio (alpha2/alpha1): 0.500.

Table 1: Various sample runs from design expert

Runs (samples)	Factor 1 $AgNO_3$ concentration (mg/mL)	Factor 2 Starch concentration (mg/mL)	Factor 3 Time (min)
A	1	10	6
B	0.5	10	4
C	0.5	80	4
D	1	80	6
E	1	10	4

https://dx.doi.org/10.4314/fuoyejet.v9i2.24 engineering.fuoye.edu.ng/journal

F	0.5	80	6
G	0.5	10	6
H	1	80	4
A	1	10	6
B	0.5	10	4

2.6 HYDROLYSIS OF STARCH BY ALPHA-AMYLASE

The process of breaking down soluble starch through hydrolysis was conducted in conditions where the temperature remained constant. Experiments were executed using tubes that held both a starch sample for comparison and a starch sample for testing purposes. A varied quantity of α-amylase was introduced into every tube. This outlines the experimental procedure. The sample F contained no nanoparticles, the sample G1 – 1 mL of nanoparticles and 1 mL of amylase', the sample G2 – 2 mL of nanoparticles and 1 mL of amylase', and the sample G3 – 1 mL of nanoparticle and 2 mL of amylase. The tubes were kept in an incubator at 37 ℃ for 5 min. Subsequently, samples were introduced into the spectrophotometer or colorimeter at a wavelength of 540 nm to measure the concentration of reducing sugars, following (Miller, 1959). The UV-Vis spectrophotometer plot was used to quantify the amount of reducing sugars generated from each sample.

3. RESULTS AND DISCUSSION

The aqueous silver nitrate ($AgNO_3$) solution was a coluorless solution, but after the synthesis of the silver nanoparticle in the autoclave there was a brown colour formation (Pani et al., 2016) which indicated the formation of silver nanoparticles. This colour formation agreed with the study reported by (Pani et al., 2016; Kumar, et al., 2022). UV-Vis spectrophotometry studies, Transmission electron microscopy, and X-ray diffraction techniques were employed to confirm the existence of AgNPs and to assess their shape, structure, and constituent elements before applying the produced nanoparticles in the process of starch hydrolysis. Figure 1, is the spectrophotometry plot for sample G, this shows the relationship between the absorbance and wavelength of the silver nanoparticle produced. In Figure 1, the sample with the best yield was selected (this was the sample which had the highest peak of absorbance between the wavelength of 400 nm-500 nm) and used for further analysis and hydrolysis of starch. The absorbance spectrum of silver nanoparticles typically falls within the range of 400 nm to 500 nm, as indicated by the formation of pure and spherical silver nanoparticles with a sharp peak around 400 nm in the UV-vis spectra (Khudair *et al.,* 2023). Additionally, the resonant nonlinear optical absorption properties of silver nanoparticles were studied using laser pulses at 400 nm, showing saturable absorption and reverse saturable absorption behaviours (Jiang *et al.,* 2021). Furthermore, the absorption spectrum of metallic nanoparticles, including silver nanospheres, is crucial for characterizing properties like size and shape imperfections, with the absorption spectrum being particularly sensitive to these factors (Wang *et al.,* 2021). These studies collectively emphasize the importance of understanding the absorbance characteristics of silver nanoparticles within the specified wavelength range for various applications in fields like nanotechnology and optical devices. Figure 2, depicts the XRD plot of the silver nanoparticle. Figure. 2 shows that the silver nanoparticles are crystalline and this confirm the structure of silver nanoparticle present in the sample. Figures.3 (a) and (b) show the TEM image for the silver nanoparticle produced. This verifies that the Sample is Nano-sized. Figures.3(a) and (b) showed nanoparticle sizes at a magnification of 20 nm and 100 nm used respectively.

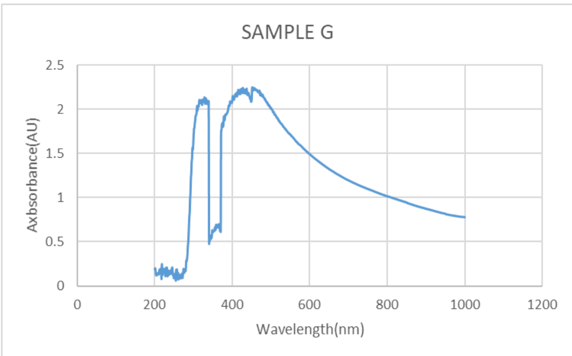
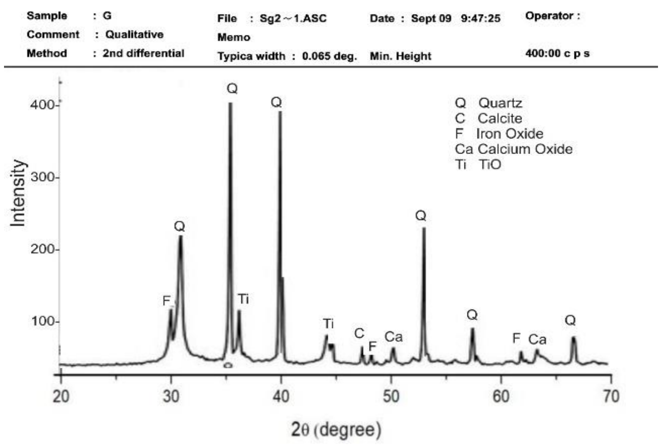


Figure 1: Graph of Absorbance Versus Wavelength UV-Vis for the synthesized AgNPs (sample G)



https://dx.doi.org/10.4314/fuoyejet.v9i2.24 engineering.fuoye.edu.ng/journal

Figure 2: Plot of the XRD results for the synthesized silver nanoparticle

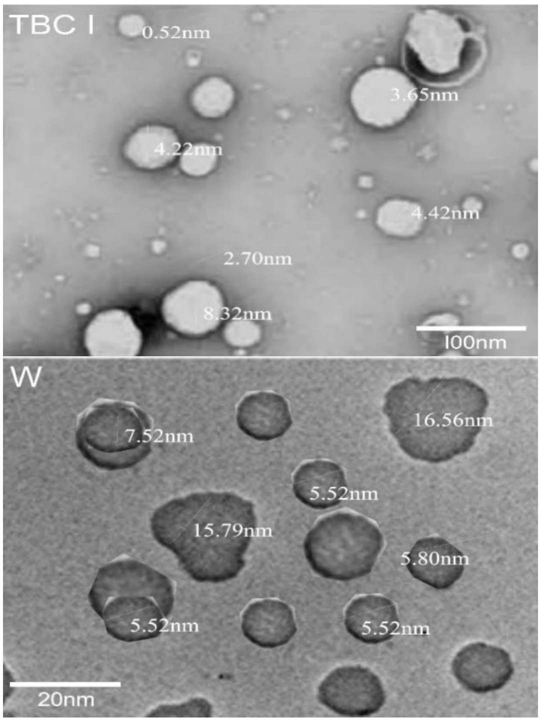


Figure 3: TEM image of silver nanoparticles synthesized from starch

Also, the UV-Vis spectrophotometer was utilized for the assessment of glucose levels in the samples at a wavelength of 540 nm to show which of the combination ratios gave the best yield of glucose. The glucose content of the syrup synthesized by using AgNPs produced, and $AgNO_3$ free hydrolyzed wastestarch were analyzed by using the UV-vis spectrophotometer instrument. Fig 4 shows glucose standard curve. Figure.5, depicts graph of absorbance versus wavelength for the syrup produced. The glucose concentration of the samples produced was determined, using the glucose standard curve shown in Figure 4 by applying the equation derived from the curve. Figure.5, was used for the determination of the syrup produced by using different combination ratios of the sample G and alpha-amylase, and the (AgNPs free-syrup). Sample G2 has a concentration of 2.41 mg/mL, sample G3 has a concentration of 3.44 mg/mL, sample F has a concentration of 2.03 mg/mL and sample G1 has a concentration of 5.23 mg/mL (which implies a percentage conversion of approximately 52.3%). This agrees with the work reported by (Falkowska & Molga, 2014).

4. CONCLUSION

In summary, the analysis carried out on the starch-synthesized AgNPs gave a very good result in terms of size, shape, etc. and this showed that the nano- particle formed could be used for the hydrolysis; the UV-vis spectrophotometer analysis carried out, showed that the sample of starch that was hydrolyzed with AgNPs and alpha-amylase gave a better yield of syrup than the sample hydrolyzed with only alpha-amylase (i.e. In the presence of AgNPs, the degradation of starch digestion kinetics led to a rapid increase in the production of a higher quantity of reducing sugars); Sample G1 had a concentration of 5.23 mg/mL, sample G2 had a concentration of 2.41 mg/mL, sample G3 had a concentration of 3.44 mg/mL, and

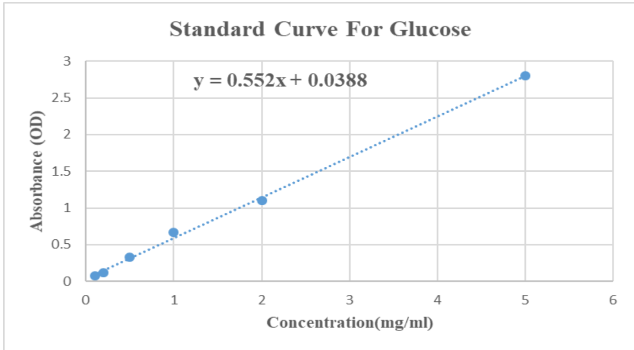


Figure 4: Glucose Standard Curve

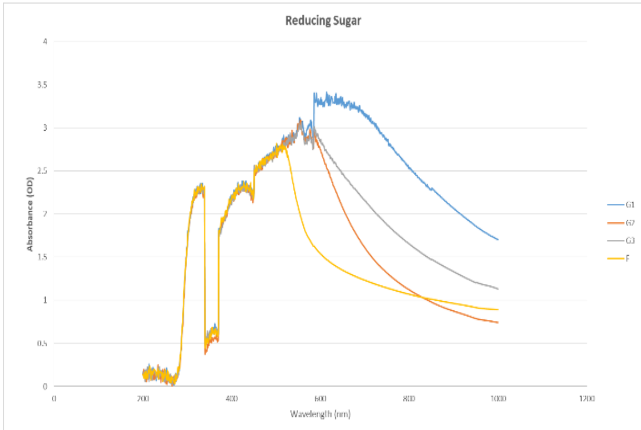


Figure 5: Graph of Absorbance Versus Wavelength UV-Vis for the syrup produced by using different combination ratios of the sample G and alpha-amylase, and the syrup produced (free of $AgNO_3$)

sample F had a concentration of 2.03 mg/mL. This study revealed that nanoparticles could exert a noteworthy impact within the realm of Nano-catalysis, offering a potential avenue for their application in starch industries to expedite the breakdown of intricate molecules into simpler ones by attaching enzymes to

https://dx.doi.org/10.4314/fuoyejet.v9i2.24 engineering.fuoye.edu.ng/journal

the nanoparticle surfaces. Additionally, the technique used for silver nanoparticle synthesis was noted for its stability, simplicity, and cost-effectiveness, not necessitating a significant financial investment. More research is needed to optimise conditions for enzyme activity, substrate concentration, and stability. Furthermore, selection of the appropriate enzyme combination is crucial for enhancing the conversion of cassava fibrous residue to glucose syrup, highlighting the importance of enzyme synergy in the green synthesis process.

ACKNOWLEDGMENTS

The authors express their gratitude for the authorization provided by the Department of Chemical Engineering at the University of Ilorin to access and use their laboratory facilities.

CONFLICT OF INTEREST

The authors state that there is no known conflict of interest.

REFERENCES

Aritonang, H.F., H. Koleangan, &A.D. Wuntu (2019): Synthesis of silver nanoparticles using aqueous extract of medicinal plants (Impatiensbalsamina and Lantana camara) fresh leaves andanalysis of antimicrobial activity.*International journal of microbiology, 2019. DOI: 10.1155/2019/8642303*

Basiri, S., A. Mehdinia, & A. Jabbari(2018): A sensitive triple colorimetric sensor based on plasmonic response quenching of green synthesizedsilver nanoparticles for determination of Fe2+, hydrogen peroxide, and glucose. *Colloidsand Surfaces A: Physicochemical and Engineering Aspects*, 2018. **545**: p. 138-146. DOI: 10.1016/j.colsurfa.2018.02.053

Bhosale, M. A. & Bhanage, B. M. (2015): Silver nanoparticles: Synthesis, characterization and their application as a sustainable catalyst for organic transformations.*Current Organic Chemistry,* **19**(8): p. 708-727. DOI: 10.2174/1385272819666150207001154

Chopra, Radhika; Sharma, Kamaldeep, Kumar, Vandana (2016). Pentacenequinone-Stabilized Silver Nanoparticles: A Reusable Catalyst for the Diels–Alder [4+ 2] Cycloaddition Reactions. *The Journal of Organic Chemistry,* 2016. **81**(3): p. 1039-1046. https://doi.org/10.1021/acs.joc.5b02495

Ernest, V., Shiny, P.J., Mukherjee, A. & Chandrasekaran, N. (2012): Silver nanoparticles: a potential nanocatalyst for the rapid degradation ofstarch hydrolysis by α-amylase.*Carbohydrate Research,* 2012. **352**: p. 60-64. https://doi.org/10.1016/j.carres.2012.02.009

Falkowska, M. & E.J. Molga (2014): Nanosilver: a catalyst in enzymatic hydrolysis of starch.*PolishJournal of Chemical Technology,* 2014. **16**(3): p. 111-113. DOI: 10.1016/j.carres.2012.02.009

Jiang, J., Wang, J., Wu, T., & Gao, Y. (2021, June). Nonlinear Absorption and Ultrafast Dynamics of Ag Nanoparticle. In Photonics (Vol. 8, No. 7, p. 233). MDPI. http://dx.doi.org/10.3390/photonics8070233

Kumar, A., Kumar, B.,Ghosh A., Tiwari, M. & Reyaz, M. (2022). Microbial production of silver nanoparticles. (Agnps) by some bacteriial isolates. IOSR Journal of Biotechnology and Biochemistry. Vol. 4, issue 4, pp 26-38. http://dx.doi.org/10.9790/264X-0404022638

Khudair, Z. F., Abass, Z. A., Salem, K. H., Mohammed, K. A., Zabibah, R. S., Alkhafaji, M. A., & Saxena, K. K. (2023). Wavelength Role in Synthesis and Study of the Structure of Silver Nanoparticles Prepared by Pulsing Laser Ablation in Liquid. International Journal of Nanoscience, 22(04), 2350032. https://doi.org/10.1142/s0219581x23500321

Kumar, Ajeet; Kumar, Santosh; Saxena, Amit; De Arnab & Mozumdar, Subho (2008): Selective Protection of Carbonyl Compounds over Nano-sized Nickel Catalysts. *Catalysis Letters,* 2008. **122**: p. 98-105. http://dx.doi.org/10.1007/s10562-007-9349-5

Madhavan, S., & Okamoto, S. (2018) Silica-Supported Silver as a Green aSustainable Catalyst for the [3+ 2]-Cycloaddition Reaction of Azomethine Ylides with 2′-Hydroxychalcone Derivatives. *ChemCatChem*, *10*(9), 2014-2018. http://dx.doi.org/10.1002/cctc.201702035

Matheson, N.K. & B.v. McCleary (1985): Enzymes metabolizing polysaccharides and their application to the analysis of structure and functionof glycans.*The polysaccharides,* 1985. **3**: p. 1-105.

Miller, G.L (1959): Use of dinitrosalicylic acid reagent for determination of reducing sugar *Analytical chemistry*, 1959. **31**(3): p. 426-428.

Munawar, A., Shaheen, M., Ramzan, S., Masih, S. A., Jabeen, F., Younis, T., & Aslam, M. (2023). Diversity and enzymatic potential of indigenous bacteria from unexplored contaminted soils in Faisalabad. Heliyon, 9(4). https://doi.org/10.1016/j.heliyon.2023.e15256

Nangare, G., Tegeli, V., Ingle, S., Matole, V., Birajdar, A., & Adlinge, S. (2021): UV Spectrophotometric Analysis and Validation of Dapsone in Semisolid Dosage Form. *Research Journal of Pharmacy and Technology*, 2021. **14**(9): p. 5007-5009. http://dx.doi.org/10.52711/0974-360X.2021.00872

Pani, A., J.H. Lee, &S.I. Yun (2016): Autoclave mediated one-pot-one-minute synthesis of AgNPs and Au–Ag nanocomposite from Melia azedarach bark extract with antimicrobial activity against food pathogens.*Chemistry Central Journal,* 2016.**10**: p. 1-11. DOI: 10.1186/s13065-016-0157-0

Selina, O. K., William, O. A., & Orua, A. O. (2023). Determination of Optimum Reaction Velocity for Glucose Production from Cassava Starch Using Gluco Amylase Sourced from Rice. Journal of Engineering Research and Reports, 25(6), 66-76. https://doi.org/10.9734/jerr%2F2023%2Fv25i6923

Vigneshwaran, N., Nachane, R., Balasubramanya, R., & Varadarajan, P. (2006): A novel one-pot 'green synthesis of stable silver nanoparticles using soluble starch.*Carbohydrate research,* 2006. **341**(12): p. 2012-2018. https://doi.org/10.1016/j.carres.2006.04.04

Wang, J., Shao, Y., Chen, C., Wu, W., Kong, D., & Gao, Y. (2021). Wavelength-dependent optical nonlinear absorption of Au-Ag nanoparticles. Applied Sciences, 11(7), 3072. https://doi.org/10.3390/app11073072