IPS Journal of Nutrition and Food Science
ISSN: 3115-5391
IPS J Nutr Food Sci, 6(2): 866-870 (2026)
https://ipsintelligentsia.com/

Edible Cookies Obtained from Wheat, Cocoyam and Pigeon Peas (*Cajanus cajan* L.) Composite Flour Blends Possessed Enhanced Antioxidant and Acetylcholinesterase Inhibitory Abilities

Bosede Oluwatoyin Ogungbesan[1], Adetinuke Olajumoke Ayoola-Bobola[1] and Praise Olusegun Oloye[2]

[1]Department of Hospitality Management Technology, Rufus Giwa Polytechnic, Owo, Nigeria.
[2]Department of Food Technology, Rufus Giwa Polytechnic, Owo, Nigeria.
*Corresponding author: ogungbesantoyin1@gmail.com; +2348066247789

Abstract
This study investigated the antioxidant and acetylcholinesterase inhibition properties of cookies produced from wheat-cocoyam-pigeon pea composite flour blends at different ratios. The cocoyam and pigeon peas were thoroughly processed into flours and mixed with the commercial wheat flour to obtain the WCP 1, 2, 3 and 4 (100:0:0, 80:10:10, 70:10:20, 60:10:30) (wheat:cocoyam:pigeon pea) flour blends, respectively used to bake cookies. The antioxidant and acetylcholinesterase (AChE) inhibitory activities were examined. The sample WCP 3 had improved antioxidant (~14-69%) and AChE-inhibitory (65%) properties when compared to WCP 1 (14-38%; 22%), WCP 2 (24-53%; 40%), WCP 4 (26-58%; 50%) and standards (GSH (~80%) and neostigimine (~84%), respectively. The addition of cocoyam and pigeon pea flours to wheat flour produced cookies loaded with better antioxidants and effective AChE inhibitory activities.

Keywords: Pigeon pea, cocoyam, acetylcholinesterase, inhibition, antioxidants, cookies

Article History
Received: 25 Apr 2026
Accepted: 29 May 2026
Published: 18 Jun 2026

Scan QR Code to view

License: CC BY 4.0
Open Access article

How to cite this paper: Ogungbesan, B. O., Ayoola-Bobola, A. O., & Oloye, P. O. (2026). Edible cookies obtained from wheat, cocoyam and pigeon peas (*Cajanus cajan* L.) composite flour blends possessed enhanced antioxidant and acetylcholinesterase inhibitory abilities. *IPS Journal of Nutrition and Food Science, 6*(2), 866–870. https://doi.org/10.54117/qgkt3r88

1. Introduction

Free radicals have been implicated to cause oxidative stress, in the disease pathogenesis and management systems, giving rise to unacceptable state of health that reduce the quality of life through development of several chronic diseases (Malomo *et al*., 2020). Therefore, it is important to inhibit or scavenge these radicals by using antioxidants. Although, the body possessed its natural self-defense mechanisms (such antioxidants like gluthathione and superoxide dismutase) to fight the free radicals, but there existed a concern about the ineffective amount being released during illness and old age developments (Thammarat *et al*., 2015). The serine hydrolase, Acetylcholinesterase (AChE), has long been known to play a very crucial physiological role in maintaining the major neurotransmitter, acetylcholine (ACh) in the body by rapidly clearing free ACh from the synapse (Kumar & Chowdhury, 2014). ACh is the neurotransmitter involved in cholinergic neurotransmission, released by presynaptic cholinergic terminals and activating nicotinic and/or muscarinic receptors to modify postsynaptic cell function (cognitive memory). Therefore, the dysfunction of cholinergic neurotransmission in the brain, the formation and growth of brain amyloid lesions, the senile plaques, is widely believed and reported (Willcox *et al*., 2014) to be contributing to a crucial event in the pathogenesis of Alzheimer's disease (AD). As of today, the current available therapy for AD consisted of the administration of AChE inhibitors due to their clinical efficacy in prolonging the half-life of ACh (Kumar & Chowdhury, 2014). Recently, there is a great awareness in the food industry and in preventive health care with respect to the development of natural nutraceuticals and functional foods ingredients from plant materials due to their high biodegradable/ digestion rate with no toxicity (residual effects or negative side effects). Example of such crops were pigeon pea and cocoyam, which were being purposefully grown for flours with high protein and fibre contents towards utilization in the production of snacks (cookies) and other baked products.

Cocoyam originated from Asia and about forty (40) species are mostly grown in West Africa (Efezino *et al*., 2023). Cocoyam contained a high carbohydrate content (70– 88% on dry basis), principally in the form of starch, which not only imparts desirable functional properties to foods, but also provides energy and promotes satiety in consumers. Cocoyam is highly medicinal for diabetic patients because it has low starch content, it is easily digestible and contains protein more than

the other root tubers. Its leaves have been shown to be a rich source of folic acid, riboflavin, vitamin A and C, calcium and phosphate (Efezino *et al*., 2023). The leaves are consumed because they are rich in protein and vitamins while the root is rich in carbohydrates and minerals. The corms and cormels are boiled, baked and tubers are sometimes ground to produce paste for use in stews and soups (Efezino *et al*., 2023).

Legumes are rich in protein and essential minerals but contain small quantities of fats that are mostly unsaturated. For instance, pigeon pea (*Cajanus cajan* L.) is relatively cheap and contained a high amount of protein (23%) that is a rich source of lysine but is usually deficient in sulphur-containing amino acids especially methionine and cystine. It is relatively high in protein and can be used to fortify bread (Adeola.*et al.*, 2011). The composite flour from cocoyam, pigeon peas and wheat flour is considered advantageous in the developing countries as it reduced the importation of wheat flour and encouraged the use of locally grown crops (Olugbuyi *et al*., 2022). Therefore, the cookies from indigenous crop like cocoyam and pigeon peas with low amount of wheat flour is expected to meet the demand of most coeliac and Alzheimer's disease patients (Bhatt & Rajinder, 2015). Therefore, the objective of this study is to explore the potential cognitive-health-related bioactivities in cookies produced from cocoyam, pigeon peas and wheat composite flour blends.

2. Materials and Methods

2.1 Materials

The cocoyam and pigeon peas were obtained from Shasha's market, Akure, Nigeria and authenticated at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria. Wheat flour and other baking ingredients were purchased from a commercial baking store in Akure, Nigeria. All chemicals used were of analytical grade and obtained from Sigma-Aldrich, London, United Kingdom.

2.2 Preparation of cocoyam and pigeon pea flour and formulation of composite flour blends

The cocoyam flour was obtained according to the method previously described by Alawode *et al*. (2017) while the pigeon pea flour was obtained according to the method previously described by Oguntuase *et al*. (2022). The composite flour blends were formulated as stipulated in the Table 1 *viz:*

Table 1: Flour blends formulations

Samples	Wheat	Cocoyam	Pigeon pea	Total (%)
WCP 1	100	0	0	100
WCP 2	80	10	10	100
WCP 3	70	10	20	100
WCP 4	60	10	30	100

2.3 Production of cookies

Cookies were produced as previously described by Udeh *et al*. (2024) with the following ingredients, composite flour, margarine, baking powder, salt, beet, eggs and water. The dry ingredients were thoroughly mixed in a bowl for few minutes followed by adding the margarine and eggs and kneaded to form batter. The batter was then rolled on a rolling board sprinkled with flour for a uniform thickness and cut with a 50 mm-diameter cookie cutter. The cookies were placed in baking trays leaving a 25 mm space in between and baked at 180 ^{0}C for 10 min in the baking oven. After baking, the cookies were cooled at ambient temperature, packaged in polyethylene bags and stored prior to subsequent analysis.

2.4 Determination of ferric reducing antioxidant power (FRAP)

Ferric reducing or antioxidant power was determined as described by Malomo *et al* (2020). Briefly, 100 µl of the extract were mixed with 2.5 ml of 200 mmol/l phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide and incubated at 50 °C for 20 min. Then, 2.5 mlof 10% trichloroacetic acid was added, and the tubes were centrifuged at 10,000 rpm for 10 min. After this, 5 ml of the upper layer were mixed with 5.0 ml distilled water and 1 ml of 0.1% ferric chloride, and the absorbance of the reaction mixtures was measured at 700 nm. Ascorbic acid was used as a positive control.

2.5 Determination of hydroxyl radical scavenging activity

The deoxyribose assay was used to determine the hydroxyl radical scavenging activity in an aqueous medium as previously described by Malomo *et al* (2020). The reaction mixture containing $FeCl_3$ (100 µM), EDTA (104 µM), H_2O_2 (1 mM) and 2-deoxy- D-ribose (2.8 mM) at various concentrations of extracts in 1 ml final reaction volume made with potassium phosphate buffer (20 mM, pH 7.4) and incubated for 1 hr at 37ºC. The mixture was heated at 95 ºC in water bath for 15 min followed by the addition of 1 ml each of TCA (2.8%) and TBA (0.5% TBA in 0.025 M NaOH containing 0.02% BHA). Finally, the reaction mixture was cooled on ice and centrifuged at 5000 rpm for 15 min. Absorbance of supernatant was measured at 532 nm. Ascorbic acid was taken as the positive control.

$$\%\ \text{Hydroxyl Scavenging Activity} = \left[\frac{(A_c - A_s)}{A_c}\right] \text{x } 100$$

Where A_c is the absorbance of control and A_s the absorbance of the extract.

2.6 Determination of iron chelating activity

Metal chelating activity was measured as described by Malomo *et al* (2020). 0.1 mM $FeSO_4$(0.2 ml) and 0.25 mM ferrozine (0.4 ml) were subsequently added into 0.2 ml of flour sample and dough meal. After incubating at room temperature for 10 min, absorbance of the mixture was recorded at 562 nm. Chelating activity was calculated using the following formula:

$$\text{Metal Chelating Activity} = \left[\frac{(A_{control} - A_{sample})}{A_{control}}\right] \text{x } 100$$

Where $A_{control}$ is the absorbance of control reaction (without plant extract), and A_{sample} is the absorbance in the presence of a plant extract.

2.7 Inhibition of acetylcholinesterase (AChE) activity

AChE inhibition assay was performed as previously described (Malomo & Aluko, 2019) with slight modifications. Briefly,

the reaction was carried out at room temperature using 0.1 M sodium phosphate buffer (pH 7.5) and the final assay mixture was 200 µL in a 96-well microplate (Costar, Corning, NY, USA). Aliquots of 20 µL samples were added to the microplate wells that contained 130 µL of the buffer. DTNB (20 µL) and acetylthiocholine (10 µL) were added to each well. Then, 20 µL of AChE (0.5 U/mL final assay concentration) was added to initiate the reaction and the mixture incubated for 15 min at room temperature. The absorbance and the reaction kinetics were read for 2 min at wavelength of 412 nm using a Synergy H4 microplate reader (Biotek Instruments Inc., Winooski, Vermont, USA). In the uninhibited wells, 20 µL of the buffer was used to replace samples. In the background wells, 20 µL of the buffer was used to replace AChE while 40 µL of buffer was used to replace both AChE and samples in the blank wells. The concentration of sample that inhibited 50% AChE activity (IC_{50}) was calculated from a non-linear regression plot of percentage AChE activity versus sample concentration.

2.8 Statistical analysis

All determinations were carried out in triplicates. Data was subjected to analysis of variance (ANOVA) using SPSS (version 21, USA), while means was separated using Duncan New Multiple Range Test (DNMRT) at 5% level of significance ($p<0.05$).

3. Results and Discussion

3.1 Antioxidant properties of cookies

The antioxidant activities of the food samples against ferric reducing antioxidant power (FRAP), metal (Fe^{2+}) chelation and hydroxyl (OH) free radical varied with the different percentage inclusion of cocoyam and pigeon pea. Interestingly, WCP 3 has the highest inhibitory activities against oxidation of molecules and free radicals' formation as expressed by their scavenging, chelating and inhibiting abilities. The antioxidative ability of this cookie sample WCP 3 could be attributed to the synergetic effect of the flour sample combinations, that is, wheat, cocoyam and pigeon pea as previously reported (Oguntuase *et al.*, 2022). The FRAP of the cookie samples to reduce Fe^{3+} to Fe^{2+} ranged from 13.81 to 25.76% as presented in Fig 1. The reducing power of a sample is an essential anti-oxidation defense system mechanism, which the sample used to scavenge the free radicals via hydrogen atom or electron transfer (Dastmolchi *et al.*, 2007). The significantly ($p>0.05$) higher reducing power of the composite cookie samples when compared to the control sample may be linked to the bioavailable proteins present in the cocoyam and pigeon pea flours.

The antioxidant potential of the composite biscuits was also measured by assessing their Fe^{2+} chelating abilities (Fig 2). The results showed that the different percent inclusions of cocoyam and pigeon pea were able to chelate Fe^{2+} significantly ($p>0.05$) in the ranges of 62.34 to 68.99%, which although were lower when compared to glutathione, the known antioxidant (81.12%). The ability of the formulated composite cookies to chelate Fe^{2+} might indicate its potential to form sigma bond with Fe^{2+} and more efficient than Fe^{2+}-ferrozine complex. This complex, thus led to the depletion of redox potentials and thereby steadying the oxidized form of ferrous ion (Malomo *et al.*, 2020).

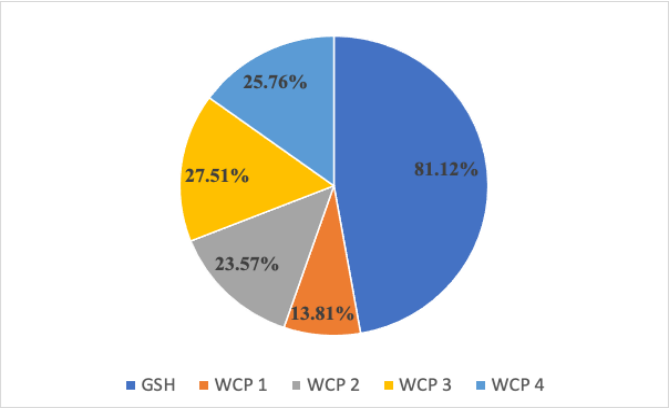


Figure 1: Ferric reducing antioxidant properties (FRAP) of cookies.
KEY: WCP 1 = 100% wheat flour (Control); WCP 2 = 80% wheat flour + 10% cocoyam flour + 10% pigeon pea flour; WCP 3 = 70% wheat flour + 10% cocoyam flour + 20% pigeon pea flour; WCP 4 = 60% wheat flour + 10% cocoyam flour + 30% pigeon pea flour

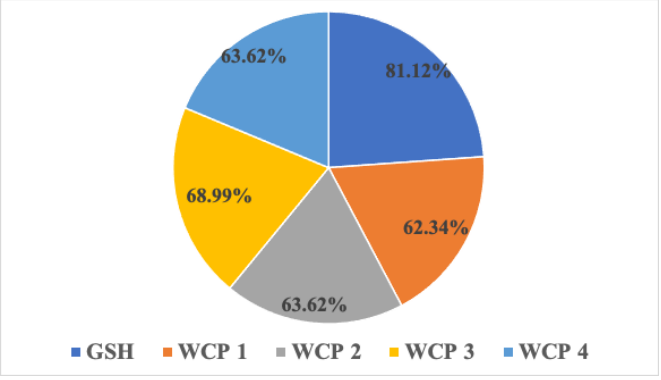


Figure 2: Metal (Iron) chelation properties of cookies.
KEY: WCP 1 = 100% wheat flour (Control); WCP 2 = 80% wheat flour + 10% cocoyam flour + 10% pigeon pea flour; WCP 3 = 70% wheat flour + 10% cocoyam flour + 20% pigeon pea flour; WCP 4 = 60% wheat flour + 10% cocoyam flour + 30% pigeon pea flour.

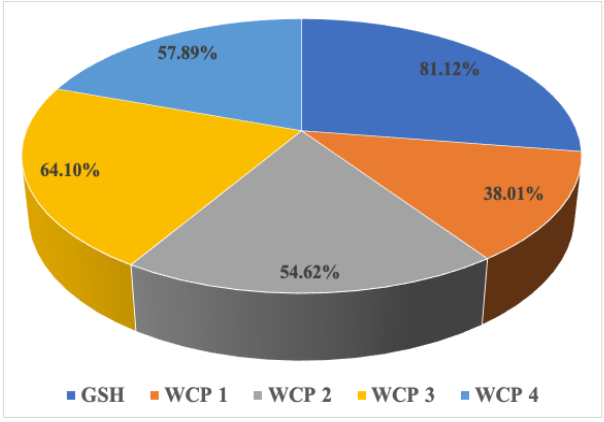


Figure 3: Hydroxyl (OH) radical scavenging potentials of cookies.
KEY: WCP 1 = 100% wheat flour (Control); WCP 2 = 80% wheat flour + 10% cocoyam flour + 10% pigeon pea flour; WCP 3 = 70% wheat flour + 10% cocoyam flour + 20% pigeon pea flour; WCP 4 = 60% wheat flour + 10% cocoyam flour + 30% pigeon pea flour

The OH* radical scavenging abilities of the composite cookies ranged from 54.62 to 64.10% and were significantly ($p>0.05$) higher when compared to 38.01% obtained for control cookie sample (Fig 3). The OH* is highly reactive and is involved in potentiating lipid peroxidation by removing electrons in poly-

unsaturated fatty acids resulting in peroxyl-radicals' formation, while the overproduction of the OH* has been implicated in oxidative and cellular damage (Oguntuase *et al.,* 2022). Therefore, the abilities of the different percentage inclusions of cocoyam and pigeon pea to scavenge the OH* might be linked to their potentials to prevent oxidative and cellular damage (Malomo *et al.,* 2020). Besides, the *in vitro* antioxidant abilities of the different percentage inclusions of cocoyam and pigeon pea could be attributed to their protein and fibers contents as earlier reported by past findings (Owheruo *et al.*, 2021).

3.2 Acetylcholinesterase inhibition properties of the cookies

The AChE inhibitory abilities of the composite cookies, as presented in Fig 4, ranged from 20.68 to 66.17% and were however, significantly ($p>0.05$) lower than 83.64% obtained for the neostigmine, a known synthetic AChE inhibitory drug. Various reports (Kumar & Chowdhury, 2014; Iannello *et al*., 2014; Saravanaraman *et al*., 2014; Ghribia *et al*., 2014) have shown the acetylcholinesterase (AChE) enzyme as an important target in research aimed at developing therapeutic tools against a wide range of human metabolic disorders. This is because AChE hydrolyzes the neurotransmitter acetylcholine (ACh) at cholinergic synapses, with a higher catalytic efficiency than other known enzymes. The dysfunction of cholinergic neurotransmission in the brain, the formation and growth of brain amyloid lesions, including the senility-promoting plaques have been widely reported as contributing factors to Alzheimer's disease (AD) pathogenesis (Willcox *et al*., 2014). As of today, the current available therapy for AD consists of the administration of AChE inhibitors due to their clinical efficacy in prolonging the half-life of ACh (Kumar & Chowdhury, 2014). Recently, there is awareness in the food industry and in preventive health care activities with respect to the development of natural nutraceuticals and functional foods ingredients from plant materials due to their high biodegradability rate, which results in little or no toxicity (residual effects or negative side effects). Besides its role as an effective modulator of the central and peripheral nervous systems, the ubiquitous process-regulatory nature of AChE such as in cell growth, locomotion or apoptosis has given it a priority over BChE during recent investigations (Kolář *et al*., 2010). Normally, AChE rapidly and efficiently degraded ACh into acetate and choline, thereby terminating the signaling action (Kolář *et al*., 2010).

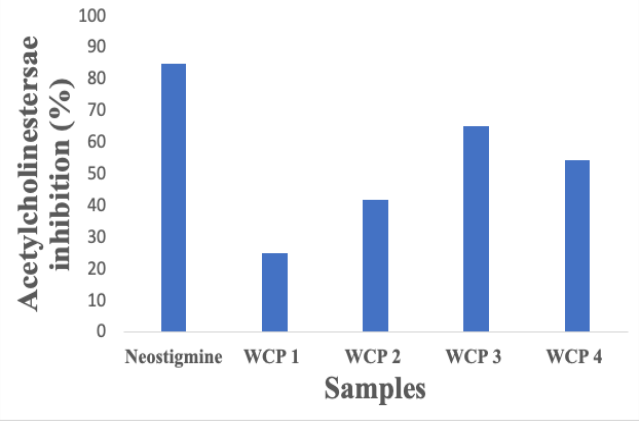


Figure 4: Acetylcholinesterase inhibition potentials of cookies
KEY: WCP 1 = 100% wheat flour (Control); WCP 2 = 80% wheat flour + 10% cocoyam flour + 10% pigeon pea flour; WCP 3 = 70% wheat flour + 10% cocoyam flour + 20% pigeon pea flour; WCP 4 = 60% wheat flour + 10% cocoyam flour + 30% pigeon pea flour

4. Conclusion

The cookies from wheat, cocoyam and pigeon pea flour blends exhibited antioxidant and bioactive anti-acetylcholinesterase activities, such as in the inhibition of AChE activities and preventing the proliferation of the generated reactive oxygen species. Most especially from this study, cookies from WCP 3 composite flour, which is 70% wheat flour + 10% cocoyam flour + 20% pigeon pea flour possessed high antioxidant and acetylcholinesterase-inhibitory properties when compared to the known ascorbic acid and AChE-inhibitors, respectively. This study, thus showed that cookies with high antioxidants and cognitive health-management (anti-acetylcholinesterase) qualities can be produced from combinations of wheat, cocoyam and pigeon pea flours. Besides, the *in vivo* study on the composite cookies is recommended, so as to ascertain the findings currently obtained from this present *in vitro* study.

Conflict of Interest

The authors declared no conflict of interest in the submission of this manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

Authors contributions

All authors performed the experiments, wrote the manuscripts, read and approved the final manuscript.

References

Adeola AA, Olunlade BA, Ajagunna AJ. Effect of pigeon pea or soybean substitution for maize on nutritional and sensory attributes of Kokoro. *Annals of Science and Biotechnology*. 2011; 2(1):61-66.

Alawode, E. K., Idowu, M., *et al*. 2017. Some quality attributes of complementary food produced from flour blends of orange flesh sweet potato, sorghum, and soybean. *Croat. J. Food Sci. Technol*., 123-124

Bhat, A., Jyoti, K., Manish, S. and Rajinder, P. (2015). Constraints in production and marketing of citrus fruit in Jammu region of J&K State. *Economic Affairs J*., 60(2), 331-337

Efezino, S. A., Ejiroghene, R. E., & Ogechi, T. C. (2023). Evaluating the proximate chemical composition and sensory properties of composite bread from wheat and cocoyam flours. African Journal of Food Science, 17(5), 74–84. doi: 10.5897/AJFS2022.2197

Ghribia, L., Ghouilaa, H., Omrib, A., Besbesb, M., & Janneta, H. B. (2014). Antioxidant and anti-acetylcholinesterase activities of extracts and secondary metabolites from Acacia cyanophylla. *Asian Pacific Journal of Tropical Biomedicine*, 4(Suppl 1), S417-S423.

Iannello, C., Pigni, N. B., Antognoni, F., Poli, F., Maxia, A., de Andrade, J. P., & Bastida, J. (2014). A potent acetylcholinesterase inhibitor from Pancratium illyricum L. *Fitoterapia*, 92, 163–167.

Kolář, D., Wimmerová, L., & Kádek, R. (2010). Acetylcholinesterase and Butyrylcholinesterase inhibitory activities of Berberis vulgaris. *Phytopharmacology*, 1(1) 7-11.

Kumar, S., & Chowdhury, S. (2014). Kinetics of acetylcholinesterase inhibition by an aqueous extract of *Cuminum cyminum* seeds. *International Journal of Applied Science and Biotechnology*, 2(1), 64-68.

Malomo, S.A., Nwachukwu, I.D., Girgih, A.T., Idowu, A.O., Aluko, R.E., and Fagbemi, T.N. 2020. Antioxidant and renin-angiotensin system (RAS) inhibitory properties of cashew nut and fluted-pumpkin protein hydrolysates. *Polish Journal of Food and Nutrition Science.* 70(3), 275-289.

Malomo, S.A. and Aluko, R.E. 2019. Kinetics of the inhibition of acetylcholinesterase by hemp seed protein-derived peptides. *Journal of Food Biochemistry*, e12897, 1-11.

Oguntuase, S. O., Ijarotimi, O. S., Oluwajuyitan, T. D. & Oboh, G. (2022). Nutritional, antioxidant, carbohydrate hydrolyzing enzyme inhibitory activities, and glyceamic index of wheat bread as influence by bambara groundnut substitution. *SN Applied Sciences*, 4(4), 1-16.

Olugbuyi, A.O., Oladipo, G.O., Malomo, S.A., Ijarotimi, S.O., and Fagbemi, T.N. 2022. Biochemical ameliorating potential of optimized dough meal from plantain (*Musa AAB*), soycake (*Glycine max*) and rice bran (*Oryza sativa*) flour blends in Streptozotocin induced diabetic rats. *Applied Food Research*, 1-11. https://doi.org/10.1016/j.afres.2022.100097

Owheruo, J. O., Oluwajuyitan, T. D., Ifesan, B. O. & Bolade, M. K. (2021). Extruded breakfast meal from malted finger millet (Eleusine coracana) and watermelon (Citrullus lanatus) seed flour: in-vivo nutritional qualities study. *Bulletin of the National Research Centre*, 45(73): 1-9. https://doi.org/10.1186/s42269-021-00533-x

Saravanaraman, P., Chinnadurai, R. K., & Boopathy, R. (2014). A new role for the non-pathogenic non-synonymous single-nucleotide polymorphisms of acetylcholinesterase in the treatment of Alzheimer's disease: A computational study. *Journal of Computational Biology*, 21(8), 632-647.

Thammarat K, Leena N, Punnanee S, & Soottawat B. (2015). Functional and antioxidative properties of Bambara groundnut (Voandzeia subterranea) protein hydrolysates. *International Food Research Journal*, 22(4), 1584 – 1595

Udeh CC, Malomo SA, Ijarotimi OS. *In vitro* antioxidants and anti-inflammatory potentials of high protein-fibre cookies produced from whole wheat, sweet potato, rice bran and peanut composite flour blends. *Food Production, Processing and Nutrition*, 2024; 1-11.

Willcox, D. C., Scapagnini, G., & Willcox, B. J. (2014). Healthy aging diets other than the Mediterranean: A focus on the Okinawan diet. *Mechanisms of Ageing and Development*, 1-15. doi.org/10.1016/j.mad.2014.01.002