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Effects of Isolated Cola *nitida* (Kolanut) Caffeine on Metoclopramide-Induced Parkinsonism in Male Wistar Rat

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Abstract:
This study investigated the effect of isolated cola nitida caffeine on metoclopramide-induced parkinsonism in male Wistar rat. Thirty (30) male Wistar rat weighing between 140 - 160 grams (g) were used for the research. They were divided into six (6) groups of five (5) rats each. Parkinsonism was induced in the male Wistar rats by chronic administration of Metoclopramide (MCP) dissolved in saline and injected intraperitoneally at a dose of 20 mg/kg body weight once daily for 14 days. The normal control group (group A) received standard pellet diet and distilled water ad libitum. Group B was induced with parkinsonism and untreated. Groups C, D and E, were induced with parkinsonism and received 50mg/kg, 100mg/kg and 200mg/kg, respectively, of Cola nitida caffeine extract orally. Group F, also induced with parkinsonism, was treated with 100/25mg/kg Pardopa (levodopa-carbidopa) - the standard drug. This study showed a measurable amelioration of parkinsonian symptoms in the groups treated with Cola nitida caffeine. However, it was concluded in this study that though the potential utility of Cola nitida caffeine as a neuroprotective agent is established, there is need for further research into the implications of this Cola nitida caffeine in the aftermath. The efficacy of Cola nitida caffeine appears to be fairly modest and not without limitations. The standard drug remains a superior choice in managing parkinsonism as it presents with minimal side effects compared to Cola nitida caffeine.

Keywords: Parkinsonism, levodopa, caffeine.

INTRODUCTION

Parkinsonism is a clinical syndrome that manifests as varied degrees of stiffness as well as a number of classical motor symptoms such as bradykinesia, tremor, rigidity and unstable posture (Balestrino & Schapira, 2019). They are commonly due to primary neurodegenerative disease, resulting in the loss of dopaminergic nerve terminals along the nigrostriatal pathway, similar to idiopathic Parkinson's disease, dementia with Lewy bodies and other neurodegenerative disorders (Ogawa *et al.,* 2018). It is a constellation of neurological abnormalities, including sluggish or delayed movement, resting tremor, muscular stiffness, and issues with balance and coordination. It is important to note that Parkinsonism is not the same as Parkinson's disease but because it is the most common cause of Parkinsonism, it is often used in place of the term "Parkinsonism." Parkinsonism typically affects both sides of the body, whereas Parkinson's disease generally affects one side more than the other (Jankovic, 2018). Parkinsonism is characteristically present in Parkinson's disease. The symptoms can also result from other

neurodegenerative disorders, as well as specific brain lesions, head trauma, medications, metabolic conditions, and toxin exposure (Wang *et al.,* 2023). Development of Parkinsonism has been linked to certain factors; Age, the prevalence and risk of development increases with age, affecting 1–2% of the population over the age of 65 years, and 3% of those over the age of 85 years (Ostrem and Galifianakis, 2010). Environmental toxins; Chronic manganese (Mn) exposure has been shown to produce Parkinsonism-like illness characterized by movement abnormalities (Guilarte & Gonzales 2015). Other environmental toxins such as pesticides, herbicides (paraquat) have been associated with an increased risk of developing Parkinsonism (Liu *et al.,* 2021).

Parkinson's disease is considered a degenerative process, affecting the nigrostriatal dopaminergic system and manifests with Parkinsonism symptoms (Bonnet *et al.,* 2012). The most common cause of Parkinsonism is Idiopathic Parkinson's disease, a degenerative brain disorder primarily caused on by the loss of dopaminergic neurons within the substantia nigra of the midbrain that releases dopamine in the striatum, which reportedly accounts for about 80% of all cases. The motor symptoms of Parkinsonism result from the loss of the inhibitory influence of dopamine on the basal ganglia, a group of brain structures that are involved in the control of movement. The basal ganglia normally receive signals from the cerebral cortex, which passes through the striatum, a part of the basal ganglia which then sends signals to the substantia nigra, which produces dopamine that is sent back to the striatum (Bear *et al.,* 2023). This feedback loop helps to regulate movement and ensure that it is smooth and coordinated. There is a disruption of this feedback loop in Parkinsonism due to loss of dopamine-producing neurons. As a result, there is an inconsistency in the levels of dopamine in the basal ganglia, which leads to the characteristic motor symptoms of Parkinsonism such as tremors, stiffness, postural disturbances and difficulty with movement. Dopamine is a catecholamine neurotransmitter that has an important role in motor behavior and is implicated in numerous mental conditions and emotional states (Milyavsky *et al.,* 2020). The dietary source and precursor for dopamine is the amino acid tyrosine. Prior to storage within vesicles, Dopamine synthesis occurs at the synaptic terminal and as dopamine is synthesized, it is released by dopaminergic neurons, and it then travels across synapses to bind to dopamine receptors. This binding cause signals to be sent to the receiving cell, which has a variety of physiological and behavioral implications (Korshunov *et al*., 2020).

The main motor features of Parkinsonism (i.e., tremor, rigidity, and akinesia) are associated with a deficiency of dopamine in the posterior putamen and the motor circuit (Jeong *et al*., 2023). The loss of dopamine-producing neurons in the pars compacta region also leads to changes in other parts of the brain including the basal ganglia and cerebral cortex (Kochar *et al.,* 2022). This has been shown to significantly affect the quality of life of people with Parkinsonism, causing significant morbidity, and reducing life expectancy. It also has significant financial repercussions, such as loss of earnings, increased healthcare expenses, and increased cost of drugs globally. Drug-induced extrapyramidal illnesses are becoming more common, but certain drugs can cause Parkinsonism to show up by obstructing dopamine receptors in the brain. One of such drugs is metoclopramide, which will in the course of this study, be the inducer for Parkinsonism in a male Wistar rat.The Food and Drug Agency (FDA) has approved Metoclopramide to treat nausea and vomiting in patients with gastroesophageal reflux disease (in patients who fail to respond to established therapy) and diabetic gastroparesis by increasing gastric motility (Shakhatreh *et al*., 2019; Rettura *et al*., 2021). In simple terms, Metoclopramide is an anti-sickness medicine taken by prescription to help you stop feeling sick mostly after an operation or chemotherapy. It has therefore been developed as an anti-emetic for a variety of gastrointestinal diseases, such as nausea and vomiting, but it may also impact dopamine levels in the brain and by implication,

reduces dopamine availability in the brain, leading to a decrease in motor function and development of Parkinsonism symptoms. Older people and those who use Metoclopramide for a prolonged period are at a higher risk of developing Parkinsonism from the drug (Jankovic, 2018).

Presently, some available drugs do provide beneficial symptomatic relief, but with sustained use, they are frequently linked to manifestations of adverse effects. Levodopa, among others is one of the therapies for Parkinson's disease, and can improve motor function for years, but subsequently results in uncontrollable muscular spasms such as dyskinesia and dystonia (sustained muscle contractions). These prompted the quest for alternative measures that can be adopted to manage Parkinsonism. Some chemical compounds available as a supplement or in a person's diet also have been shown to have a neuroprotective effect for Parkinsonism. Caffeine (tea, kola) has been shown to reduce the loss of dopamine-producing neurons in the brain (Alasmari, 2020).

Caffeine, 1,3,7-trimethylxanthine, is a natural stimulant that is found in various plants, including coffee beans, tea leaves, and cocoa beans and is also added to some soft drinks and medications (Giada, 2019). It is both water- and lipid-soluble, it readily crosses the blood–brain barrier that separates the bloodstream from the interior of the brain. The caffeine molecule is structurally similar to adenosine, and therefore competes with adenosine for binding sites on the cell surface without activating them (Giada, 2019), Once in the brain, caffeine acts as an agent that reduces the effects of adenosine. The European Food Safety Authority (EFSA) reported that up to 400 mg of caffeine per day (around 5.7 mg/kg of body mass per day) does not raise safety concerns for non-pregnant adults, while intakes up to 200 mg per day for pregnant and lactating women do not raise safety concerns for the fetus or the breast-fed infants. It is possible for caffeine to be good for your health as well as bad. It may result in undesirable consequences including restlessness, anxiety, trembling, and an accelerated pulse. However, studies have shown that moderate caffeine use has been linked to a lower chance of developing certain conditions, including Parkinsonism. This further suggests that caffeine may have a protective effect against the development of Parkinson's disease, and that it may also have some potential as a treatment for the symptoms of Parkinson's disease, including Parkinsonism. One possible mechanism by which caffeine may reduce Parkinsonism is through its effects on the brain's dopamine system. Caffeine has been shown to increase dopamine release and block the activity of adenosine, a neurotransmitter that inhibits dopamine release. These effects may help to compensate for the loss of dopamine in the brain and reduce the severity of Parkinsonism symptoms. In addition to other therapeutic options, this study hopes to explore the potential protective and therapeutic effects of isolated Cola *nitida* caffeine in metoclopramide-induced Parkinsonism on Male Wistar rat.

MATERIALS AND METHODS

Drugs and Chemicals

The drugs used in this study were Reglan (Metoclopramide), Pardopa (levodopa and carbidopa drug combination). Reglan was manufactured by ANI Pharmaceuticals Inc, U.S.A and Pardopa was manufactured by Schwarz Pharmaceuticals, U.S.A. They were both purchased from Donaville Pharmacy, Chime Avenue, New Haven, Enugu Nigeria. The chemicals used in this study were ethanol, dichloromethane, hexane, nitric acid, ammonium molybdate, formalin, ethyl acetate, silica gel, sodium sulfate and standard solutions of caffeine. All chemicals were of analytical grade and obtained from Ogbete Main Market, Enugu, Nigeria.

Plant Collection, Identification and Authentication

Fresh Cola *nitida* seeds were locally sourced and obtained from new market in Enugu, Nigeria and used for this study. It was identified and authenticated as a viable cola nut fruit (Cola *nitida*) by Mr. J.I. Enyi at the Department of Crop Science, University of Nigeria, Nsukka.

Sample Preparation (Aqueous Extraction) of Cola *nitida*

Approximately 3.5kg of the Cola *nitida* seeds were weighed using a weighing balance. The seeds were properly washed and then transferred into a thermostatic oven maintained at 80°C for 48 hours. At the end of 48 hours, it was removed and reweighed to obtain the dry weight of the Cola *nitida* as 1.86kg. The 1.86kg of dry Cola *nitida* was transferred into an electric kenwood blender and pulverized to further increase the surface area. The powdered specimen was soaked in 250mL of 70% ethanol for 72 hours with occasional shaking. The ethanol extract was filtered using a Whatman filter paper grade 1 (with pore size of 11 microns and a filtration rate of 150 seconds/100 mL) and evaporated to dryness at temperature 40-45 °C and stored at 4 °C until further use. (Ani et al., 2020)

Extraction of Caffeine from Aqueous Cola- nitida Extract Using Column Chromatography:

The extraction of caffeine from aqueous Cola *nitida* extract using column chromatography was performed as following the method of column chromatography by Ani et al., 2022. The aqueous extract was dissolved in a small amount of water and transferred to a separator funnel. Dichloromethane was added and the funnel was shaken gently. The layers were allowed to separate and the lower organic layer was collected. The extraction was repeated with fresh dichloromethane until no more caffeine was extracted. The organic layers were combined and dried over anhydrous sodium sulfate. The solvent was filtered and evaporated to obtain a yellowish solid, which was the crude caffeine. A glass column was packed with silica gel and wetted with hexane. The crude caffeine was loaded on top of the column and eluted with a mixture of hexane and ethyl acetate at a ratio of 9:1. The fractions were collected and monitored by TLC (Thin layer Chromatography) using a UV lamp. The fractions that contained pure caffeine, as indicated by a single spot on the TLC plate, were combined and the solvent was evaporated to obtain white crystals of caffeine (Nyamien *et al.*, 2015).

Recovery Procedure:

The recovery procedure was performed to determine the percentage yield and the purity of the extracted caffeine. The percentage yield of the extract was calculated by dividing the mass of the extracted caffeine by the mass of the original extract and multiplying by 100. The purity was determined by measuring the peak area of the extracted caffeine and comparing them with those of authentic caffeine standard (Umeda & Puyate, 2020).

Thin Layer Chromatography:

Thin layer chromatography (TLC) was used to monitor the progress of the extraction and purification of caffeine from Cola *nitida* extract. TLC was also used to identify any other compounds that may be present in the extract, such as tannins and phenols.

Tests for identification of Caffeine:

Tannic Acid Test:

This test is based on the formation of a white precipitate between caffeine and tannic acid. A small amount of the caffeine extract is dissolved in water and a few drops of tannic acid are added. The formation of a white precipitate indicated the presence of caffeine (Umeda & Puyate, 2020).

Murexide Color Reaction Test:
This test is based on the formation of a purple-colored complex between caffeine and ammonium molybdate in the presence of nitric acid. A small amount of the caffeine extract is dissolved in water and a few drops of nitric acid are added. The solution is heated until it becomes yellow and then cooled. A few drops of ammonium molybdate were added and the solution was heated again. The formation of a purple color indicated the presence of caffeine (Umeda & Puyate, 2020).

Experimental Animals
A total of thirty (30) male Wistar rats weighing between 140 - 160 grams (g) were used in this experiment. These animals were purchased from the Animal House, Department of Physiology, Faculty of Basic Medical Sciences, Enugu State University College of Medicine (ESUCOM), Enugu, Nigeria. The rats were accommodated in the Animal House after purchase and they were kept in cages (27 × 30 × 42 cm) in a well-ventilated space at room temperature 25 ± 1 °C. They were fed with standard pellet diet produced by Guinea Feed Nigeria Limited and water given was given *ad libitum*. The rats were allowed to acclimatize to the laboratory environment for two weeks before the experiment commenced. Proper hygiene was maintained by constant cleaning and removal of feces and spilled feed from the cages daily.

Method of Induction of Parkinsonism:
Parkinsonism was induced in the male Wistar rats by chronic administration of metoclopramide (MCP), which is an antiemetic drug that blocks dopamine receptors in the brain and induces Parkinsonism-like symptoms in rats. MCP-induced Parkinsonism is a well-established animal model that mimics the motor symptoms seen in movement disorders like in Parkinson's disease. MCP was dissolved in saline and injected intraperitoneally at a dose of 20 mg/kg body weight once daily for 14 days. This dose and duration were selected based on previous studies that reported optimal induction of Parkinsonism by MCP (Vaidya *et al.*, 2022).

Experimental Design:
- Group 1 (n=5): Normal control (NC), received saline orally
- Group 2 (n=5): Negative control received Metoclopramide (MCP) 20mg/kg + saline orally
- Group 3 (n=5): 20mg/kg MCP+ 50mg/kg Cola *nitida* caffeine extract orally
- Group 4 (n=5): 20mg/kg MCP + 100 mg/kg Cola *nitida* caffeine extract orally
- Group 5 (n=5): 20mg/kg MCP + 200 mg/kg Cola *nitida* caffeine extract orally
- Group 6 (n=5): Levodopa-carbidopa (LC) group, 20mg/kg MCP + Pardopa 100/25 mg/kg of LC respectively orally

The treatments were given once daily for 14 days after induction of Parkinsonism by MCP. The doses of Cola *nitida* caffeine extract was selected based on previous studies that reported its pharmacological effects. The dose of levodopa-carbidopa was selected based on previous studies that used it as a standard drug for anti-Parkinsonism (Erukainure *et al.*, 2017).

Measurement of Serum Dopamine Levels
Sample Collection and Storage:
In other to measure serum dopamine levels, blood samples were collected through the orbital sinus of the control and experimental rats. Anesthesia (Ketamine Hydrochloride Injection, USP) was administered intraperitoneal before the blood collection and, alcohol swabs were placed around the site of puncture to reduce bleeding after collection. The blood samples were collected

using serum separator and was allowed to clot overnight. After which, it was centrifuged for 20minutes at approximately 1000xg. The freshly prepared serum samples were assayed immediately at room temperature.

Reagents and Materials Provided:

1. Pre-coated, ready-to-use well strip plate
2. Plate sealer
3. Standard (2)
4. Standard Diluent (1x20mL)
5. Detection Reagent A (1x120uL)
6. Assay Diluent A (1x12mL)
7. Detection Reagent B (1x120uL)
8. Assay Diluent B (1x12mL)
9. TMB (Tetramethylbenzidine) Substrate (1x9mL)
10. Stop Solution (1x6mL)
11. Microplate reader with 450nm filter
12. Absorbent paper for blotting the microplate
13. Wash Buffer (30x concentrate, 1x20mL)

Dopamine Assay Procedure:

1. Prepare wells for diluted standard, blank, and samples. Use a microplate shaker for gentle mixing.
2. Add dilutions of standard, blank, and samples, then Detection Reagent A and cover with plate sealer, Incubate at 37°C for 1 hour.
3. Wash wells thoroughly and remove residual liquid.
4. Add Detection Reagent B and cover with plate sealer, incubate at 37°C for 30 minutes.
5. Repeat washing process.
6. Add Substrate Solution, incubate at 37°C for 10-20 minutes. Protect from light and the liquid turns blue on application of substrate.
7. Add Stop Solution and the liquid turns yellow, mix gently by tapping.
8. Ensure no water, fingerprints, or bubbles on the plate. Run microplate reader at 450nm immediately

Test Principle:

The assay employs a competitive inhibition enzyme immunoassay, involving a microplate pre-coated with a monoclonal antibody specific to dopamine. A competitive reaction occurs between biotin-labeled and unlabeled dopamine (standards or samples). After incubation, unbound conjugate is washed off. Avidin-HRP (Horseradish Peroxidase) is added and incubated. Bound HRP conjugate inversely correlates with dopamine concentration. Substrate solution produces color intensity inversely proportional to dopamine concentration.

Calculation of Results:

The assay follows a competitive inhibition enzyme immunoassay principle, where dopamine concentration inversely correlates with assay signal intensity. Calculate averages of duplicate readings. Create a standard curve (log-log or semi-log) with dopamine concentration vs. absorbance. The dopamine concentration is then determined via regression analysis or plot software.

Histological Analysis

Rats from group A and group B were euthanized and perfused with a fixative solution- formalin, to preserve the tissue and prevent degradation. Their brains were removed from the skull and post-fixed in the same fixative solution before the analysis began. The brain was sliced into thin sections (about 20-40 µm thick) and collected in a buffer solution and stored at 4°C until further processing. The sections were stained with hematoxylin and eosin (H&E) stain to show the general morphology and nuclei of cells. The stained sections were mounted on glass slides and cover slipped with a mounting medium, such as glycerol or xylene. The slides are then ready for examination and analysis with the aid of an electronic microscope.

Ethical Approval

All experimental procedures were conducted in accordance with National Institute of Health Guide for the care and use of Laboratory Animals as stated in the "guide to the care and use of Laboratory Animals Resources". Ethical approval was obtained from the Research and Ethics committee of the College of Medicine, University of Nigeria Enugu Campus, Enugu State.

Statistical Analysis

The data obtained from the study were expressed as mean ± standard deviation (SD). The differences between the groups were analyzed by using one-way analysis of variance (ANOVA) and Tukey Post- hoc test for multiple comparisons. The level of significance was set at $p < 0.05$. The statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software version 25.

RESULTS

Table 4.1: Effects of isolated Cola *nitida* (Kolanut) caffeine on serum dopamine concentration (pg/ml) in male Wistar rat

Groups	Day 1	Day 14	Day 28
A	19.53 ± 1.58	19.84 ± 1.66	19.91 ± 1.71
B	20.08 ± 2.03	16.12 ± 1.77*	11.90 ± 1.12*
C	20.65 ± 0.14	16.50 ± 1.10$^{*\beta}$	17.52 ± 1.16$^{*\beta}$
D	19.76 ± 0.16	15.56 ± 0.91$^{*\beta}$	17.25 ± 0.74$^{*\beta}$
E	20.61 ± 0.17	16.43 ± 1.20$^{*\beta}$	18.62 ± 1.36$^{*\beta}$
F	20.50 ± 0.15	16.46 ± 1.68$^{*\beta}$	20.18 ± 2.06$^{*\beta}$
P-values	0.8420	0.0031	0.0027

Results were expressed as Mean ± Standard Deviation. $^{*\beta}$ $P<0.05$ showed a statistically significant difference compared with groups A and B respectively using Tukey Post-hoc test for multiple comparisons.

Keys: Group A= Normal control and received standard pellet diet and distilled water *ad libitum;* Group B= metoclopramide-induced Parkinsonism untreated; Group C, D and E= metoclopramide-induced Parkinsonism + 50, 100 and 200 mg/kg respectively and Group F= metoclopramide-induced Parkinsonism + Parcopa (levodopa-carbidopa) 100/25 mg/kg orally.

Table 4.1 shows a typical result of the antiparkinsonian effect of isolated Cola *nitida* caffeine on dopamine concentrations in male wistar rats. There was a statistical increase in the concentrations of dopamine in groups C, D, E and F indicating a recovery of dopaminergic function by treatments while group B has a significant decrease in dopamine concentrations when

compared to the normal control group A. The increase in the concentrations of dopamine followed a regular incremental pattern and therefore suggests that the increase is dose-dependent. Group F had a significantly higher dopamine concentration than group C, D and E, indicating a superior effect of Pardopa- the standard drug over isolated Cola *nitida* caffeine.

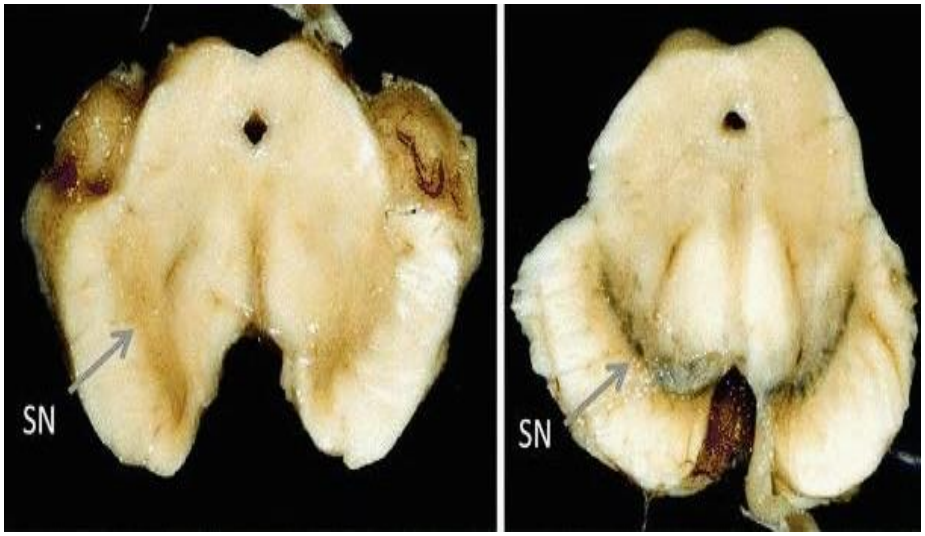

Fig 1: Cross section of mid brain showing the substantia nigra (SN) of rats with metoclopramide-induced parkinsonism vs normal control rats

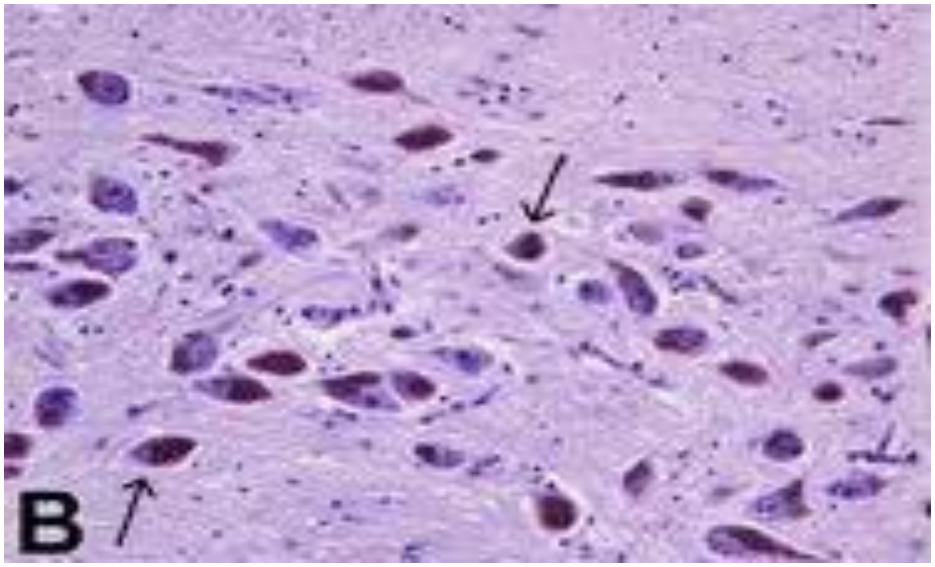

Fig 2*: Histological section of the Substantia nigra of the normal control group (H & E stain, 400× magnification).* ↑

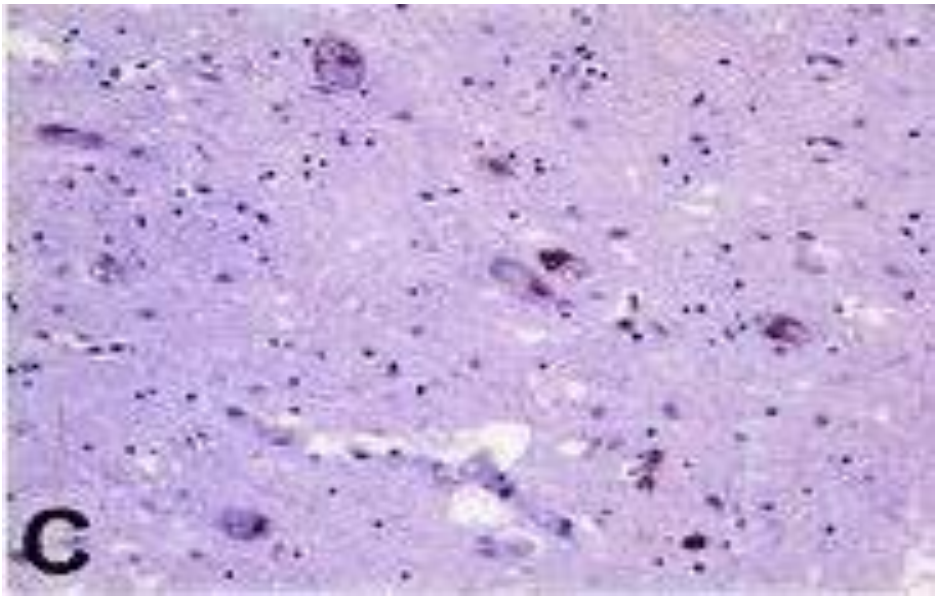

Fig 3: Histological section of the Substantia nigra of rats with metoclopramide-induced Parkinsonism (H & E stain, 400× magnification).

Figure 1: Loss of substantia nigra (SN) neurons causes Parkinsonism. In Fig 1, Pathological examination of a rat in normal control group reveals typical pigmented Dopaminergic, DA neurons in the Substantia nigra (arrows). In contrast, loss of Substantia nigra (SN) neurons leads to pigment disappearance in the brain of rats with Parkinsonism (arrows).

Magnification of the SN area reveals a dense network of melanin-pigmented SN neurons in the healthy brain (Fig 2) while most of SN neurons are lost in rats with Parkinsonism (Fig 3). The melanin-containing granules have a red-brown hue and are distributed in the cytosol of all Substantia nigra neurons (Fig 2-3).

Table 4.2: Differences in the body weight (g) of rats in the control and experimental groups.

Groups	Day 1	Day 14	Day 28
A	137.30 ± 6.78	142.02 ± 6.47	146.98 ± 6.36
B	138.46 ± 8.10	125.30 ± 9.30*	114.72 ± 6.56*
C	139.96 ± 5.98	122.52 ± 6.46$^{*\beta}$	120.20 ± 6.67$^{*\beta}$
D	137.20 ± 4.40	114.60 ± 3.70$^{*\beta}$	117.34 ± 4.67$^{*\beta}$
E	140.48 ± 7.30	116.48 ± 5.08$^{*\beta}$	122.32 ± 5.21$^{*\beta}$
F	142.12 ± 8.09	129.10 ± 7.53$^{*\beta}$	137.68 ± 8.30$^{*\beta}$
P-values	0.8460	0.0010	0.0021

Results were expressed as mean ± SD of percentage of body weight of six rats per group. $^{*\beta}$ P<0.05 showed a statistically significant difference compared to groups A and B respectively using Tukey Post-hoc test for multiple comparisons.

Keys: Group A= Normal control and received standard pellet diet and distilled water *ad libitum;* Group B= metoclopramide-induced Parkinsonism untreated; Group C, D and E= metoclopramide-induced Parkinsonism + 50,100 and 200 mg/kg respectively and Group F= metoclopramide-induced Parkinsonism + Pardopa (levodopa-carbidopa) 100/25 mg/kg orally.

Table 4.2: Differences in the body weight (g) of rats in the control and experimental groups.
The results from the table showed that there was a significant difference in the body weight of rats among the control and experimental groups. The normal control, group A, showed gradual and consistent increase in body weight throughout the study, indicating normal growth and development. The negative control, group B, showed a consistent decrease in body weight throughout the study, indicating adverse metoclopramide effect on health and metabolism of the Wistar rats. The C, D, and E groups showed a dose-dependent attenuation of weight loss throughout the study in a dose-dependent manner, indicating a reversal of malnutrition by the treatments but they were still lower than the normal control group. The administration of Pardopa (group E) also prevented or reversed the weight loss induced by metoclopramide, and it was statistically significant to the normal control group on day 28. The results suggest that caffeine intake may cause weight loss. This is in agreement with Uhuo *et al.,* 2021 who claimed that caffeine may cause weight loss by increased energy expenditure and metabolism, while Levodopa increases dopamine levels in the brain, reducing dyskinesia and improving mobility.

DISCUSSION AND CONCLUSION

This study investigated the possible neuroprotective effects of caffeine isolated from Cola *nitida* (Kolanut) with respect to parkinsonism in Wistar rat. This syndrome typically affects the dopaminergic neurons of the substantia nigra pars compacta thus reducing dopamine availability. In this study, outlined in Table 4.1, there was a measurable elevation of dopamine concentration in the treated groups following administration of Cola *nitida* caffeine. Dopamine levels were also observed to be low in the untreated group, to that effect. This indicates the active degradation of the dopaminergic neurons as a result of the disease. There was a relatively increased dopamine concentration in the group treated with a higher dose of caffeine. This study is in agreement with the research carried out by Soliman *et al*., 2016 which concluded that the effect of caffeine on parkinsonism is dose-dependent. However, there was a more significant increase in dopamine concentration in the group treated with the standard drug, Pardopa, suggesting that though caffeine may provide antiparkinsonian benefits by modulating the dopaminergic system and enhancing its function (Erukainure *et al*., 2017), the standard drug remains a superior choice; confirming the standard drug; levodopa-carpidopa as the 'gold standard' of treatment according to Pathan and Alshahrani, 2018.

Parkinsonism is characterized by the presence of at least two of four fundamental pointers; tremor, rigidity, bradykinesia, and postural instability (Faiz and Pihlstrøm, 2017) with its major cause being Idiopathic Parkinson's Disease or simply Parkinson's Disease. Metoclopramide was used to induce parkinsonism in the group of Wistar rats for the research. Metoclopramide, an antiemetic commonly used in patients with gastric motility disorders, acts as a dopamine receptor antagonist, thus following long term use; it can induce parkinsonian symptoms – bradykinesia, tremor, rigidity and postural instabilities.

The substantia nigra of the normal control group appears pigmented as a result of neuromelanin present in the dopaminergic neurons of the substantia nigra (Fig 1). As the degeneration of the dopaminergic neurons progresses in the groups induced with parkinsonism, the pigmentation in the substantia nigra is lost. Viewed under a microscope, the substantia nigra of the normal control group was heavily pigmented (Fig 2). This indicates the presence of neuromelanin. Histology of group B, examined under a microscope shows a scantier substantia nigra with reasonably low pigmentation as dopaminergic neurons degenerates and neuromelanin is lost (Fig 3).

Throughout the experiment, there were a lot of differences in the body weight of the Wistar rat. There was a gradual and statistically significant increase in the body weight of the normal control group as seen in Table 4.2. The untreated group exhibited consistent and statistically significant decrease in body weight throughout the experiment. Metoclopramide in high doses over a prolonged period of time leads to progressive decrease in body weight which is in agreement with the work of Wang *et al.,* 2016 which suggests that chronic administration of Metoclopramide for 14 days resulted in a significant decrease in body weight and food intake in rat. When treated with Cola *nitida* caffeine, rats in groups C, D, E, showed a little improvement in body weight and this is because caffeine has the tendency to increase metabolism in the body therefore influencing some level of weight loss. Group F treated with Pardopa displayed a measurable increase in body weight. The standard drug reverses the influences of metoclopramide on the rats and thus enhances weight restoration, and overall mobility improvements.

Essentially, parkinsonism as a result of Parkinson's Disease cannot be cured but can, in fact, be managed. So many approaches have emerged through the years to manage parkinsonian symptoms. The most common is the standard drug; Levodopa (Pardopa), which enhances dopamine delivery to the CNS and dopamine replacement. Caffeine has been studied widely because of the significant effects it has on the central nervous system; from mediating the release of certain neurotransmitters in the brain to sleep regulation and general cognition. Caffeine exerts some neuroprotective effects and can reduce the incidence of parkinsonism and increase the availability of dopamine in the substantia nigra of the brain. This is in agreement with previous studies carried out by the Honolulu Heart Program between 1965 and 1998 in the study of cardiovascular diseases, where caffeine from coffee was found to reduce the incidence of Parkinsonism. In this study, caffeine isolated from Cola *nitida* showed some ameliorative effects on the brain of the rats induced with parkinsonism. This sheds light on the possible therapeutic route that could transform our approach to preventing and managing parkinsonism. This study presents positive indications that Cola *nitida* caffeine treatment over a period of time improved motor and neurochemical impairments caused by metoclopramide-induced parkinsonism. However, though the potential utility of Cola *nitida* caffeine as a neuroprotective agent is established, there is need for further research into the implications of this Cola *nitida* caffeine in the aftermath. The efficacy of Cola *nitida* caffeine appears to be fairly modest and not without limitations. Caffeine can be harnessed for its positive attributes but must be used mindfully to prevent adverse effects. Administration of isolated Cola *nitida* caffeine over a longer period of time may cause varying physiological changes viz concomitant weight loss, loss of appetite and water imbalance. Therefore, at this point, it may not be the best choice when approaching Parkinsonism but it does present some neuroprotective benefits. This research suggests a balanced approach to the use of naturally occurring substances in the quest for effective treatment. The variation in the efficacy of isolated Cola *nitida* caffeine calls for further research to explore potential enhancements in its therapeutic application. Exploring alternative avenues and innovative strategies to managing Parkinsonism is important, especially in clinical practice.

Based on the findings of this study, the following recommendations are therefore suggested Since the standard drug Pardopa showed more significant benefits, it might be worthwhile to explore the potential of combining Cola *nitida (*kolanut*)* with other compounds which have neuroprotective effects and other existing medications to enhance therapeutic outcomes. Further research on the optimal dose, duration and frequency of Cola *nitida* caffeine administration for the treatment of parkinsonism and to compare its efficacy and safety with other sources of caffeine, such as tea, coffee, etc.

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Digital Switch Over: Essential for the Creation of a Viable Digital-Tv Sector and Enhancing Creativity in Nigeria, A Review

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Abstract:
A study of the system of information dissemination in Nigeria presents a set of emerging issues that needs addressing by the modernization of existing frameworks. A key enabler in this study points to digital switch over for a healthy and sustainable media ecosystem. This study has shown that Nigeria with about 155 analogue television stations that operate mostly on regional and state basis, has no truly national spine for TV channels; and that 70 per cent of the over 200 million population have access to only 4 or fewer TV broadcast channels. The study also reveals that the analogue TV contents currently being disseminated are relatively weak, and the pay as you use digital terrestrial broadcasting platforms offer a few new digital – only Nigeria channels primarily for entertainment. Hence, getting the digital broadcast market to work is essential for the creation of a viable terrestrial broadcasting industry in Nigeria.

Keywords: Bandwidth, Digitization, Quantization, Switchover, Terrestrial standards.

INTRODUCTION

The key to information transformation from analogue to digital demands digitization. Digitization is the process of converting analogue information of any form e.g., text, photographs, voice, etc., to digital form with suitable electronic devices such as scanner or specialized computer chips, so that the information can be processed, stored, and transmitted through digital circuits, equipment, and networks [1]. According to [2], digital switch over (DSO) is described as the process of launching the digital terrestrial broadcasting platform and switching off analogue terrestrial broadcasting platform. A digital television is a TV broadcasting system that can transmit images with about 720 to 1080 horizontal lines of resolution as compared with about 480 lines of the ordinary (analogue) television system [3]. Digital television offers interference free, CD-quality sound and has the capacity of multiplexing at least 6 broadcast channels under one transmission bandwidth.

Nigeria keyed into achieving the mandate of digitization of broadcasting initiated by the International Telecommunication Union, ITU mandate, to transmit from analogue to digital broadcasting by June 17, 2012. This followed the treaty agreement that was signed at the Regional Radio Communication Conference (RRC-06) in Geneva, Switzerland in 2016, that ushered in an "all-digital terrestrial broadcast service for sound and TV. This was aimed at creating a more equitable, just and people centered information specifically for connecting underserved population and remote communities, thereby bridging the digital divide. The realization of this had not been easy for some countries including Nigeria. Part of the problems is that digital frequency plans must be put in place and coordinated with regional, states and even neighbouring

countries. Secondly, viewers must change their television reception equipment (set-top boxes, etc.) and many transmission sites must be upgraded over a relatively short period of time [4]. On her part, Nigeria is a large country and the number of viewers affected by the analogue switches over is enormous.

One of the greatest challenges surrounding digital switchover in Nigeria and indeed some developing countries is that nations that do not undertake the switchover now and in the nearest future will find it extremely difficult and expensive to source and repair their analogue equipment. This is because in future, the status of digital switchover would change rapidly. The statistics of the countries that have successfully switched over has it that in 1995, the USA became one country that completely switched over from analogue through ISDS and had raised a switch over standard known as ATSC. China in 2006 followed suit with its own digital terrestrial standard also known as DTMB. In North America, Europe and some parts of Asia, analogue switch over has been completed [5]. It must be noted that Nigeria depends to a large extent on these countries for the repair and supply of her needed spare parts. Hence, there is therefore the need for Nigeria to keep pace with the changing times.

Drawing from the UK experience, the Visual Baseline Studies opined that the switch over to digital television in the UK was the biggest single change to broadcasting. The switch made more choices available for millions of viewers and made way for new services that could confirm its role as one of the global leaders in broadcasting and creative industries [5] [6]. It is believed that with a large viewing population, Nigeria would achieve more dividend when she eventually transits to digital broadcasting. Nigerian broadcasters will no doubt enjoy an era of cost effectiveness as a station could be made to carry up to six or more channels of signal on the same frequency. Since digital programming are flexible and faster than analogue, stations may gradually rely on syndicated programmes because the digitization process encourages equal opportunities that results in healthy competition.

There are two primary aims of this study: 1) to review transmission bandwidth in digital transmission and to show that bandwidth in digital systems can multiplex many more transmission channels, unlike in analogue transmission. 2) to emphasize other benefits of digital switchover in Nigeria.

DSO TECHNIQUES

The major techniques of digital TV broadcasting involve the following stages, Signal sampling, Signal quantization, Signal encoding, Transmission of encoded pulses, Decoding of encoded pulses, etc. These techniques are all typified in pulse code modulation principle, PCM.

Pulse code modulation PCM is a technique used to convert the amplitude of an analogue signal to binary value and thereby digitally represent the binary values into streams of quantized amplitude with regular intervals. The use of PCM for TV signal transmission is the fastest method of getting information from one place to another. This is because PCM lends itself comfortably to time division multiplexing TDM, and also it is adoptable to microprocessor/microcontroller-controlled communication equipment, and to the transmission of digital data. Because of this, PCM is used in the distribution of live TV broadcasts from one end of a country to the other. This is made more possible by the use of microwave towers usually positioned with about 32 – 48Km interval to relay the TV signals right around the country. Each tower station contains a receiver that receives the signal, reamplifies it and transmits it to the next tower location at a reasonable

line-of-sight. However, since each reamplification of signal and noise can only aggravate the signal-to-noise ratio, it is only critical that PCM be employed in TV transmissions because it has the most noise immune system of modulation possible.

TRANSMISSION BANDWIDTH IN A PCM SYSTEM

At the rate determined by the original information content (which may be analogue in nature e.g., speech or picture content), the information signal is sampled at regular interval by the digital carrier to produce sampled pulses that could be rounded off (quantized) to the nearest approximatable amplitude values. These amplitude values help to shape up the quantized levels, that it introduces some noise at these stages.

If we assume that the quantizer uses ν number of binary digits to represent each quantized level, then the number of levels that may be represented by ν digits will be. $q = 2^{\nu}$, where q = total number of digital levels of a q - level quantization [7].

Again, let us assume the number of samples of the analogue content per second = f_s

Therefore, the number of bits/secs can be expressed as

Number of bits/secs = Number of bits per sample x number of samples per second = ν bits/sample x f_s samples per seconds.

As a matter of fact, the number of bits/secs = signalling rate of pulse code modulation (PCM) and could be denoted by " τ ". Hence, for signalling rate in PCM, $\tau = \nu f_s$, where $f_s \geq 2 f_i$.

Also, since bandwidth needed for PCM transmission is given by half of the signalling rate, transmission for bandwidth in PCM, $Bandwidth \geq \frac{1}{2}\tau$. But $\tau = \nu f_s$. Therefore, $Bandwidth \geq \frac{1}{2}\nu f_s$. Again since $f_s \geq 2 f_i$. Hence, $Bandwidth \geq \nu f_i$ for PCM digital TV transmission. The interpretation of the above equation is that within a given bandwidth of a digital TV transmission, for instance, a ν number of extra channels of signal can be multiplexed without and inter-channel interferences. This goes to explain why the DSO TV platform, often referred to as free TV, could offer its viewers with more than about 6 digital channels, including sports, music, movies, and news. In PCM TV transmission, higher bit codes are required at 10MHz sampling rate or more [7] [8]. This ensures better picture resolutions resulting from increased number of quantized signal levels. It also gives rise to excellent fidelity of TV signal that is not discernibly different from a standard continuous modulation transmission.

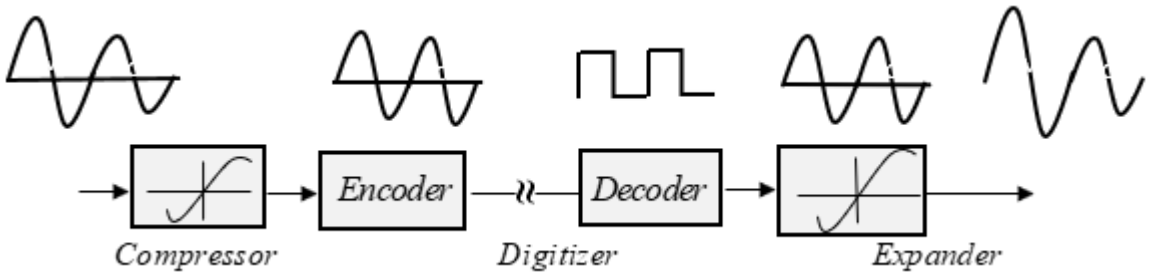

Figure 1: Companding Process

QUANTIZATION ERROR

Quantization error is as a result of the approximation made in rounding off signal levels in order to enable signal amplitudes to be easily encoded into binaries. The amount of this error can be minimized by increasing the number of quantization levels, which of course lessens the space between each other. For instance, a 4-bit code has a minimum of 16 sample levels, while a 5-bit code has 32 levels. This higher bit code decreases the error at the expense of transmission time and bandwidth of transmission. The sample rate is also critical and must be greater than twice the highest significant frequency [7] [8] [9].

The quantization error of PCM systems can be made negligible for strong signals but the very weak signals result in very significant errors regardless of the number of levels used. This problem can be overcome by the use of companding. Companding is the process of volume compression before transmission and expansion after detection [8]. This can be illustrated below.

From Figure 1, it can be observed that the weak portion of the input signal was made strong and nearly equal to the strong portion by the compressor but restored to proper level by the expander. This process is very essential in transmitting quality signals using PCM.

By the use of time-division multiplexing (TDM), PCM transmission for telephones has proved its ability to package more signal messages into short-haul cables, especially in digital PCM television transmission. This has found use in phone-in programmes, and outside broadcasting and reporting in TV transmission. Once digitalized, these voice signals can be electronically switched and restored without degradation. Because PCM could comfortably lend itself to time-division multiplexing, it is very adaptable to microprocessor–controlled communications equipment and to the transmission of digitalized data.

CATEGORIES OF DIGITAL TERRESTRIAL TV SERVICES IN NIGERIA

Licensing of DDT services in Nigeria can be grouped as below.

Digital TV Program Channel Service

This service consists of the provision by any type of TV program for the purpose of broadcasting digital form for general reception [10]. There are two main business modes of this type of TV program, namely:

Free-to-Air (FTA)

In free to air television, viewers do not have to pay to watch the television service. The stations depend largely on advertising and sponsorship to generate revenue. Organisations that seek to provide FTA services require to obtain an authorization for each digital program service. All FTA channels are usually classified as public, commercial or community and shall be carriered by a common carrier

Pay/Subscription Based

In this class, television viewers have to pay a subscription fee to watch the television service. The model requires the television to be encrypted and subscribers have to obtain a module that encrypts the service in their service. This is sometimes called conditional access (CA) module. Most pay TV operators often provide a banquet of channels to their subscribers. Hence, organizations that seek to provide pay TV services shall be authorised to provide a collection of programs services which shall be stated in the Pay TV Authorization [3].

THE BENEFITS OF DIGITAL SWITCH OVER

There are a lot of benefits derivable from switching from analogue to digital TV broadcasting in Nigeria. Some of them include

1. **Freeing of Spectrum Band:** It is expected that when the process of DSO is completed in Nigeria, some frequency bands of the spectrum would be vacated by broadcasters and would be ceded to the telecommunication regulatory agencies for sale and used in the mobile broadcast industries. The spectrum according to [11] which is expected to worth over one billion dollars would change the entire creative industry ecosystem. This is also capable of creating millions of jobs in the years to come.
2. **Utilization of Broadband:** Everywhere in the world, video consumption has been the key driver for majority of homes acquiring broadband. Consequently, the commercialization of broadband requires homes to consume the large pipes of data which uniquely video can do. An example of such commercial utilization is the thriving local film industry with lots of home-grown contents. Most other uses do not require much data. FreeTV and DSO can provide the best of local content to drive purchase of last mile home data. Also, the home equipment used for the home can be the FreeTV boxes which already have the data port to bring data to the home [11] [12].
3. **Encourages Multiplexing:** Analogue broadcasting provides a limited choice of programming due to restricted bandwidth, and it is no longer an efficient technology. The same transmission channel used to broadcast a single analogue programme could carry a multiplex of up to 10 or more digital programs of equivalent quality. Free TV is available nationwide on both digital terrestrial TV DTT and direct-to-home DTH transmission [11] [12] [13].
4. **Value Added Services:** In addition to broadcast services, Free TV also provides value added services such as enforcement and collection of TV licenses, Premium PayTV channels, Push video on Demand, Information Service Audience Measurement, etc [14].
5. **Job Creation:** The DSO is not just about high-fidelity sound, and picture, it is about creating jobs, especially for our teeming youth, stimulating local content and empowering channel owners. It is estimated [14] that in the next three years, the DSO in Nigeria will be capable of creating more than one million jobs in the manufacturing of set-top boxes or decoder manufacturing, TV production, film production, distribution (supply of STBs, TVs, and dongles for the internet), as well as TV and Online advertising.
6. **Payment of One-a-year Access Fee:** Another major advantage of the DSO is that viewers will not pay subscription fee. Once a subscriber has acquired the set-up box and pays the once-a-year access fee, which of course is a token, the subscriber is connected for free viewing all the way. By this, millions of Nigerians who cannot afford to pay the rising subscription fees being charged by the Pay Tv platforms are assured of free digital viewing. This is the meaning of bridging the digital divide [15].

CONCLUSION

This study has reviewed bandwidth, DSO, and the benefits of introducing it to the overall economy of Nigeria. In it also, the bandwidth of digital transmission especially PCM has been assessed. The most important limitations found in the study lies in the fact that most developing countries and including Nigeria, could not key into the DSO for universal implementation, either due to lack of commitment or lack of the needed funds. Overall, this study has strengthened the idea that many benefits exist in switching from analogue to digital transmission of signals (especially TV and radio) and has also encouraged Nigeria (and other nations that have not switched) to fully key into the program for their national growth and development. It will be

interesting to assess the long-term effect of DSO to cover these arears: the economy of the nations that have not recently switched over; local content development; creative works in tertiary institutions; management of the spectrum dividend and re-establishment of public broadcasting.

Barring some exception, any TV set (be it black and white analogue or coloured) can be switched to digital. All that is needed is to attach a set-top-box to the existing television, or alternatively programs can still be viewed, if the TV has a built-in TV digital tuner. However, while acquiring a set-top-box, it is appropriate to ensure that a certified product that supports the DVB-T2 standard is acquired. This makes the TV type compatible with the generation of new TV technologies to come. The DVB-T2 is the next development of the digital video broadcast terrestrial standards.

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Effects of Multi-Modal Approach on Vibration Perception Threshold (VPT) in Trigeminal Neuralgia Using Neurothesiometer

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Abstract:
This case study describes a 50 years old woman diagnosed as a case of trigeminal neuralgia with no remarkable findings on CT scan. She presented with clinical signs and symptoms of trigeminal neuralgia and depression. The objective of this study was to determine the effect of multi-modal approach on pain, vibration perception threshold and depression. She was assessed for pain on numerical pain rating, vibration perception threshold from ophthalmic, maxillary and mandibular branches of trigeminal nerve on neurothesiometer and depression on beck's inventory of depression. Study was conducted from June to September, 2022 at Zubaida Khanum Medical Center Gujar Khan, THQ Gujar Khan, THQ Sohawa, Pakistan. Detailed history was taken using an 18-item questionnaire, designed by the Pain Panel of the European Academy of Neurology (EAN). Patient had 15 sessions based on a multi-modal approach which included Medications, Physical therapy and Cognitive behavioral therapy. Data was collected before and after the intervention. Vibration Perception Threshold not also improved on the affected side (p-value 0.033) but also on the unaffected side (0.038). Pain decreased significantly (≥30%) as well as symptoms of burning sensation, numbness, muscular pain, difficulty in chewing and tinnitus were also relieved. Depression was decreased from a score of 29 to 12 on Beck's Inventory of depression.

Keywords: Trigeminal Neuralgia, Neurothesiometer, Vibration Perception Threshold (VPT)

INTRODUCTION

Trigeminal neuralgia also known as tic douloureux, is a sudden in onset and manifest as severe, stabbing and recurrent pain in the domain of one or more branches of trigeminal nerve lasting for short duration from seconds to few minutes.[1] Primary trigeminal neuralgia is supposed to be caused by the compression of trigeminal nerve in the region of head where the brain connects the spinal cord.[2] Primary trigeminal neuralgia is diagnosed on the basis of symptoms of the patient and the description of the pain. There are no specific tests for the diagnosis of trigeminal neuralgia.[3] The symptoms of this condition in the cheek and jaw region includes; numbness or tingling sensation, burning sensation or electric shock like pain on the affected side of the face, regular aches, difficulty opening mouth, difficulty in chewing and short burst of intense pain.[4] Multi-modal approach for TN management must be individualized comprising of all available treatment options based on bio-pscyo-social model.[5] Quantitative sensory testing is a diagnostic

tool for the determination of somatic sensory system which depicts precise sensory disorders that may not be identified in regular clinical examination.[6] Samaira Younis et al (jul,2016) reviewed patients of trigeminal neuralgia without having any sensory disorder in neurological examination, possessed sensory disorders at Quantitative Sensory Testing.[7] That is why we have also utilized neurothesiometer to assess vibration perception threshold (quantitative sensory testing) before and after multimodal approach for management of trigeminal neuralgia.

Although neurothesiometer has been used in assessing improvement in peripheral neuropathies[8]; to the best of our knowledge this study is the first one to assess the effect of multi-modal approach on vibration perception threshold using neurothesiometer. In our case study, we used a multi-modal (comprising of medication, cognitive behavioral therapy and physical therapy) approach as the treatment protocol for trigeminal neuralgia that gave synergistic effects.

CASE DESCRIPTION

This case study describes a 50 years old woman, already a diagnosed case of trigeminal neuralgia with no remarkable findings on CT scan. She presented with the chief complaints of sudden electric shock like pain, burning sensation on left side of the face, difficulty in opening jaw, stretching of upper eyelid, numbness of lips, altered taste sensations and left side facial weakness that was impacting her functional status. She also had the history of depression which has been aggravating as per assessment using Beck's Inventory of Depression. The case was notably peculiar because she presented with pain in all the three branches of the trigeminal nerve over the complete left side of the face which made her symptoms more excruciating. The patient rates her pain during neural attacks as 9-10/10 on the Numerical Pain Rating Scale (NPRS). Multi-modal approach was suitable to reduce her symptoms, depression and to improve her functional status and quality of life. She was treated under the supervision of a doctor, physiotherapist and psychiatrist at Zubaida Khanum Medical Center. Her detailed management is described in (Table-1).

Table-1: Treatment Protocol for TN

Medications	**Physiotherapy**	**Cognitive Behavioral Therapy**
Prescribed medications: ➢ Tablet Deltacortil 1x TDS for 5 days ➢ Capsule Pregabalin 75 mg 1x H.S ➢ Tablet Surbex Z 1x OD ➢ Syrup Ginkgo Biloba 2tsp x OD for 1 month. ➢ Tablet Carbamazepine* 100mg 1x BD was also advised with the above regimen. *The carbamazepine is a first line, gold standard treatment for the management of trigeminal neuralgia but the patient did not bother to take carbamazepine without informing the doctor because she believed that she might develop addiction to it.	**Short-term goals:** reducing pain and number of neural attacks **Long-term goals:** reducing the symptoms and improving the functional status of the patient. The physiotherapy management included the following: ➢ **Manual therapy techniques:** 20-25 mins/session to reduce tenderness and relax muscles. ➢ **TENS:** 5 days/week for pain relief.	**Short-term goals:** reducing depression and frequency of panic attacks **Long-term goals:** to improve patient's quality of life by decreasing. The cognitive behavioral therapy included the following: CBT sessions were done with a therapist twice a week. Total 10 sessions were done with each session lasting for 30 to 45 minutes. ➢ **Patient's counseling and Education:** psychotherapeutic intervention using neurodevelopmental techniques and education regarding taping technique, nerve desensitization using cotton, fork and other

	➢ **Therapeutic ultrasound:** 5 days/week over trigger points. ➢ **Superficial moist:** 20minutes/session to reduce muscular tension in the cervical region and shoulder muscles especially trapezius. ➢ **Isometric neck exercises and PNF technique:** 3 sessions/day with 10 rep/set and 2 set/session. ➢ **Acupressure:** Using magnets on alternate days. ➢ **Facial gun:** nerve desensitization to promote the reduction of nervous system to constant afferent input. ➢ **Cardiovascular exercise:** Walking to improve overall health status and fitness level. ➢ **Self-massage therapy** was also educated to the patient.	materials to reduce hypersensitivity. ➢ **Deep breathing exercises and distraction techniques:** provide relaxation and to divert the attention of the patient from her pain respectively.

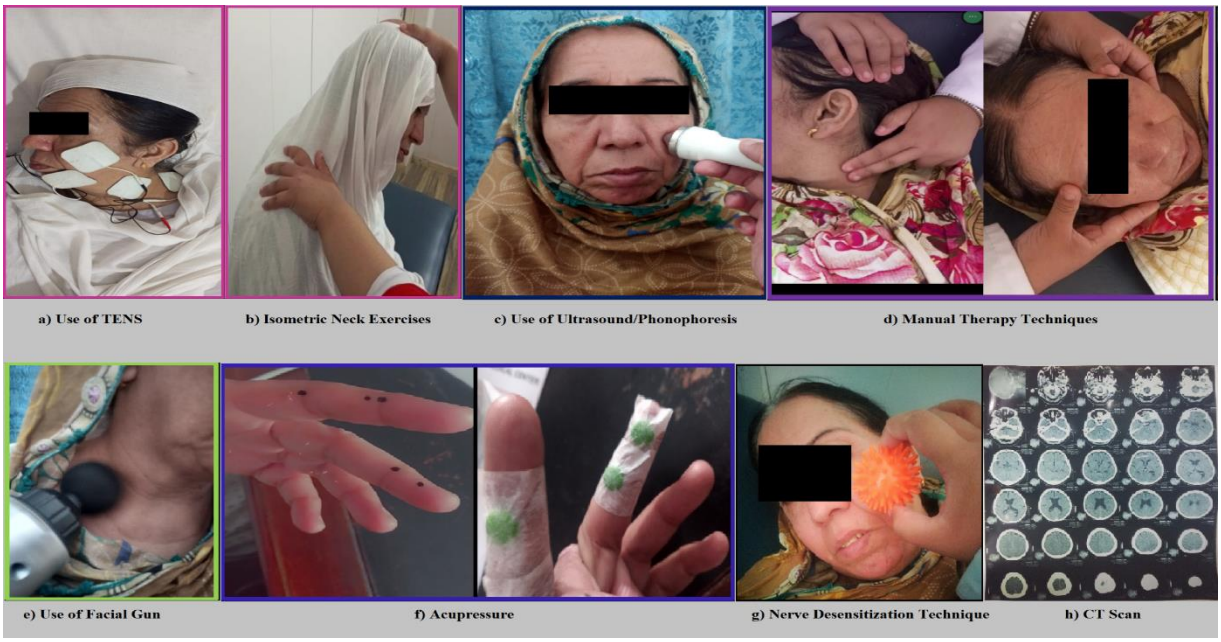

Figure1: Physical Therapy Treatment Protocol

DISCUSSION & RESULTS

Neurothesiometery was done before and after the intervention. Vibration perception threshold improves statistically in all branches of trigeminal nerve bilaterally i.e., congruent effect on the other side.[9] So it was very surprising that the treatment protocol given on the affected side also enhanced Vibration perception threshold on the contralateral side which opens a new platform for the researchers.[10] Pain neuroscience education adjunct with TENS improves pain, sensory abnormalities, jaw function and psychosocial factors.[11]

Not only frequency of episodes but also symptoms of headache, neuralgic pain (MCID: reduction of 30% / 2 points)[12], altered sensations, tinnitus, nasal congestion and depression (MCID: reduction of 29.64%)[13] also reduced remarkably. TN is associated with increased risk of depression but no study has evaluated the effect of cognitive behavioral therapy (CBT) in trigeminal neuralgia.[14] X. Moisset proposed in the study that psychotherapy and other complementary therapies which includes use of neurostimulators i.e., TENS proved to be beneficial for the management of trigeminal neuralgia.[15]

Our results are supported by Alfio Spina et.al proposed notion of personalized multimodal approach for Trigeminal neuralgia in improving chronic pain.[5]

Table-2: Comparison of Bilateral VPTs

Variable	Mean±SD(Hz)	P Value
Pre-Right Ophthalmic	15.6±0.7	0.038
Post Right Ophthalmic	13.1±1.0	
Pre-Right Maxillary	15.6±1.5	0.048
Post Right Maxillary	12.0±0.5	
Pre-Right Mandibular	17.1±1.2	0.007
Post Right Mandibular	12.0±0.5	
Pre-Left Ophthalmic	18.0±1.0	0.006
Post Left Ophthalmic	11.5±0.5	
Pre-Left Maxillary	25.3±1.0	0.005
Post Left Maxillary	12.5±0.5	
Pre-Left Mandibular	20.8±0.7	0.007
Post Left Mandibular	10.8±0.7	
Pre-Total Mean of Right Side	16.1±0.8	0.038
Post Total Mean of Right Side	12.3±0.6	
Pre-Total Mean of Left Side	21.3±3.6	0.033
Post Total Mean of Left Side	11.6±0.8	

Table-3: Comparison of Symptoms

Symptoms in Patient	Before Intervention	After Intervention
Facial burning sensation	Present	Absent
Facial numbness	Present	Absent
Pain in SCM	NRPS: 8/10	NRPS: 2/10
Pain in upper trapezius	NRPS: 7/10	NRPS: 1/10
Difficulty in chewing/drinking	Present	Absent
Stretching of eyelid	Present	Absent
Tinnitus	Present	Absent
Nasal decongestion	Present	Absent

Headache	Present	Rarely
Depression	Scoring: 29 (Beck's Depression Inventory)	Scoring: 12 (Beck's Depression Inventory)
Frequency of neural attacks	7-10times/week	1-2times/week

TN is associated with increased risk of depression but no study has evaluated the effect of cognitive behavioral therapy (CBT) in trigeminal neuralgia.[14] This study showed remarkable decrease (29.64%) in depression after multimodal approach comprising of CBT.

Mauro Barone stated in the study that pain neuroscience education adjunct with TENS improves pain, sensory abnormalities, jaw function and psychosocial factors.[11]

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Technological Innovation is a Change of a Technical or Scientific Nature

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Abstract:
Technological innovation is a change of a technical or scientific nature that introduces a good or service offered by a company or organization into the processes that develop within it in order to achieve greater competitiveness. In other words, this type of innovation corresponds to any technological modification of the product offered by the organization within its processes. Technological innovation is a type of innovation that relates exclusively to technology.

Keywords: AI, DL, ML, IoT, IT Infrastructure, Technology

INTRODUCTION

Artificial intelligence (AI) is a branch of engineering that has been round for the reason that Forties and specialises in the use of records, probabilities, and diverse styles of uncertainties to remedy issues that traditional laptop scientists locate tough [1]. AI is assessed as computer intelligence, which isn't the same as human beings or different residing class intellect. AI is likewise called the evaluation of clever robots, or any item or tool which could recognize and recognise its surroundings and take suitable motion to enhance its probabilities of succeeding in its desires. In each laptop imaginative and prescient and clinical imaging, machine learning (ML) is a method that fosters many AI packages. However, the use of this method blindly, specially for clinical packages, can also additionally bring about negative results. As a end result, capacity pitfalls and related demanding situations in ML stages which include pre-processing, gaining knowledge of, and assessment should be understood. There's lots of desire that making use of AI to healthcare and biomedical software will bring about massive modifications in all fields, from analysis to treatment. Biomedical engineering is in excessive call for and many nations are going through a health practitioner shortage. Healthcare firms are nonetheless suffering to hold up with all the latest technical advances and the excessive needs of clients in phrases of provider levels.

TECHNOLOGY

Technological innovation is a mixture of the brain's computing strength and the preference to mimic human intelligence [2]. The development of generation is the end result of the human preference to remedy issues and enhance the exceptional of lifestyles. Electronic gadgets are technological improvements that correlate with our efforts to remedy issues. The computer is an electronic device that accepts enter, tactics records with the aid of using editing or manipulating it, shops the records for similarly reference, and produces output, all consistent with a sequence of commands or commands. This organization of tactics is the von Neumann structure, or von Neumann idea, posted in 1945 with the aid of using John von Neumann, which describes a proposed structure for a computer. The idea depicts a saved-application computer with instruction and application records saved withinside the identical memory. "The layout includes a crucial manipulate, the crucial mathematics and good judgment unit, memory, and enter and

output." This idea changed into so effective and treasured that our computer systems have always accompanied this version.

All traditional computing gadgets nonetheless function thru those 4 simple functions: receiving input, processing data, storing data, and producing output. There were many technological advances withinside the fields of AI and ML over the past decade [1]. The fast increase of AI equipment and technology, which includes the sector of biomedical and healthcare, has been facilitated with the aid of using a great association of progressed computer processing speed, advanced records collection, and a sturdy AI skills pool. This will end result in a massive extrade withinside the step of AI generation, in addition to its acceptance. ML algorithms can become aware of developments related to sicknesses and health situations. ML strategies have the capacity to enhance healthcare get admission to in evolved nations in addition to innovation in most cancers detection and treatment.

Deep learning (DL) is a relatively novel and hastily increasing outlet of ML. It makes use of multi-layered deep neural networks (DNNs) to version abstraction from massive records, permitting it to make experience of records which include images, sounds, in addition to texts. DL permits the invention of members of the family that had been formerly tough to discover the use of conventional ML algorithms. In the 1980s, artificial neural networks (ANNs) had been used to assemble the early structure for DL, and the true effect of DL changed into visible in 2006. DL has been utilized in an intensive variety of packages considering that then. ANN is one of the strategies that is utilized in biomedical engineering packages. In assessment to preceding neural networks, which simplest had 3 to 5 layers of connections, ANNs and DL networks have extra than 10 layers of connections.

As the reputation of AI, ML and DL are developing because of their reliability and flexibility. There are numerous techniques and technics withinside the location of biomedical imaging, which can be famous over time. Nowadays, the maximum famous device in biomedical software is convolution neural network (CNN). CNN may be used with different mixtures of DL structure like CNN with auto-encoder, support vector machine (SVM) for classification, and K-approach set of rules in photo segmentation; likewise, there are severa approaches and the structure to be had for resolving the real-lifestyles and different studies issues. There are positive CNN fashions present with diverse layers and structures, like Visual Geometry Group (VGG), AlexNet, GoogLeNet, Residual Networks (ResNet), Highway nets, DenseNet, Wasserstein Generative Adversarial Network (WGAN) and plenty of extra.

IT INFRASTRUCTURE

As we all recognise, the a great deal-dissected and discoursed cloud paradigm has laid a stimulating basis for compactly gratifying the grand imaginative and prescient of IT infrastructure optimization thru a continuing synchronization of numerous proven, enterprise-scale, and venture-essential technology which include compartmentalization (virtualization and containerization), grid, on-call for, utility, and autonomic computing models, provider orientation, and multitenancy [3]. This groundbreaking evolution and elevation withinside the IT subject have brought in innumerable and insightful influences on commercial enterprise in addition to IT domain names those days. Clouds are being located and proclaimed because the relatively consolidated, converged, virtualized, shared, and automatic IT environments for hosting and compactly turning in a galaxy of various IT answers and commercial enterprise offerings. The cloud generation guarantees anytime, anywhere, any community, and any tool get

admission to to data and provider. That is, the a great deal-predicted ubiquitous provider shipping is being completely facilitated with the arrival, articulation, and adoption of the effective cloud idea. The fashion is that every one forms of commercial enterprise and IT offerings, packages, and records at the moment are being modernized accordingly and adroitly migrated to cloud structures and infrastructures so that it will reap all of the at first estimated benefits (technical, user, and commercial enterprise cases).

The cloud paradigm has grow to be a flexible IT phenomenon and a fantastic fertile ground that has stimulated many withinside the international to pop out with some of newer cloud-centric offerings and structures that facilitate rankings of human beings-centric, multifaceted, and wealthy cloud packages to attain out to many on this related international. Besides, there were quite a few typical in addition to precise improvements withinside the shape of pragmatic tactics, styles, nice practices, key guidelines, metrics, and so forth for moderating the growing IT complexity, for boosting IT agility, autonomy, and affordability, and for heightened IT productivity. The strong and resilient cloud version is directly supporting out international commercial enterprise companies to reap the venerable venture of extra with less. Thus, cloud because the core, crucial, cheap, and cognitive infrastructure for implicitly looking after all styles of commercial enterprise modifications, concerns, and demanding situations portends and portrays a brighter and completely satisfied future for commercial enterprise businesses so that it will surge beforehand and to hold up their edge earned of their offerings, outputs, and outlooks.

With a legion of resource-constrained, embedded, and networked gadgets becoming a member of withinside the IT panorama and with the seamless synchronization with the remote, on-call for, and elastic clouds (typical clouds which include public, private, and network or precise clouds which include garage, understanding, science, records, sensor, tool, and mobile), there abound hordes of real-time and complex packages and offerings.

CLOUD COMPUTING

Cloud computing as in-structures, networks, or packages wherein the most strength of human creativity may be accessed, can supply those equipment to the IoT [4]. The records created, the device used and the technique of creating complicated visualizations are hid on this context. Cloud computing is a programming version that supplies on-call for software program to cease customers from more than a few computing centers, which includes database offerings and laptop infrastructure. The key equipment presented with the aid of using cloud computing are Infrastructure as a Service (IAAS), Application as a Service (PAAS), and Software as a Service (SAAS) Mell and Grance. Many of those providers provide computing centers which include garage and records processing on request. Cloud computing concentrates at the active optimization of disbursed assets with the aid of using manifold consumers. Western clients, for example, are given a cloud garage provider (for instance, email) primarily based totally on their time zone. The identical area is sent with the aid of using cloud garage to Asian clients, typically relying on their time zone.

As a bendy and preordained innovation, cloud computing has lately emerged and is growing hastily. According to client wishes, the cloud computing platform gives relatively versatile, open, and programmable digital servers, area, computer strength, and virtual communication. A answer bundle for the digital transition of data can also be furnished if it's miles designed for IoTs and coupled with cutting-edge records garage, distribution and preservation technology. In addition, so long as the computing area allows, the user generates understanding very quickly. The client,

who wishes to be to be had easily, can nonetheless use a great deal of the data. One of the important thing sides of cloud infrastructure is media control, because the cloud enables the garage, coping with and sharing of big portions of digital data. For virtual media related to IoTs, this functionality can show a key function. In the future, many extra multimedia offerings might be viable for people at the move, for instance, smartphones, capsules and computer operators, advert hoc car structures, separate ambulances, and rescue operations. Cloud infrastructure plans to show an real sturdy duty withinside the employer of packages and in-shape for the ones assets which can be relevant. The server might be used extra regularly, specially with Fog Computing, additionally called Edge Computing or Micro-Datacentre, an extended network (MDC). A on hand method for coping with content material in disbursed environments is cloud garage. Without the duty of coping with big garage and processing instruments, it gives streamlined get admission to to data. Another function cloud companies provide is to percentage a large wide variety of on-line media. In addition to social networks, conventional cloud computing carries extra sharing and content material control capability. Similarly, it isn't always viable to import man or woman documents one after the alternative if cloth is to be shared. This difficulty is addressed with the aid of using cloud computing so all data may be accessed straight away with the aid of using all entities for whom the records is transmitted. Also, cloud garage can offer extra context-conscious offerings, as IoT and sensor networks aren't effectively thick in belongings to behavior such operations. To generate similarly tailor-made and purposeful software program, records deposited withinside the cloud can also additionally also be completely processed.

SOFTWARE DESIGN

With the emergence of software program computing improvements, making sure the device's reliability has grow to be a difficult mission for which industries and allied software program builders were making important efforts [5]. Identifying software program layout, structure, in all likelihood fault situations, maintainability, computer virus probability, smells, replication, etc., can save you a software program device from becoming corrupted or failing all through the software program improvement lifestyles cycle (SDLC). In such a case making sure Quality of Service (QoS) shipping with computationally green software program improvement may be massive. To observe the efficacy of software program structures assessing the complete device's refactoring may be of superb significance as it is able to keep away from any fault or layout problems withinside the future, hence making the software program device extra green. When it involves item-orientated software program, refactoring is the procedure of creating modifications to a device's inner shape with out affecting its capability or performance. It approach that the software program device might be converted into smooth code and layout. Software device exceptional and protection may be progressed with the aid of using imposing this method. Refactoring can be executed on the technique, class, or bundle degree, relying at the scenario. After every extrade, the refactoring procedure plays step-with the aid of using-step to make sure that the entirety maintains to run easily and efficiently. Our supply code metrics want to be computed primarily based totally on class-level and technique-level surveys to forecast the proportion of techniques or lessons to be refactored withinside the gadget gaining knowledge of framework. Using software program, refactoring can enhance OOP's (Object-oriented programming) inner shape whilst nonetheless maintaining its outside conduct and capability. Because code is continuously being changed to satisfy new requirements, the supply code deviates from its unique plan shape. Finding insects in software program code is tough due to the fact the code will become multifaceted, tough to study and debug, or even too tough to enhance upon due to this. Software refactoring techniques allow us to take away odors that damage the application's operation whilst making it extra understandable and extensible. The identity of code

that calls for refactoring is a massive task withinside the context of refactoring. It will increase the issue of the mission for the researchers.

BUSINESS

While the arena of commercial enterprise is hastily evolving and the commercial enterprise corporations are winding up an increasing number of greater difficult to make it step by step tough for administrators to have far-accomplishing knowledge of commercial enterprise scenarios [6]. Elements which include globalization acquisitions and mergers, deregulation, mechanical and competition advancement, have limited corporations to rethink their strategies in commercial enterprise and plenty of large businesses have became toward the techniques of BI (Business Intelligence) so that it will permit themselves to recognize and control commercial enterprise approaches to feature to top hand. Business Intelligence is mainly followed to enhance the expediency and records nature, and permit chiefs to higher realize the scenario in their company whilst compared to their competitors. Innovations and packages of Business Intelligence help businesses with the aid of using analyzing various styles in correlation to the general enterprise; modifications withinside the behavior of customer and expenditure on designs; the inclination of the customer; abilities of the employer; and monetary situations. It is used to encourage directors training session which changes can higher react to various styles. It evolved as a layout for investigating amassed data to permit simple management gadgets to permit improvement in tremendous understanding of responsibilities of an association, and therefore assist to discern out higher commercial enterprise strategies and choices.

ML (Machine Learning) is an detail of a promising AI (Artificial Intelligence) understanding that has been followed notably over time in a developing wide variety of fields and branches so that it will automate the hassle fixing and figuring out complicated choice techniques. ML is a shape of records evaluation that permits the computer systems to examine from the records so that it will collect stories and understanding approximately the records to remedy real-international issues. ANN (Artificial Neural Network) is the maximum famous technique in ML that is inspired with the aid of using the human brain's organic neural community and works at the precept of human gaining knowledge of aspects. Some different strategies are case-primarily based totally reasoning, NLP, genetic algorithms, inductive gaining knowledge of, etc.

GADGETS

A wide variety of demanding situations save you the securing of IoT gadgets and making sure cease-to-cease protection in an IoT surroundings [7]. Since structures management machines and exclusive objects are fairly new, protection has now no longer usually been regarded as pinnacle want all through an object's plan stage. Furthermore, in mild of the truth that IoT is a starting commercial enterprise sector, severa object designers and makers are step by step eager on getting their objects to show off hastily, as hostile to locating a manner to manufacture protection in from the starting. A noteworthy difficulty noted with IoT protection is the usage of hardcoded or default passwords, that may spark off protection breaks. Regardless of whether or not passwords are converted, they're frequently now no longer sufficiently capable of ward off penetration.

Another simple difficulty confronting IoT devices is that they're regularly asset obliged and do now no longer incorporate the procedure belongings critical to actualize stable protection. All matters taken into consideration, severa devices do now no longer or can not provide propelled protection highlights. For instance, sensors that display screen dampness or temperature can not

address current encryption or different protection efforts. Besides, the identical wide variety of IoT devices are "set it and overlook it"—set withinside the subject or on a gadget and left till a part of the bargain—they slightly ever get protection updates or fixes. From a producer's perspective, constructing protection in from the starting may be expensive, avoid improvement, and purpose the device now no longer to paintings because it should. Interfacing history assets now no longer intrinsically meant for IoT community is some other protection task. Supplanting history basis with related innovation is cost-restrictive, and such large numbers of advantages might be retrofitted with extraordinary sensors. In any case, as history assets that likely have now no longer been refreshed or ever had safety from cutting-edge dangers, the attack floor is prolonged. IoT protection is also tormented with the aid of using a lack of enterprise-recounted fashions. While severa IoT protection structures exist, there may be no unmarried settled upon shape. Enormous businesses and enterprise institutions can also additionally have their very personal precise norms, whilst positive sections, for example, present day IoT, have restrictive, inconsistent models from enterprise pioneers. The collection of those gauges makes it tough to verify frameworks, but further assure interoperability among them.

STANDARDS

Innovation and generation improvement are accelerating [8]. Strategic plans and roadmap are had to assist make sure that the marketplace is certainly served with nice practices which can be pertinent to the desires and context of this very big marketplace. The requirements guide our want to stability agility, openness, and protection in a fastmoving surroundings. The requirements offer us with a dependable platform from which we will innovate, differentiate, and scale up our generation improvement. They assist us manipulate critical protection and combine the proper level of interoperability. Standards assist make sure cybersecurity in ICT and IoT structures. The respective ecosystems of clever infrastructure, clever manufacturing, health, training, banking, management, governance, etc. would require an extraordinary integration of structures throughout domain names, hierarchic boundaries, and lifecycle stages. System requirements might be wanted for the automation and digitalization of our structures and answers.

The international has by no means been as aggressive as today, but cooperation is a should to supply answers for an increasing number of complicated structures. No technical committee and no requirements employer can unmarried-handedly broaden all of the requirements that are wanted. We all want to paintings together. However, requirements or even SDOs are now no longer at the vanguard of cities', utilities, or customers' minds. There are misconceptions on what requirements are for, and the case to be used of requirements has now no longer been made. Liberalization and markets have lots of superb virtues, however they can not create their personal situations of existences: they should be designed!. Furthermore, requirements want to be supported with the aid of using permitting technical rules and policies to make sure that the evolved requirements are furnished a conducive coverage and regulatory framework to be seamlessly followed with the aid of using all of the stakeholders.

DEVELOPMENT

The maximum growing innovation withinside the coming duration relies upon on man-made reasoning [9]. The innovation impacts the customer cooperation with and with the aid of using the Internet. Computer-primarily based totally intelligence has the impending to extrade how human beings interact with the excessive-tech international in addition to with each other thru their paintings and socially. As the human cerebrum is related, but the machines are computerized. The massive evaluation among human and man-made brainpower is that the

human beings regulate to various weather with the aid of using making use of various steady cycles whilst man-made reasoning paperwork machines that replicate human behavior and perform human-like activities. Half breed smart framework characterizes a product framework that participates in identical a mixture of techniques and techniques from synthetic understanding as subfields, for example, neuro fluffy frameworks. Artificial intelligence has been implemented to exclusive territories as follows:

- Intelligent family
- Newfangled clinical offerings
- Programmed driving
- Emotional interactions

SOCIETY

The technological revolution is reworking hastily many stuff whilst human beings are now residing in a brand new virtual society which creation is taking region daily, primarily based totally on ideas which include digital truth, massive records, social networks, customers, geolocation or virtual divide amongst many others [10]. The use of huge records units and more than one digital gadgets with the aid of using residents is a truth that can not be stopped. The intake of offerings primarily based totally on those new technological infrastructures represents every day a better percent of the financial sectors withinside the evolved nations. A new virtual lifestyle is being created. Technological changes are affecting more than one dimensions at the herbal, social and financial structures wherein the arena is prepared and studies is produced.

During ultimate decades, we've got step by step moved toward the understanding society wherein lifelong gaining knowledge of will become a should in phrases of competitiveness and wellbeing. Knowledge has been diagnosed because the motive force of financial increase and productivity with the aid of using the Organization for Economic Cooperation and Development (OECD). The understanding-primarily based totally economic system has been associated with training and innovation and to the use of Information and Communication Technologies (ICTs). Creation and manufacturing of recent understanding is a consistent want of humans so that it will attain a better knowledge of factors and tactics on the social, biophysical, psychological, natural or technological spheres to higher explain complexity of truth. The acceptances of worldwide interactions wherein natural and human ecosystems are fuzzily related at exclusive spatial scales are breaking the boundaries of the conventional device theory. A new comprehension of truth seems whilst acknowledged and new factors, tactics and technology that had been now no longer covered earlier than in our interpretations grow to be now taken into consideration rationally under modern approaches. The generalization of this exercise right into a collaborative surroundings can take us to co-introduction and co-manufacturing tactics as new techniques of wondering and learning capable of generate new understandings.

CONCLUSION

Innovation is any procedure that reduces production and administration costs, increases productivity or the use of equipment or time, improves the quality of products or services, increases safety, reduces scrap, improves placement, etc., that is any measure that leads to increased competitiveness. A special type of innovation are technological innovations, from the category of useful proposal, through technical improvements to inventions, which can provide their owner with a significant competitive advantage, i.e. a monopoly. In developed economies, special attention is paid to innovations and innovators, and inventiveness ranks high on the scale

of the value system. The most valuable resource of the company is not material goods such as buildings, plants or capital, which can always be found on the market, but the knowledge, experience and inventiveness contained in the nation's industrial tradition and quality personnel.

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Investigating Innovative Methods for Improving the Shelf Life of Meat

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Abstract:
Over the past few decades, various approaches have been employed for meat preservation. With advancements in technology, the meat industry is actively pursuing modern and cost-effective methods for preserving meat and meat products. In this regard, several unconventional methods, such as Super-chilling, Hydrofluidization freezing, Impingement freezing, electrostatic freezing, and Pressure-shift freezing, have demonstrated their potential for effective meat preservation. Additionally, active and intelligent packaging options will prove beneficial for meat packaging, although their capabilities require further improvement before implementation. Furthermore, these new technologies are more environmentally friendly, requiring reduced energy and water consumption while generating minimal waste. As a result, this article will prove valuable to the meat industry in considering these techniques for more efficient commercial preservation of meat and meat products.

Keywords: Meat preservation methods, Super-chilling, Hydrofluidization freezing, Impingement freezing, environmentally-friendly, active packaging.

INTRODUCTION

With increasing cognizance and demand of consumers for wholesome, fresh and safe meat products, scientists are untiringly engaged in developing and discovering numerous innovative and progressive food preservation techniques for potential commercial application. A vast range of novel thermal and nonthermal meat processing and preservation technologies has gained much attention over the past few years. Several benefits are allied with the use of these techniques which include high process efficiency, enhanced product quality, improved safety, and prolonged shelf stability of meat products.

METHODS

Advancements in Thermal Meat Processing Techniques

Thermal techniques have been preferably used by the food industry to inactivate several spoilage indicators and pathogenic microorganisms and ensure product stability. Nutritional profile of meat and meat-based products attracts microorganisms to grow readily and deteriorate the quality of meat. Therefore, thermal treatment is thought to be the more appropriate method to prevent meat from getting spoiled. However, application of heat to prevent microbial spoilage adversely affects the nutrients present in the muscle foods. Alternatively, low temperature methods have been utilized to slow down or seize the microbial activities. Low temperature preservation methods are claimed to be more beneficial in comparison with the high temperature methods because they usually do not disturb the nutritional contents of food. With the passage

of time, several advancements have been made in low temperature processing techniques for process improvement that are discussed in the following section.

Super-Chilling:

The method of super-chilling is used to describe the process where some portion of the water present in a particular product is frozen. During super-chilling, the product temperature is decreased up to 1–2 ˚C for initial surface freezing. After some time, the product attains the uniform temperature by equilibration of ice distribution which is maintained during the storage and distribution of the product. This process has been mostly used for seafood preservation but unfortunately, the process is not effectively expanded for chilled meat storage. Super-chilling is useful for inhibiting microbial growth and can reduce the use of freezing/thawing thereby reducing energy cost, labor and weight loss. The ice prevents super-chilled meat products from temperature elevation in poor cold chains; though, increased drip loss is recorded during the storage of meat. Super-chilling is claimed to prolong the shelf life of meat products for 1.4–4 times as compared to the conventional meat-chilling.

Freezing:

The size and structure of ice crystals depends on the rate of freezing. Fast freezing leads to the formation of small ice crystals and causes less cellular damage to product being frozen. Reports conclude that cell integrity of muscle foods has better preserved by rapid freezing methods in contrast to slow freezing. Ultra-rapid freezing involves freezing food between -218 up to - 225 ˚C. Temperature of the product is rapidly decreased to desired level which results in the formation of smaller ice crystals; hence, flavor and textural attributes of the food are not disturbed. This is the reason why the fast-freezing technology is increasingly being used in food industry.

Immersion Vacuum Cooling:

Immersion vacuum cooling (IVC) is another method that is claimed to attain high cooling rate and less cooling losses in comparison with traditional cooling methods. Additionally, further advancements have been made in order to improve the competitiveness and safety concerns. These strategies include combining this technology with other cooling methods, pressure control, inclusion of agitation, applying altered condensation temperatures, and using water with different initial temperatures. IVC is mainly used for meat preservation and involves the combination of water evaporation, conduction, and convection.

Hydrofluidization Freezing:

Hydrofluidization freezing (HFF) is a modified form of immersion freezing which involves a circulation system that pump refrigerating liquid in a cooling vessel through nozzles, in a thereby creating agitating jets which forms a fluidized bed having a turbulent liquid and moving products. HFF offers high heat transfer coefficients that provides paced freezing.

Impingement Freezing:

The use of impingement technology is one of the latest techniques that are commercially introduced for the preservation of meat and other food products. The main advantage of this method is higher heat transfer rate through coupling impingement technology with cryogenic freezing system (Winney, 2012). This technique involves directing high speed (50 ms21) jets of fluid to the surface of a solid product that breaks up the static layer of gas surrounding the food surface. This process enhances the efficiency of heat transfer which results in rapid freezing compared to conventional blast freezing systems. Reports have shown that impingement

freezing is more effective for those foods having high surface area to weight ratio, that is, meat cuts and fish fillets

Pressure-Shift Freezing:

Freezing foods using high pressure (200–400 MPa) has attracted researchers in recent years with special reference to pressure-shift freezing (PSF). In PSF, high pressure is applied while cooling the food to subzero temperatures which prevents the phase change and then food is frozen after releasing this pressure. When the pressure of the system is released, uniform and small ice crystals are formed in food due to rapid nucleation.

Comparison of meat thermal preservation methods

Techniques	Advantages	Disadvantages
Super-chilling	Reduced labor and energy cost, less product weight loss, improved meat safety	Complex calculations are required for effective heat transfer and temperature control
Ultrarapid freezing	Less cellular damage, better textural quality	Cause mechanical cracking, higher drip loss
Immersion vacuum cooling	Improved tenderness, less cooling losses,	Safety risks due to inclusion of water
Hydrofluidized freezing	Fast freezing rates, improved quality	No literature available
Impingement freezing	Effective heat transfer	Mechanical damage

Novel Nonthermal Meat Preservation Methods

Apart from the traditional preservation methods that usually involve heat or chemical interventions, many nonthermal preservation techniques have been emerging for preserving solid foods particularly meat products. The most widely used methods include OP, ultraviolet (UV) radiation, ultrasound treatment, HPP, electrolyzed oxidized water with high-pressure carbon dioxide treatment, PEF technology, and plasma technology. The potential of some techniques for meat preservation is summarized in this section.

Acidic Electrolyzed Water Coupled with High Hydrostatic Pressure:

Use of acidic electrolyzed water (AEW) has gained attention of researchers in recent years for effectively controlling the bacterial spoilage of meat-based products. AEW has shown better bactericidal effect in contrast to other conventional sanitizers. Previous studies have depicted that AEW can be used synergistically with other antimicrobial interventions for controlling microbial spoilage of meat and other food products. Used electrolyzed water in combination with other antimicrobial agents on fresh poultry surface and noticed a significant reduction in number of pathogenic bacteria particularly Salmonella. Recently, has reported that using AEW in synergism with high hydrostatic pressure can significantly reduce the bacterial load from shrimp surface without disturbing the quality of meat and can be used as a tool for preservation of other meat-based products.

HPP in Improving Meat Quality and Safety:

Several novel nonthermal techniques have been investigated for effective meat preservation. Literature on these methods highlights that these techniques can assist meat processors to meet the consumer demand of meat with good nutritional value, superior quality, and esteemed safety as well to fulfill the criteria of energy efficient meat processing and preservation. In this regard,

HPP is reported as an effective tool in improving meat safety and quality. Some researchers suggested that HPP (100 MPa) improves texture, color, and water holding capacity of meat in addition to provide microbial safety and extended shelf life. HPP technology has shown its potential for inactivating the growth of microorganisms from meat and meat products during the processing and storage.

Use of UV Treatment in Meat Safety and Preservation:

UV light has wide applications in decontaminating the surface of solid foods by inhibiting the growth of microorganisms and by destructing the pre-existing microbes on the food surface. Short-wave UV light has been deployed to treat the surface of meat fillets and reported to curb the growth of Pseudomonas species.

Plasma Technology in Meat Preservation:

Among various nonthermal processing techniques, plasma technology has also been extensively reviewed to extend shelf life of meat and meat products. Nonthermal plasma is composed of reactive photons comprising ions or free electrons which have significant bactericidal effect Plasma contains charged species, which when applied to the bacterial cell, cause protein denaturation, enzyme inhibition, oxidation of amino acids, breakage of bonds on the cell membrane due to bombardment of radicals, perforation of cell membranes due to the diffusion of ionized species, oxidation of the membranes, DNA damage and reduction of cell membrane resulting in loss of functionality and cell death. Several investigations have affirmed the competence of plasma technology for effective meat decontamination.

Advances in Meat Packaging Technology

With the advent of self-service meat cases for raw chilled and processed meats, the need for meat packaging to fulfill multiple functions has resulted in a variety of materials and systems that are available and each can be tailored to specific needs and applications. Application of several new and more efficient approaches is of no use without proper packaging. Packaging is of prime importance as it protects food from harmful environmental effects by acting as an inert material. So, proper packaging plays a dynamic role in preserving quality, safety, and sensory attributes of the product. Several advancements have been made in packaging technology of which active meat packaging stands out as most important emerging technology.

THE IMPACT OF NON-CONVENTIONAL MEAT STORAGE METHODS ON HEALTH AND THE ENVIRONMENT

These methods not only make it possible to obtain meat and meat products with a longer shelf life, safer for health and without preservatives, but also are more environment-friendly in comparison with traditional methods. With the use of alternative methods, it is possible to obtain meat products that are microbiologically safer, whilst also high quality and free from chemical additives. Moreover, these new technologies are also more ecological, do not require large quantities of energy or water, and generate less waste.

CONCLUSION

It does not seem that using these meat preservation methods would lead to changes that result in the formation of toxic compounds, which could pose health risks. The choice of using the described methods varies depending on the costs and the availability of related technological resources. Innovative meat packaging also plays a significant role in ensuring the safety, convenience, and quality of meat.

Modern approaches to meat processing and preservation have demonstrated the potential benefits of high-quality and long-lasting meat products, aligning with consumer demand. These innovative technologies not only reduce energy and water consumption but also decrease the production of wastewater. However, the widespread adoption of these technologies globally poses challenges for food industry innovators and researchers. Consumer perception of these technologies is not always well-defined, necessitating further clarification and explanation.

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Chemical and Sensory Properties of Maize Ogi Enriched with Flours and Protein Isolates from Bambaranut and Soybean

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Abstract:
Background: Ogi is an infant complementary food in Nigeria that is starchy in nature, a bulky gruel with decreased nutrient density like protein and essential amino acids. Flours and protein isolates from bambaranut and soybean were prepared to enrich maize ogi. Objective: The objective of this study was to evaluate the chemical and sensory quality of maize ogi enriched with flours and protein isolates from bambaranut and soybean. Methods: Standard procedures were used for the processing of flours and protein isolates. Ogi made from 100 % maize flour served as control. The other samples include; maize ogi /soybean isolate (B), maize ogi /bambaranut isolate (C), maize ogi/soy flour (D) and maize ogi/bambaranut flour (E) in different blend ratio which were assessed for pasting analysis, amino acid, micronutrient, antinutrient and sensory evaluation. Result: Pasting properties of the maize-based ogi flour was altered significantly ($p<0.05$), the protein isolates enriched ogi had higher (79.22, 74.97 oC) pasting temperature over the flour enriched ogi (71.86, 66.31 oC). The micronutrients were greatly improved by the addition of flours from bambaranut and soybean while the anti-nutritional contents were minimal. In general, the ogi samples scored above 6 on a 9-point hedonic scale, suggesting that acceptable protein enriched ogi could be made from maize flours and either bambaranut or soy flour as well as their protein isolates. Conclusion: The protein, amino acids, micronutrient, composition of the maize-based ogi were enhanced significantly following the addition of flours and protein isolates from bambaranut and soybean with decreased antinutritional contents which could help in alleviating protein energy malnutrition among under five children.

Keywords: Maize ogi, bambaranut, soybean, bambaranut and soybean protein isolates.

INTRODUCTION

Ogi is a smooth, free flowing thin porridge obtained from wet-milled, fermented cereal grain popularly served as breakfast cereal and infant complementary food in Nigeria (1). *Ogi* is produced from cereals such as sorghum, maize and millet or their combinations (2). A major disadvantage with cereal-based gruel such as maize *ogi* is its starchy nature which makes them bind so much water, thus yielding a bulky gruel with decreased nutrient density (3).

Maize *ogi*, like most cereal products is low in protein as well as in essential amino acids such as lysine, tryptophan and threonine (4). Therefore, solitary and consistent consumption of maize *ogi* could predispose individuals to protein energy malnutrition which essentially is a state of inadequate dietary protein and energy (5).

Soybean (*Glycine max*), a legume, is one of the nutritious and affordable sources of plant protein that have been employed to improve the diets of millions of people, especially low-income

earners in developing countries owing partly to its functional properties (6). Soybean contains; proteins (40%), lipids (20%), minerals (5%), and B vitamins for human nutrition (7). Processing is necessary to destroy or remove some of the undesirable constituents of legumes such as soybean and bambaranut and to improve their palatability ((8)

Compared to soybean, Bambaranut (*Vigna subterranea L. Verdc*) is an under-utilized legume of the African origin and it is the third most important legume after groundnut (*Arachis hypogeal*) and cowpea (*Vigna unguiculata*) in Africa (9). Bambaranut contains about 24 % protein with a good balance of the essential amino acids and relatively high proportions of lysine (6-8%) and methionine (1-3%) (10). Bambaranut minor constituents include minerals, vitamins as well as anti-nutritional factors such as trypsin inhibitors and polyphenols (11).

Protein isolates are the most refined form of protein products containing the greatest concentration of protein (90 %) on a dry weight basis (12). It's high concentration of protein with the advantages of colour, flavour and functional properties makes it an ideal raw ingredient for use in beverages, infant and children milk food and certain types of specialty foods (12, 13). Protein isolates have been developed from a variety of legumes such as soybean, bambaranut, cowpea, peanut, canola, cashew nut, almonds, sesame, pinto and navy beans (14, 15). By combining legumes and their protein isolates with maize in *ogi* preparations, the protein value of maize *ogi* could be enhanced. The study was to evaluate the chemical and Sensory quality of maize *ogi* enriched with flours and protein isolates from bambaranut (*Vigna suberranea L*) and soybean (*Glycine max*).

MATERIALS AND METHODS

Maize, bambaranut and soybean, were obtained from Benue State Agricultural and Rural Development Authority (BNARDA), Makurdi, Nigeria. All equipment used were obtained from the Department of Food Science and Technology, Joseph Sarwuan Tarka University, Makurdi, Benue State and Kogi State University, Anyigba.

Preparation of Maize *Ogi*

Ogi was processed according to the modified method of (16). Maize grains were obtained, sorted to eliminate the bad grains, cleaned to remove debris and foreign bodies and steeped in clean tap water for 24 h at room temperature. The steeped grains were then washed with clean water, wet milled using a M6FY commercial flour milling machine and wet sieved using a 250 µm sieve. The husks were disposed while the filtrate (slurry) was allowed to ferment for 24 h to collect sediment. At the end of the fermentation period, *ogi* was recovered by using a cheese cloth to squeeze out the water. The wet *ogi* sample was then dried in the Gallenkamp (United Kingdom) moisture extraction oven (60 °C, 12 h). The dried starchy cake obtained was milled and sieved using (250 µm mesh sieve) to obtain *ogi* flour.

Processing of Defatted Bambaranut Flour

Bambaranut flour was prepared according to the method described by (17). The Bambaranut was sorted to remove extraneous materials and damaged seeds. Soaked (10 h at 1:2 w/v), boiled for 20 min, manually dehulled (by rubbing in between the palms) and oven dried in the Gallenkamp (United Kingdom) moisture extraction oven at 60 °C for 24 h. The dried bambaranut was milled in a M6FY commercial flour milling machine, followed by sieving through a 250 µm mesh and packaging in airtight plastic containers which were placed in shelves at room temperature until used. The flour was defatted using a modified method of (18). The flours were defatted using n-

hexane (flour to solvent ratio 1:5 w/v) with constant magnetic stirring provided for 4 hours. The trace of residual hexane was removed by placing the defatted flours inside a fume cupboard for 6 h to dry. Flours obtained were packed in plastic tubes until further use.

Processing of Defatted Soybean Flour

Soybean flour was processed according to the modified method described by (19). The soybean seeds were sorted to remove pebbles, stones and other extraneous materials. They were wetted, cleaned and steeped for 10 h. The steeped soybean seeds were drained and precooked for 15 min followed by dehulling (by rubbing in between the palms) and the hulls were removed by rinsing with clean water. The dehulled soybean seeds were oven dried at 60 °C for 12 h and dry milled into fine flour. The soybean flour was sieved using 250 µm mesh sieve to obtain smooth flour. The flour was defatted using a modified method of (18). The flours were defatted using n-hexane (flour to solvent ratio 1:5 w/v) with constant magnetic stirring provided for 4 hours. The trace of residual hexane was removed by placing the defatted flours inside a fume cupboard for 6 hours to dry. Flours obtained were packed in plastic tubes until further use.

Processing of Bambaranut Protein Isolate

Protein was extracted from defatted bambaranut flour using a modified isoelectric precipitation procedure (19). The defatted flour was dispersed in distilled water at 1:10 (w: v) ratio. This was followed by adjustment to pH 7.5 with 1.0 M NaOH to solubilize the protein. The resulting mixture was stirred using a magnetic stirrer for 4 h and centrifuged at 3,500 xg for 30 min using the labnet spectrafuge 6C low speed centrifuge. The residue was discarded and the supernatant filtered with cheesecloth and adjusted to pH 4.5 using 1.0 M HCl to precipitate most of the proteins. Thereafter, the mixture was centrifuged (3500 x g, 30 min). The resultant precipitate was re-dispersed in 25 ml distilled water, frozen at 0 °C and then freeze dried at -52 °C to yield a free-flowing powder. The bambaranut protein isolates was stored in a sealed tube at 4°C until analyzed.

Processing of Soy Protein Isolate

Protein was extracted from defatted soy flour using a modified isoelectric precipitation procedure (20). The defatted flour was dispersed in distilled water at 1:10 (w:v) ratio. This was followed by adjustment to pH 8.5 with 1.0 M NaOH to solubilize the protein. The resulting mixture was stirred using a magnetic stirrer for 4 h and centrifuged at 3,500 xg for 30 min. The residue was discarded and the supernatant filtered with cheesecloth and adjusted to pH 4.5 using 1.0 M HCl to precipitate most of the proteins. Thereafter, the mixture was centrifuged (3500 x g, 30 min). The resultant precipitate was re-dispersed in 25 ml distilled water, frozen at 0 °C and then freeze dried at -52 °C to yield a free-flowing powder. The soy protein isolate was stored in a sealed tube at 4 °C until analyzed.

Product Formulation

Five products were formulated as shown in Table 1. The products comprised A (100 % maize ogi), B (90.65 % maize flour + 9.35 % soy protein isolates), C (89.81 % maize flour + 10.19 % bambaranut protein isolates), D (75.07 % maize flour + 24.93 % soybean flour) and E (70.03 % maize flour + 29.97 % bambaranut flour). Each product was formulated to give 16g protein/100 g. The amounts of the materials required to meet the protein target were achieved through material balance from their respective protein contents using the procedure outlined by (21).

Table 1: Flour Blends Formulation to Achieve 16 % Protein

Samples	Maize Flour (MF)	Bambaranut Flour (BF)	Soybean Flour (SF)	Bambaranut Protein Isolate (BI)	Soy Protein Isolate (SPI)	Total
A	100	-	-	-	-	100
B	90.65	-	-	-	9.35	100
C	89.81	-	-	10.19	-	100
D	75.07	-	24.93	-	-	100
E	70.03	29.97	-	-	-	100

Pasting Properties of Maize-Based *Ogi* Enriched with Bambaranut, Soybean Flours and their Isolates

The Rapid Visco Analyser (RVA model 3D, Newport Scientific, Sydney, Australia) was used to determine the pasting properties of the samples. Exactly 2.5 g sample was weighed into a dried empty canister and 25 ml of distilled water was dispensed into the canister containing the sample. The solution was thoroughly mixed and the canister was well fitted into the RVA as recommended. The slurry was heated from 50 to 95 °C with a holding time of 2 min followed by cooling to 50 °C with 2 min holding time. Peak, trough, breakdown, final and set back viscosities as well as peak time and pasting temperature was read from the pasting profile with the aid of thermocline for windows software connected to a computer.

Determination of Micronutrients Composition of Maize-Based *Ogi* Enriched with Bambaranut, Soybean flours and their Protein Isolates

Determination of Potassium Content:

The method of (22) was used in determining the potassium content of samples. One gram of the sample was dissolved in 20 ml of acid mixture (650ml of concentrated HNO_3; 80ml PCA; 20ml conc H_2SO_4) and aliquots of the diluted clear digest were taken for photometry using Flame analyser (Sherwood Flame Photometer 410, Sherwood Scientific Ltd. Cambridge, UK).

Determination of Phosphorus Content:

The method of (22) was used in determining the potassium content of the samples. Two gram of food samples was ashed for 4h at 60 °C and five millilitres of 6N HCl and several drops of nitric acid was added. It was then heated to dissolve the ash completely, cooled and transferred to a 100 ml volume flask and diluted to volume with the volume flask. An aliquot was pipetted which contained five milligrams of phosphorus into a 100ml volume flask. The sample was added to molydovanadate reagent to 100 ml and the colour was allowed to develop for 10 minutes. The absorbance was read at 400nm against a phosphorus standard curve.

Determination of Sodium Content:

The method of (22) was used. Weight of 0.2542 g of NaCl was dissolved in 1 litre of distilled water to give 100ppm sodium. This working standard solution was diluted to produce a range containing 0 – 10ppm sodium and made up to 100 ml mark and 2 ml sample aliquot (sample stock solution) was read using a JENWAY PFP7 flame photometer. The concentration of the test mineral in the sample was calculated with reference to the graph (standard curve) and obtained as follows:

$$Na\ (mg/100g) = 100 \times Xx\ VF \times D\ \text{W} \times 100 \times \text{Va}$$

W = Weight of the sample analysed
X = Concentration of Na obtained from the standard curve

VF = Total volume of digest/extract (100ml)
Va = Volume of extract used
D = Dilution factor

Determination of Calcium Content:
Calcium was determined using the atomic absorption spectrophotometer described by (22). Calcium carbonate (2.495 g) was dissolved and diluted to 100ml with de-ionized water. This solution contained 1000 mg Ca^{2+} ions and from this stock solution, calcium standard of the following concentration levels 0.0, 3.0, 6.0, 9.0 were prepared. The absorbance of both the sample and the standard working aliquot were determined in the AGILENT (Model 5805, Agilent Spec England) atomic absorption spectrophotometer at 239.9 nm.

The concentration of the test mineral in the sample was calculated with reference to the graph (standard curve) and obtained as follows:

$$Ca\ (mg/100g) = 100 \times XxVf \times D\ W \times 100 \times Va$$

W = Weight of the sample analyzed
X = Concentration of Ca obtained from the standard curve
Vf = Total volume of extract
Va = Volume of extract used
D = Dilution factor

Determination of Thiamine Content:
The method of (22) was used. Five gram of homogenized sample was poured into 100 ml volumetric flasks and 0.1 N HCI was added and mixed. It was autoclaved for 30 minutes at 121 °C. The samples were allowed to cool and interfering substances was precipitated by adjusting the pH to 6.0 followed immediately by readjusting the pH to 4.5. This was then diluted to volume with water and filtered. Five millilitres of 6% enzyme (mylase) were added and incubated for 3 hours at 45-50 °C. This was then cooled and pH adjusted to 3.5 and diluted with water to volume, mixed and filtered. Ten millilitres of the diluted extract were oxidized by passing it through a SepPak C_{18} cartridge followed by 5ml 0.01 M phosphate buffer at pH 7.0. The Vitamin was separated by HPLC using a 4.6 mm X 25 cm ultraphere ODS, 5 columns and detected by fluorescence at 360 nm/415 nmex/em. The thiamine content was measured by the calculation below:

$$\mu g/g = CX\ VX\ (DF/WT)$$

Where,
C= Concentration of vitamin in µg/ml obtained from peak height or area of sample and standard
V= Sample Volume, ml
DF= Dilution factor
WT= Sample weight, g.

Determination of Riboflavin Content:
The method of (22) was used to determine the riboflavin content. Riboflavin was extracted with dilute acids and after removing the interfering substances by treatment with $KMno_4$, it was determined in a fluorimeter at 450-500 mm wavelength. Two grams of the sample and 10 mg of riboflavin were poured into conical flask, 50 ml of 0.2 NHCI was boiled on a water bath for an hour,

allowed to cool and the pH adjusted to 6.0 using NaOH, also 1N HCl was added to lower the pH to 4.5, it was filtered in a 100 ml measuring flask and volume was made up to the mark. Two test tubes were marked as 1 and 2 to remove interference. 1 ml of acetic acid (glacial) was added to each test tube, it was mix and 0.5ml of 3% $KMno_4$ was added. The fluorimeter was adjusted to zero deflection against $0.1NH_2SO_4$ and 100 against tube no 2. 20mg of sodium hydrogen sulphate was added to test tubes and fluorescence was measured within 10 seconds and was recorded as blank.

The riboflavin content was measured by the calculation below:
W=Weight of sample.
X= (reading of sample 1) - (reading of sample blank)
Y= (reading of sample + standard tube 2)- (reading of sample + standard blank)

$$\text{Riboflavin (mg per g of sample)} = \frac{x}{Y - X} = \frac{1}{w} \quad (10)$$

Determination of Niacin Content:
The method described by (22) using High performance liquid Chromatography was used in determining the niacin content. The first step was the use of alkaline digestion on food sample. Niacin derivatives such as coenzymes and niacinamide was converted into total niacin by alkaline digestion with aqueous calcium hydroxide.

Following alkaline extraction of food, niacin was purified and concentrated using C18 and cation exchange cartridge (SCX). The purified extract was determined by HPLC at a detection wavelength of 254 nm using C8 column and PIC A reagent in 15 % methanol. Food samples was finely grounded and mixed well before taking sample aliquot, an analytical balance was used to weigh accurately 1 g of sample and put into a 50 ml PP centrifuge tube, Alkaline Extraction was by weighing 0.75 g $Ca(OH)_2$ and added into the centrifuge tubes which contained the 1.0 g sample. Including a 'duplicate' sample, a 'control' sample, a 'recovery' sample, a 'niacin standard' and a 'blank'. and to the 'recovery' sample, 1.0 g of food sample was added in 1.0 ml niacin stock standard of 100 ug/ml and then 0.75 g $Ca(OH)_2$. To the 'niacin standard' tube was added 1 ml stock standard (100 ug/ml) and then 0.75 g of Ca (OH). The 'blank' sample which contained only water and Ca (OH). 25 ml measuring cylinder was used to add 10 ml UHQ water into all tubes and finally, a glass rod to mix each tube well, and then 10 ml of UHQ water was added. The glass rod was rinsed as well.

Determination of Selected Antinutritional Composition of Maize-Based *Ogi* Enriched with Bambaranut, Soybean Flours and their Protein Isolates

Determination of Phytate:
Phytic acid content was determined as described by (23). The test sample was extracted with 0.2N hydrochloric acid. The extract (0.5 ml) was transferred into a test tube fitted with a ground glass stopper. Ferric solution (1 ml) was added, the tube was covered and heated in a boiling water bath for 30 minutes. After cooling, the content of the tube was centrifuged (3000 xg) for 30 min. The supernatant (1 ml) was transferred to another test tube and 1.5 ml of 2, 2-bipyridine solution was added. Absorbance of the solution was measured at 519 nm against distilled water and the concentration was obtained from a calibration curve.

Determination of Tannins:

Tannins were determined as described by (23). Each sample (1g) was dispersed in distilled water (10ml) and agitated. This was left to stand for 30 minutes at room temperature and centrifuged. The supernatant (2.5 ml) was dispersed into a 50 ml volumetric flask. Standard tannic acid solution (2.5 ml) was transferred into a separate 50ml flask. Folin-Denis's reagent (1 ml) was measured into each flask, followed by 2.5 ml of saturated sodium carbonate (Na_2CO_3) solution. The mixture was diluted to the 50 ml mark and left for 90 min at room temperature. The absorbance was measured at 250 nm and readings were taken with the reagent blank at zero. The tannin content was derived from an (absorbance of test sample), As (absorbance of standard solution), C (concentration of standard solution), W (weight of sample used), Vf (total volume of extract) and Va (volume of extract analyzed), as shown below

$$\%\,Tannin = \frac{An}{As} X C X \frac{100}{w} X \frac{Vf}{VaA} \quad (11)$$

Trypsin Inhibitor Assay:

Trypsin inhibitory activity (TIA) was determined as described by (24). This was measured in terms of the extent to which an extract of the test sample inhibited the action of bovine trypsin on the substrate benzoyl-DL-arginine-p-nitrianilide (BAPNA). Each sample (1 g) was continuously extracted at room temperature for 3 h with 50ml of 10 mmol/L NaOH using a mechanical shaker (GallenKamp orbital shaker, Surrey, UK). The pH of the resulting slurry was adjusted from 9.4 to 9.6 with 1mol/L sodium hydroxide. After extraction, the suspension was shaken and diluted with distilled water so that 1 cm^3 of the extract produced trypsin inhibition of 40-60% at 37 °C. The respective dilutions were noted and TIA calculated in terms of milligrams of pure trypsin/g sample, using equation 2:

$$TIA = \frac{(2.632\ DA)}{S}$$

Where:
D=dilution factor,
A =change in absorbance at 410nm due to trypsin inhibition per cm^3 of diluted sample extract, and S=weight of the sample.

Determination of oxalates:

Oxalates were determined using the method of (22). Each sample (1 g) was weighed into 100ml conical flasks, 75 ml of 3 mol/L H_2SO_4 was added and the solution stirred intermittently with a magnetic stirrer for about 1hour and then filtered using Whatman No. 1 filter paper. The sample filtrate (extract, 25 ml) was collected and titrated against hot (80-90 °C) 0.1 N potassium permanganate ($KMnO_4$) until a faint pink colour which persisted for at least 30 seconds appeared. The concentration of oxalate in each sample was obtained from the conversion: 1 ml 0.1 permanganate = 0.006303 g oxalate.

Determination of Amino Acid Profile of Maize *Ogi* Enriched with Bambaranut, Soybean Flours and Their Protein Isolates (g/100 g protein)

Qualitative assessment of the essential and non-essential amino acid (profile) composition of the formulation was carried out using the method as reported by (25). The estimation of the amino acids was by the use of the guide strip technique where developed thin-layer chromatography

plates was used in locating the positions of amino acids in unsprayed plates. The squares containing amino acids was cut-out and eluted with 5 ml distilled water at 70 °C for 2 hours; the cellulose powder was removed by centrifugation at 5,000rpm for 5 min. The supernatant was decanted and kept for colorimetric analysis of amino acid profiles against FOA reference values of the essential amino acid

Sensory Properties of Maize-Based *Ogi* Enriched with Bambaranut, Soybean Flours and their Protein Isolates

Gruels which were neither too thin nor too thick were prepared by mixing 40 g of each flour sample in 80ml hot water using a graduated plastic cup. Hot water used was boiled using a cordless electric kettle (Sayona, model no. SCK-25). A semi-trained panel, consisting of 20 nursing mothers/caregivers selected from the Jos North Clinic, Plateau State, Nigerian were used. The nursing mothers/caregivers were women who had children within the ages of 6 months to 5 years. They were screened to ensure that they were familiar with the traditionally-prepared fermented maize *ogi* which is commonly used for complementary feeding. A 9-point hedonic scale (1- dislike extremely, 5- neither like nor dislike, 9- like extremely) as described by (26) was used to assess the sensory attributes of taste, aroma, appearance, mouthfeel, consistency and overall acceptability. The five coded samples were labelled as sample A-E, cooled to a lukewarm temperature, and served all together in disposable transparent plastic cups. Each panelist was provided with clean water which served as a palate cleanser in between evaluations.

Statistical Analysis of Samples

Data were subjected to Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test to compare treatment means; differences was considered significant at 95% ($p<0.05$) (SPSS V21 software).

RESULTS

Pasting Properties of Maize-Based *Ogi* Enriched with Bambaranut, Soybean Flours and their Protein Isolates

Pasting properties of maize-based *ogi* enriched with bambaranut, soybean flours and their protein isolates is presented in Table 2**.** There was a significant decrease ($p<0.05$) in the peak, trough, breakdown, final and setback viscosities and a significant increase ($p<0.05$) in the peak time and pasting temperature with the addition of flours and protein isolates from bambaranut and soybean to the 100 % maize *ogi*. The peak viscosity of the flour blends ranged from 190.80 to 294.00 RVU. The trough, breakdown and final viscosities of the unfortified and fortified samples ranged between 142.00 to 207.00 RVU, 48.86 RVU to 88.02 RVU and 200.00 to 300.00 RVU respectively. The setback viscosity, peak time and pasting temperature ranged between 115.20-200.50 RVU, 5.38-6.91 min and 60.40-79.22 °C, respectively.

Table 2: Pasting Properties of Maize-Based *Ogi* Enriched with Bambaranut, Soybean Flours and their Protein Isolates

Samples	PV(RVU)	TV(RVU)	BV(RVU)	FV(RVU)	SV(RVU)	PT (min)	PT (°C)
A	$294.00^{a}\pm0.01$	$207.00^{a}\pm0.02$	$88.02^{a}\pm0.02$	$300.20^{a}\pm0.04$	$200.50^{a}\pm0.02$	$5.38^{e}\pm0.01$	$60.40^{e}\pm0.01$
B	$190.80^{e}\pm0.05$	$142.00^{e}\pm0.02$	$48.86^{e}\pm0.06$	$200.00^{e}\pm0.01$	$115.20^{e}\pm0.01$	$6.91^{a}\pm0.01$	$79.22^{a}\pm0.02$
C	$220.90^{d}\pm0.95$	$165.00^{d}\pm0.01$	$55.87^{d}\pm0.96$	$236.00^{d}\pm0.08$	$122.30^{d}\pm0.02$	$6.32^{b}\pm0.01$	$74.97^{b}\pm0.02$
D	$244.40^{c}\pm0.42$	$179.60^{c}\pm0.03$	$64.69^{c}\pm0.43$	$250.50^{c}\pm0.01$	$130.00^{c}\pm0.02$	$5.89^{c}\pm0.01$	$71.86^{c}\pm0.02$
E	$262.00^{b}\pm0.02$	$188.60^{b}\pm0.51$	$73.40^{b}\pm0.53$	$273.20^{b}\pm0.01$	$155.50^{b}\pm0.02$	$5.65^{d}\pm0.02$	$66.31^{d}\pm0.01$

Values are means ± standard deviations of triplicate determinations.

Means within same column with different superscripts differed significantly ($p<0.05$)

Sample A (100 % maize *ogi*), Sample B (90.65 maize *ogi* and 9.35 soybean protein isolates), Sample C (89.81 maize *ogi* and 10.19 bambaranut protein isolate), Sample D (75.07 maize *ogi* and 24.93 soy flour) and Sample E (70.03 maize *ogi* and 29.97 bambaranut flour)
PV= Peak Viscosity, TV= Trough Viscosity, BV= Breakdown Viscosity, FV= Final Viscosity, SV= Setback Viscosity, PT (min)= Peak Time and PT(0C) =PastingTemperature

Amino Acid Composition of Maize-Based *Ogi* Enriched with Bambaranut, Soybean Flours and their Protein Isolates (g/100 g protein)

The amino acid composition of maize-based *ogi* enriched with bambaranut, soybean flours and their protein isolates (g/100 g protein) are shown in Table 3.

The results showed significant difference in all the samples analyzed. The leucine, lysine, tryptophan, glutamate and aspartate contents ranged between 6.36 to 7.67, 2.87 to 6.01, 0.97 to 1.37, 5.83 to 7.21 and 6.02 to 6.99 g/100 g protein respectively.

Table 3: Amino Acid Composition of Maize-Based *Ogi* Enriched with Bambaranut, Soybean Flours and their Protein Isolates (g/100 g protein)

Amino Acids	A	B	C	D	E	FAO/WHO, 1985
Valine	3.33^{e}±0.03	4.98^{a}±0.02	4.77^{b}±0.01	4.36^{c}±0.02	3.98^{d}±0.01	3.50
Threonine	2.78^{e}±0.01	4.00^{a}±0.02	3.67^{b}±0.01	3.21^{c}±0.01	2.94^{d}±0.01	3.40
Isoleucine	3.87^{e}±0.02	4.86^{a}±0.01	4.66^{b}±0.01	4.46^{c}±0.01	4.21^{d}±0.01	2.80
Leucine	6.36^{e}±0.01	7.67^{a}±0.01	7.22^{b}±0.02	6.88^{c}±0.01	6.56^{d}±0.01	6.61
Methionine	0.79^{d}±0.00	1.01^{a}±0.01	0.85^{b}±0.01	0.83^{c}±0.01	0.82^{c}±0.01	-
Phenylalanine	4.00^{e}±0.01	6.01^{a}±0.03	5.67^{b}±0.01	4.85^{c}±0.01	4.55^{d}±0.01	-
Lysine	2.87^{e}±0.01	6.01^{a}±0.02	5.89^{b}±0.12	5.68^{c}±0.01	5.44^{d}±0.02	5.80
Histidine	2.31^{e}±0.01	3.78^{a}±0.02	3.47^{b}±0.01	3.23^{c}±0.02	2.98^{d}±0.01	-
Tryptophan	0.97^{e}±0.02	1.37^{a}±0.01	1.29^{b}±0.01	1.19^{c}±0.01	1.15^{d}±0.01	1.10
Alanine	2.37^{e}±0.01	4.23^{a}±0.01	3.66^{b}±0.01	3.27^{c}±0.01	3.12^{d}±0.01	-
Aspartate	6.02^{e}±0.01	6.99^{a}±0.01	6.73^{b}±0.01	6.57^{c}±0.05	6.13^{d}±0.01	-
Glutamate	5.83^{e}±0.01	7.21^{a}±0.01	6.81^{b}±0.01	6.52^{c}±0.01	6.03^{d}±0.01	-
Serine	2.24^{e}±0.01	3.64^{a}±0.02	3.21^{b}±0.01	3.00^{c}±0.02	2.39^{d}±0.01	-
Glycine	2.52^{e}±0.02	3.69^{a}±0.00	3.50^{b}±0.02	3.24^{c}±0.01	2.90^{d}±0.01	-
Proline	4.76^{e}±0.01	6.00^{a}±0.01	5.64^{b}±0.01	5.22^{c}±0.02	4.90^{d}±0.01	-
Arginine	4.37^{e}±0.01	5.42^{a}±0.01	5.18^{b}±0.01	5.01^{c}±0.01	4.88^{d}±0.01	-
Tyrosine	1.46^{e}±0.01	1.95^{a}±0.01	1.72^{b}±0.01	1.67^{c}±0.01	1.57^{d}±0.01	-
Cysteine	1.00^{e}±0.01	1.87^{a}±0.01	1.61^{b}±0.01	1.49^{c}±0.01	1.26^{d}±0.05	-

Values are means ± standard deviations of triplicate determinations. Means within same column with different superscripts differed significantly ($p<0.05$). Sample A (100 % maize ogi), Sample B (90.65 maize ogi and 9.35 soybean protein isolates), Sample C (89.81 maize ogi and 10.19 bambaranut protein isolate), Sample D (75.07 maize ogi and 24.93 soy flour) and Sample E (70.03 maize ogi and 29.97) bambaranut flour

Micronutrient Composition of Maize-Based *Ogi* Enriched with Bambaranut, Soybean Flours and their Protein Isolates (mg/100g)

Table 4 shows the micronutrients composition of maize-based *ogi* enriched with bambaranut, soybean flours and their protein isolates (mg/100g). The sodium, calcium, phosphorus and potassium content ranged from, 8.02-43.76, 56.10-171.65, 155.90-272.00 and 178.70-644.20, respectively. Also, the thiamine, riboflavin and niacin ranged between; 0.53-0.98, 0.29-0.54 and 1.99-1.28, respectively.

Table 4: Micronutrient of Maize-Based *Ogi* Enriched with Bambaranut, Soybean Flours and their Protein Isolates (mg/100g)

Samples	Sodium	Calcium	Phosphorus	Potassium	Thiamine	Riboflavin	Niacin
A	8.02^{e}±0.01	56.10^{e}±0.01	155.90^{e}±0.01	178.70^{e}±0.01	0.53^{c}±0.01	0.29^{c}±0.01	1.99^{c}±0.01
B	10.08^{c}±0.01	61.55^{c}±0.01	196.80^{c}±0.01	212.00^{c}±0.01	0.37^{d}±0.01	0.19^{d}±0.01	1.28^{d}±0.01
C	8.98^{d}±0.01	58.21^{d}±0.01	180.30^{d}±0.01	182.60^{d}±0.01	0.25^{e}±0.01	0.14^{e}±0.01	1.23^{e}±0.01
D	43.76^{a}±0.01	171.65^{a}±0.01	272.00^{a}±0.01	644.20^{a}±0.01	0.98^{a}±0.01	0.59^{a}±0.01	2.73^{a}±0.01
E	31.52^{b}±0.01	82.23^{b}±0.01	201.50^{b}±0.01	284.90^{b}±0.01	0.66^{b}±0.01	0.34^{b}±0.01	2.22^{b}±0.01

Values are means ± standard deviations of triplicate determinations. Means within same column with different superscripts differed significantly (p<0.05) Sample A (100 % maize ogi), Sample B (90.65 maize ogi and 9.35 soybean protein isolates), Sample C (89.81 maize ogi and 10.19 bambaranut protein isolate), Sample D (75.07 maize ogi and 24.93 soy flour) and Sample E (70.03 maize ogi and 29.97 bambaranut flour)

Antinutritional Composition of Maize-Based *Ogi* Enriched with Bambaranut, Soybean Flours and their Protein Isolates

The antinutritional composition of maize-based *ogi* enriched with bambaranut, soybean flours and their protein isolates are presented in Table 5. The oxalate content for unsubstituted sample (100 % maize *ogi*) and substituted samples ranged from 0.11 to 6.14 (mg/100g), respectively. The antinutrients were significantly decreased (p<0.05) by various substitution levels of protein isolates from bambaranut and soybean while there was a significant increase (p<0.05) with the addition of flours from bambaranut and soybean. Also, phytate, tannins and trypsin inhibitors (TIA) were found to range between 1.87 to 9.50 (mg/100g), 0.22 to 7.81(mg/100g) and 0.10 to 10.77 %, respectively.

Table 5: Antinutritional Contents of Maize-Based *Ogi* Enriched with Bambaranut, Soybean Flours and their Protein Isolates

Samples	Oxalate (mg/100g)	Phytate (mg/100g)	Tannins (mg/100g)	TIA (%)
A	0.25^{c}±0.01	2.60^{c}±0.01	0.35^{c}±0.01	0.48^{c}±0.57
B	0.11^{e}±0.01	1.87^{e}±0.01	0.22^{e}±0.01	0.10^{c}±0.01
C	0.15^{d}±0.01	1.91^{d}±0.01	0.26^{d}±0.01	0.12^{c}±0.01
D	4.04^{b}±0.01	8.94^{b}±0.01	5.21^{b}±0.01	6.96^{b}±0.01
E	6.14^{a}±0.01	9.50^{a}±0.01	7.81^{a}±0.01	10.77^{a}±0.05

Values are means ± standard deviations of triplicate determinations. Means within same column with different superscripts differed significantly (p<0.05) Sample A (100 % maize *ogi*), Sample B (90.65 maize *ogi* and 9.35 soybean protein isolates), Sample C (89.81 maize *ogi* and 10.19 bambaranut protein isolate), Sample D (75.07 maize *ogi* and 24.93 soy flour) and Sample E (70.03 maize *ogi* and 29.97 bambaranut flour)
TIA (Trypsin Inhibitory Activity)

Sensory Scores of Maize-Based *Ogi* Enriched with Bambaranut, Soybean Flours and their Protein Isolates

Sensory properties of maize-based *ogi* enriched with bambaranut, soybean flours and their protein isolates are shown in Table 6. Sensory scores for aroma, mouthfeel, appearance, taste, consistency and overall acceptability ranged between 7.35 and 8.40, 6.25 to 8.40, 6.50 to 8.20, 6.00 to 8.55, 6.20 to 8.70 and 7.00 to 8.65, respectively.

Table 6: Sensory Scores of Maize-Based *Ogi* Enriched with Bambaranut, Soybean Flours and their Protein Isolates

Sample	Aroma	Mouthfeel	Appearance	Taste	Consistency	Overall acceptability
A	8.40^{a}±0.50	8.40^{a}±0.75	8.20^{a}±0.83	8.55^{a}±0.60	8.70^{a}±0.47	8.65^{a}±0.59
B	7.35^{b}±0.59	7.15^{bc}±1.57	7.30^{b}±0.66	7.80^{b}±0.70	7.85^{b}±0.59	8.00^{b}±0.79

C	7.30^{b}±1.17	7.50^{b}±1.32	7.25^{b}±0.97	7.75^{b}±0.97	7.20^{c}±0.52	7.95^{b}±0.89
D	6.40^{c}±1.14	6.50^{cd}±1.19	6.80^{bc}±0.95	6.45^{c}±0.89	6.85^{c}±0.75	7.15^{c}±0.88
E	6.10^{c}±1.55	6.25^{d}±1.29	6.50^{c}±1.36	6.00^{c}±1.08	6.20^{d}±0.89	7.00^{c}±1.08

DISCUSSION

Pasting Properties of Maize-Based *Ogi* Enriched with Bambaranut, Soybean Flours and their Protein Isolates

Pasting is the result of a combination of processes that follows gelatinization from granule rupture to subsequent polymer alignment due to mechanical shear stress during the heating and cooling of starches (27). The pasting properties of maize-based *ogi* enriched with bambaranut, soybean flours and their protein isolates showed peak viscosity were between 190.80 and 294.00 RVU which are higher than the 67.50- 86.00 reported by (28) when they substituted yellow maize flour with composites of soybean and pumpkin flour. Peak viscosity is often correlated with the final product quality; it provides an indication of the viscous load likely to be encountered during mixing (29). Peak viscosity is also an index of the ability of starch to swell freely before their physical break down (30, 31). High peak viscosity is an indication of high starch content (32). The relatively lower peak viscosities of the protein isolates samples; B and C as compared to the flour samples; D and E indicates that the protein isolates flours will not form a very thick paste, hence, maybe suitable for product requiring low gel strength and elasticity such as infants weaning foods (28, 33). The lower peak viscosity content of the isolates samples as compared to the flour samples may also be attributed to the reduction in carbohydrate content of the protein isolates compared with the flour samples since peak viscosity is an indication of higher starch content. Differences observed in the peak viscosities of the protein isolates and flour samples also indicates that they were differences in the rate of water absorption and starch granule swelling during heating (28).

Trough, is the minimum viscosity value in the constant temperature phase of the RVA pasting profile and it measures the ability of the paste to withstand break down during cooling (30, 34). Trough, sometimes called hold period, hot paste viscosity, shear thinning, holding strength due to the accompanied breakdown in viscosity is when the samples were subjected to a period of constant temperature (usually 95 ^{o}C) and mechanical shear stress (35). There were significant ($p<0.05$) decrease with the addition of flours and protein isolates to the maize *ogi*. Trough viscosities were between 142.00 and 207.00 RVU, which are similar to the value of 177.48 - 196.26 RVU reported by (36) and higher than the value of 61.50 – 79.00 RVU reported for composites of yellow maize, soybean and pumpkin flours reported by (28). The protein isolates enriched *ogi* were found to have lower trough viscosities compared with the flour samples. Higher holding periods exhibited by the control and flour samples indicated that these formulations will withstand high heat treatments during processing than the protein isolates samples which have lower trough viscosities. High trough viscosities represent low cooking losses and superior eating quality (28). Decrease in trough viscosity is an indication of breakdown or stability of the starch gel during cooking (37, 38). The lower the trough viscosity, the more stable is the starch gel. Therefore, the lower trough values observed in the *ogi* enriched with protein isolates indicates the stability of the paste as compared to the flour samples during cooling.

Final viscosity gives a measure of the resistance of paste to shear force during stirring (31, 35, 39). The final viscosity of the maize-based *ogi* were between (200.00 and 300.20 RVU) and were lower as compared to (1895 and 1801.82 RVU) reported by (40) for kokoro from composites of maize and pigeon pea protein concentrate flours. The control and flour samples had higher final viscosities while the protein isolates enriched *ogi* had lower final viscosity values. The high value

of final viscosity observed in the control sample could be attributed to the aggregation of amylose. Final viscosity is commonly used to define the quality of particular starch-based flour, since it indicates the ability of the flour to form a viscous paste after cooking and cooling. Thus, the lower final viscosities recorded in the protein isolates samples implied that, the flours will form a low viscous paste rather than a thick gel on cooking and cooling and this is advantageous in complementary food since infants can easily swallow a low viscous paste rather than a thick gel that will require excessive dilution with water leading to nutrients and energy thinning.

Peak time is a measure of cooking time (31). The peak time also gives an indication of the ease of cooling. The shorter the peak time the higher the ease of cooling (41). The peak time showed significant ($p<0.05$) increase among the samples with the control sample having the lowest peak time (5.38 and the protein isolates enriched *ogi* (B), the highest (6.91). The peak time in this study is similar with (6.15 to 6.25 min) reported by (40) when they produced kokoro, a traditional maize-based snacks from composites of maize and pigeon pea protein concentrate. The higher peak time observed in the protein isolates enriched *ogi* as compared to the control and flour samples implied that the cooking time for the flour will also increase. Thus, *ogi* from (100 %) maize flour will cool easily as compared to other samples with higher peak time.

Pasting temperature is the temperature at which initial rise in viscosity occurs when starch granules and proteins begin to absorb water and swells as the temperature increases (42). This occurs when starch or starch-based foods are heated in water beyond a critical temperature, the granules absorb a large amount of water and swell to many times their original size, over a critical temperature, which is characteristics of a particular starch, the starch undergoes an irreversible process known as gelatinization (31). The pasting temperature is also a measure of the minimum temperature required to cook a given food sample (43) and affects the stability of other components in the food formula and also affects energy costs (44). The pasting temperature showed significant ($p<0.05$) increase among all the sample. The pasting temperature observed in this study is lower than the value of (80.20 – 82. 25 ^{o}C) reported by (40) for maize-based snack fortified with pigeon pea protein concentrate. Since, pasting temperature is a measure of the minimum temperature required to cook a given food sample, protein isolates enriched *ogi* with higher pasting temperature as compared to the flour samples may not be recommended for certain products due to high cost of energy. The lower pasting temperature noted in the control sample may be due to the higher carbohydrate content in the sample as compared to the flours and isolates samples. However, since the pasting temperatures in all the samples were found to be lower than the boiling point of water; it therefore means, it can form a paste in hot water below boiling point. Also, the variation in pasting temperatures among the flours and protein isolates enriched *ogi* showed, the formulations will not have the same cooking time since pasting temperature depicts onset of rise and gelatinization temperature of the sample (27).

Amino Acid Composition of Maize-Based *Ogi* Enriched with Bambaranut, Soybean Flours and their Protein Isolates (g/100 g protein)

Amino acids are the chemical building blocks that make up proteins and provide the structure for all living things as proteins participate in the vital chemical processes that sustain life. All amino acids in food have different roles that helps the body to grow and function optimally. However, essential amino acids are of main concern as they are not synthesized in the body and must be supplied in adequate amount through diets. The amino acid compositions (Table 3) showed that Leucine is the most abundant essential amino acid and it is more dominant in the protein isolates enriched *ogi* (B). The leucine content of the maize *ogi* increased significantly ($p<0.05$) with the

addition of flours and protein isolates from bambaranut and soybean. This increase could be as result of increase in the concentration of protein in the blends. This finding is in line with (45) who reported a progressive increase in the leucine contents of nixtamilized maize flour supplemented with sprouted soybean flour. (46) gave the reference standard for leucine to be (6.61 g/100 g protein). since the flours and isolates enriched *ogi* were higher than the reference standard, it means *ogi* made from these combinations ought to meet the leucine needs of the infants.

Lysine is a major limiting amino acid in cereal grain, this explains why the lysine content was lowest (2.87 g/100 g protein) in the control sample (100 % maize *ogi*) but was improved upon the addition of flours and isolates from bambaranut and soybean. The high protein in bambaranut and soy protein isolates could be responsible for the high increase in lysine content of the protein isolates enriched *ogi.* The low level of lysine in the control sample is not unexpected as lysine has been reported to be the major limiting amino acid in maize, (47). Although, there was a significant increase in lysine contents with increasing concentration of protein in the blends, it is noteworthy to say that only the protein isolates enriched *ogi* met the reference standard of (5.8 g/100 g protein reported by (46) implying that the protein isolates enriched *ogi* stand a better chance of meeting the lysine need of an infant when compared to the flour enriched *ogi*. (45) also reported increase in lysine content when he supplemented nixtamalized maize flour with sprouted soybean flour. The main role of lysine is to participate in protein synthesis; hence, it is important for growth and maintenance of the body.

Glutamate and aspartate were the most abundant none essential amino acids found in all the samples and ranged between (5.83 to 7.21) and (6.02 to 6.99) g/100 g protein, respectively. However, there was a significant increase with different incorporation levels of flours and protein isolates from bambaranut and soybean. The 100 % maize *ogi* had the least values but was improved upon the addition of flours and protein isolates from bambaranut and soybean. The increase in glutamate and aspartate content of the fortified samples could be likened to the addition effect of legumes from bambaranut and soy flour in the blends. Several researchers have documented increase in glutamic and aspartic acid as a result of the effects of legumes in the blends, (45, 48, 49).

Generally, the protein isolates enriched *ogi*, (B) was the highest in all the amino acids analysed. This could be as a result of soy protein influence on the composites. Soy isolates had the highest protein contents. In all, the protein isolates enriched *ogi* and the flour enriched *ogi* (D) met the reference standard for all essential amino acids. Therefore, infant foods formulated from such blends ought to meet the essential amino acid needs of the infants.

Micronutrient Composition of Maize-Based Ogi Enriched with Bambaranut, Soybean and Protein Isolates (mg/100 g)

The sodium content of the protein isolates enriched *ogi* (D) (43.76 mg/100g) was found to be higher in value than the control and the flour samples. The unfortified *ogi* (A) had the least content of sodium. There was a significant ($p<0.05$) increase with the fortified samples. The sodium content was found to be between (8.02 and 43.76 mg/100g) which was higher than (7.12 to 8.56 mg/100g) reported by (50) who worked on the nutritional evaluation of maize-millet based complementary foods Fortified with Soybean but lower than (58.60 to 67.99 mg/100g) reported by (51) who studied the nutritional characteristics of maize-based complementary food enriched with fermented and germinated *Moringa oleifera* seed flour. Sodium is normally consumed in the form of salt; it is essential in the regulation of water content and in the maintenance of osmotic

pressure of the body fluid. It also aids in the transport of CO_2 in the blood. However, sodium is one of the minerals whose intake is considered a factor in the ethology of hypertension, hence its low intake is encouraged (52).

The phosphorus content of the fortified *ogi* showed significant increase ($p<0.05$) among the samples. there was an increment in the phosphorus content upon the addition of flours and protein isolates from bambaranut and soybean implying that both bambaranut and soybean are rich in phosphorus. Comparing the flour enriched *ogi* with the protein isolates enriched *ogi* showed that those of the flour enriched *ogi* were more abundant in phosphorus than the protein isolates enriched *ogi.* This can be attributed to the presence of ash in the flours as compared to the protein isolates fours. The ash content of food material could be used as an index of mineral constituents of the food because ash is the inorganic residue remaining after water and organic matter have been removed by heating in the presence of an oxidizing agent (53, 54). The phosphorus contents in this study were higher than (159.30-182.00 mg/100 g). The physico-chemical and sensory properties of complementary foods from blends of malted and non-malted sorghum, soybean and *moringa oleifera* seed flours. The recommended dietary allowance (RDA) for phosphorus in infants' food is ≥180 mg/100 g (55, 56) and all the fortified *ogi* had phosphorus contents above this value meaning that both the flours and protein isolates enriched *ogi* are adequate in phosphorus which is fundamental to growth, maintenance, and repair of all body tissues, and is necessary, along with calcium and magnesium, for proper growth and formation of bones in infants and children (57, 58).

The thiamine contents (0.25 to 0.98 mg/100 g) were lower in the protein isolate enriched *ogi* (C) and higher in the flour enriched *ogi* (D). The thiamine content was higher than (0.20 to 0.33 mg/100 g) but lower than (2.3 to 3.0 mg/100 g) reported by (58). The fortified and unfortified *ogi* met the Adequate Intake (AI) of infants 0-1 year (0.2 to 0.3 mg/day) reported by the (59) suggesting that *ogi* can be made from any of the combinations with the assurance that the niacin need of the infants will be taken care of. The lower contents of thiamine in the protein isolates enriched *ogi* (B and C) could be attributed to the processing techniques employed right from the raw materials to the isolation of bambaranut and soybean which were used in the blends. Thiamine serves as a cofactor for key enzymes involved in carbohydrate metabolism. Mild thiamine deficiency is a significant public health problem across the world (60). Severe deficiency causes beriberi, a disease that has been associated with high consumption of refined rice and cereals and low intakes of animal and dairy products (61).

The riboflavin contents (0.14 to 0.59 mg/100 g) are within (0.29 to 0.64) reported by (62) for nutrient composition and suitability of four commonly used local complementary foods in Akwa Ibom state, Nigeria. Addition of flours from bambaranut and soybean to maize *ogi* lead to a corresponding increase in their riboflavin contents while the addition of isolates from bambaranut and soybean lead to decrease in their riboflavin contents. This could be attributed to the fact that legume flours have appreciable level of vitamins in them. According to the Institute of Medicine, Food and Nutrition Board, the Adequate Intake (AI) for infants 0-1 years is (0.3 to 0.4 mg/day) and the flour enriched *ogi* (D and E) met this standard. Riboflavin plays a central role in the catabolism of carbohydrates. It provides the reactive moieties of the flavin coenzymes (FMN and FAD) which serve as electron carriers in redox reactions (63). Deficiency causes impaired growth, impaired vision, dermatitis, cracked and red lips and Inflammation of the lining of mouth and tongue (64).

Antinutritional Composition of Maize-Based *Ogi* Enriched with Bambaranut, Soybean Flours and their Protein Isolates

Cereals and legumes are rich in nutrients but the bioavailability of these nutrients is usually low due to the presence of antinutritional factors such as phytate, oxalate, tannin and trypsin inhibitor (65). Soaking, boiling, fermentation and sprouting are among the traditional processing methods usually employed to reduce or eliminate these anti-nutritional factors in foods (66). The total oxalate content of this study showed that the protein isolates enriched *ogi* (B) had the lowest content of total oxalate while the flour enriched *ogi* (E) had the highest value. The relatively high amount of oxalate in the flour samples could be attributed to the presence of bambaranut and soybean in the blends. The oxalate contents in this study values are within (0.20-7.10mg/100g) reported by (67) who worked on the production and quality evaluation of *ogi* from fermented maize and horse eye bean. Oxalates affects calcium and magnesium metabolism and react with proteins to form complexes which have an inhibitory effect in peptic digestion (68). Kidney stone patients who form calcium oxalate-containing stones are advised to limit their intake of foods which contain 410 mg oxalate per serving, with total oxalate intake not exceeding (50–60 mg/day) (69). since none of the *ogi* samples have oxalate content up to 50 mg/g, it can be inferred that both the fortified and unfortified *ogi* in this present study when consumed is safe and will not pose any adverse effect on the bioavailability of nutrients. Phytate as a very stable and potent chelating food component is considered to be an anti-nutrient by virtue of its ability to chelate divalent minerals and prevent their absorption (70). The Phytate contents (1.87 to 9.50 mg/100 g) are lower than (2.54 to 13.36) reported by (71) who worked on the nutritional composition and antinutritional properties of maize *ogi* cofermented with pigeon pea but higher than (2.16 to 2.45 mg/g) presented by (72) who worked on the anti-nutrient and mineral properties of complementary food from malted red sorghum and defatted soybean flour blend. The low Phytate content of the unfortified *ogi* (100% maize flour) as compared to the flour enriched *ogi* could be attributed to the leaching effect of the soaking and dehulling employed on maize grain before milling. The reduction in the phytate content of the 100 % maize *ogi* may be due to hydrolysis of phytate by the enzyme phytase into lower inositol phosphates which are believed to be activated during the fermentation process by organisms (yeasts) whose hydrolysing ability is enhanced by fermentation (73). Phytates are known to form complexes with iron, zinc, calcium, and magnesium making them less available and thus inadequate in food samples especially for children, (71). It is known that (10–50 mg) phytate per 100 g will not cause a negative effect on the absorption of zinc and iron (74). Hence, the Phytate contents of the various *ogi* obtained from this study showed the porridges are within the safe limit and will not cause health hazards.

Tannins are naturally occurring plant polyphenols. Their main characteristic is to bind and precipitate protein thereby interfering with its digestion and absorption (74). The tannin contents (0.22 to 7.81 mg/100g) are lower than (0.92 to 8.70 mg/100g) reported by (67) for *ogi* from fermented and unfermented horse eye bean. (75) reported lethal dose of tannins to be 90 mg/100g, the tannin contents in this present study are by far, lower than the lethal dose. therefore, the fortified and unfortified *ogi* can be said to be within safe limits. The low level of tannins recorded in the protein isolates enriched *ogi* (B and C) as against the flour enriched *ogi* could be due in part to the protein isolates inclusion in those blends. (76) reported antinutrients to be higher in flours but, were significantly reduced on isolation as a result of the processes involved in the isolation of protein. Also, the low content of tannin in the 100% maize flour (A) could be attributed to fermentation. Food processing like fermentation, sprouting, decanting etc. reduces the anti-nutritional content of food thereby activating the hydrolytic enzyme (α and β

amalyses) and proteolytic enzyme (69). The presence of tannins can cause browning or other pigmentation problems in both fresh foods and processed products, (72).

Generally, the anti-nutritional contents were seen to be higher in the flours when compared to the protein isolates samples. This may be due to the processes involved in the isolation of protein. The nutritional effect of these anti-nutrients is related to their interaction with protein and minerals, (76). It is of essence to note that, despite the increase in the anti-nutritional contents in the flour enriched *ogi* (D and E), their values are within the recommended safe limits. All the samples were low and may not pose any adverse effect on bioavailability of the nutrients.

Sensory Properties of Maize-Based *Ogi* Enriched with Bambaranut, Soybean Flours and their Protein Isolates

Sensory evaluation is usually carried out towards the end of product development or formulation cycle and this is done to assess the reactions of Consumers about the product to determine the acceptability of such product. It is also an important criterion for assessing quality in the development of new products and for meeting consumer requirements (77). The sensory characteristics of food products is important as it plays an important role in determining the final acceptability by consumers. Industries and food developers have embraced sensory evaluation as an invaluable tool for creating successful products and understanding the sensory properties of materials. The attributes evaluated in this research includes aroma, mouthfeel, appearance, taste, consistency and overall acceptability.

In terms of aroma, the control sample (100 %) maize *ogi* was rated highest followed by the protein isolates enriched *ogi* (B), 8.40 and 7.35, respectively. Significant differences existed among all the samples. The lower aroma value observed in the flour enriched *ogi* (E) as compared to the rest samples maybe attributed to the higher proportion of bambaranut flour that was used in substituting the blend. The panellist preference for the control sample (A) over the other formulations maybe due to their familiarity with the smell of maize *ogi*. Aroma is an important parameter of food (6). Good aroma from food excites the taste buds, making the system ready to accept the product. Poor aroma may cause outright rejection of food before they are tasted. The aroma ratings of the evaluated samples are within acceptable limits and therefore would not be objectionable to the infants, but could be further improved by adjusting processing conditions.

The result of the mouthfeel showed, the samples were significantly reduced ($p<0.05$) by the addition of flours and protein isolates from bambaranut and soybean. However, the protein isolates enriched *ogi* (B) had the highest mean score of (8.40) whereas the flour enriched *ogi* (E) had the lowest mean value of (6.25), implying that the protein isolates enriched *ogi* was most preferred in terms of mouthfeel. This could be attributed to the soft texture of soy protein isolates used in the blend. The observed differences in mouth feel in the protein isolates sample B as compared to the other samples could be attributed to the higher proportions of maize *ogi* used in the blend or simply due to the coarseness of maize *ogi* in the protein isolates blend.

The taste of the maize-based *ogi* decreased with the addition of flours and protein isolates. there was however, no significant difference between the flour samples (D and E) as well as the protein isolates samples (B and C). The scores for taste ranged between 8.55 to 6.00. Taste is the sweet sensation caused in the mouth by contact with sweetening agent and it is an important sensory attribute of any food. Porridges of samples A (100% maize ogi) and B of 90.65:9.35 of maize *ogi* blended with soy protein isolate had the highest taste scores. Differences in taste could be

attributed to molecular changes in the flour due to different processing conditions (soaking, Dehulling, drying and defatting) that the raw materials were subjected to (28).

The consistency results of *ogi* produced from (100 % maize flour), flours and protein isolates from bambaranut and soybean revealed that, the control sample A had the best rating of 8.70 followed by the protein isolates sample B 7.85. The differences in the consistency rating of sample A as compared to others may be due to constitutional variations. This consistency is very important, as it would determine the amount of food an infant can swallow, because infants can swallow a smooth gruel and not a coarse product. However, consistency of the composite diets was within acceptable limits. Water absorption capacity and swelling index are important parameters which determine the consistency of the flour. A very thick consistency would need increased efforts to swallow and therefore may limit the food intake in young children who have not fully developed their swallow ability (78).

The sensory scores for overall acceptability of the porridge samples ranged between 7.00 and 8.65. There were significant ($p<0.05$) differences among the samples. Sample A had the highest mean score of 8.65 followed by the protein isolates sample E 8.00, respectively. All the sensory scores evaluated were more than the minimum acceptable score of six (6) (79). The results revealed high mean scores in all the sensory attributes evaluated as all the samples maintained a high level of acceptability by the panelists suggesting that acceptable protein enriched *ogi* could be made from maize flour and either bambaranut or soy flours as well as their protein isolates.

CONCLUSION

The protein, amino acids, micronutrient, composition of the maize-based *ogi* were enhanced significantly following the addition of legume flours and protein isolates from bambaranut and soybean with decreased in antinutritional content which could help in alleviating protein energy malnutrition among under five children.

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Accelerated Stability Indicating: Method Development of UV-Visible Spectrophotometric Analysis and its Validation for Vitamin-D3 Estimation in Tablets

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Abstract:
Purpose: A facile approach to develop and validate a precise accurate, and stable method over UV-visible spectrophotometer (UV-vis) for Vitamin D3 (VD3) tablets. Methods: In this study, three brands of VD3 tablets from various companies were employed. UV-vis was used to test the short-term stability research of vitamin D3. In short-term stability research, an assay of vitamin D3 is evaluated on UV-vis after one month of their manufacturing that is known as zero month then after 3 months and lastly after 6 months. Conditions of short-term stability study for six months consists of temperature 40 °C within upper-lower limit of 2 % and moisture content of 75 % within upper-lower limit of 5 %. For identification of VD3 in tablet dosage form, a simple UV-vis procedure was formulated that had an appropriate susceptibility according to accuracy, precision, and linearity. Guidelines of International Council of Harmonization of technical requirements for pharmaceuticals of Human use and United States Pharmacopeia were followed for validation of these parameters. Results: Out of three brands, one brand of vitamin D3 (VD3) tablet dosage form by UV-vis method was found to be stable at the appropriate temperature and moisture content in comparison to other two brands of VD3 that started to lose their efficiency at elevated temperature and moisture content from the normal range. VD3 shows its absorbance in deionized water at the wavelength of 265 nm, linearity range of 0.65-9.94 µg ml-1, molar absorptivity of 4.32 x 104 L/molcm-1 with a correlation coefficient equation of y=0.0503×-0.4988 and regression of R2= 0.9991. Conclusion: It was found that VD3 demonstrated the best result in climate atmosphere as compared to an accelerated parameters which lead to the breakdown of VD3 when tested with the help of UV-vis method.

Keywords: Vitamin-D3, drug degradation, accelerated stability study, UV-spectrophotometric method, Validation and calibration.

INTRODUCTION

Cholecalciferol, or Vitamin D3 (VD3) is also known as sunshine vitamin because it is obtained directly from the sunlight (Hoffmann-La Roche 1989). VD3 plays a fundamental role for healthy bone by the breakdown of calcium and its absorption in the body. Calcium and phosphorous that are involved in biological processes are sustained by VD3. The more stable form of VD3 is produced by giving an appropriate temperature (Holick *et al.*, 2000, ICH 2003). The tropical zones with high temperatures are not good for pharmaceutical products (Belsey, *et al.*, 1974). When pharmaceuticals are dispersed in these areas, the test results turned out to be lowered due to the breakdown of active pharmaceutical ingredients at high temperatures that affect their assay

(Brander *et al.*, 1991, Connors *et al.*, 1986). Physical properties of a product are of prime importance that are altered due to improper handling as well as manufacturing. Variability in temperature and humidity of the environment will affect the activity due to the lowering of active constituents of drug product. All these environmental changes should be checked through stability analytical methods (Dean *et al.*, 2000, Grady *et al.*, 1980).

Humid air has more susceptibility for VD3 (ICH 2003). The storage temperature for VD3 is 56 °C at high temperature while with its breakdown occurs (Iqbal 2011). Our aim was to explore the breakdown of the samples of VD3 tablets through a new UV-visible spectrophotometric (UV-vis) method with quick, precise, accuracy and robustness. In previous findings, VD3 was analyzed by thin layer Chromatography, Liquid Chromatography (LC), Liquid extraction, and Reverse-Phase High-Performance Liquid-Chromatography (RP-HPLC) methods (Iqbal 2011, Merck 1976). Herein, we took the VD3 commercial tablets from various companies that subjected them to stability testing to analyze its breakdown at elevated temperatures and correlated it with VD3. USP (USP 2004) and the ICH (Q_2 (R_1)- 2005) has provided specification and master formulae for method validation, so all the analytical results were validated according to their recommendations.

MATERIAL AND METHODS

Chemicals and Reagents

VD3 tablet dosage form of three different companies were used as a sample. Formic acid, Methanol, Glacial Acetic Acid, Dinitrophenylhydrazine, and Distilled water were purchased from BDH/Lab Scan Asia/RCI and were of analytical grade**s.**

Instrumentation

Shimadzu-1600, Shimadzu-1800 and Hitachi-UV were supplied by LabX, Canada. UV-vis was used to measure VD3 solution samples. For 6 months, vitamin D3 was kept at humidity of 75 percent ± 5 percent and temperature 40 ± 2 °C in the climatic chamber. By using the UV-vis procedure that is currently developed and not mentioned in official pharmacopeias, the test result of active pharmaceutical substance (VD3) was accomplished for different brands. The maximum wavelength for VD3 is 265 nm and test results were performed thrice and average was calculated (Temora *et al.*, 2016).

Standard Preparation

Standard solution of VD3 was prepared by taking 100 mg of VD3 and 10 ml of formic acid in 50 ml of the volumetric flask by placing it in Sonicator for some time while methanol was used as a blank. After, the addition of methanol, 2 ml from the stock solution was taken into a 100 ml volumetric flask while 1 ml of 2, 4 dinitrophenyl hydrazine compound was added to it and volume was made up to the mark to get absorbance at 265 nm.

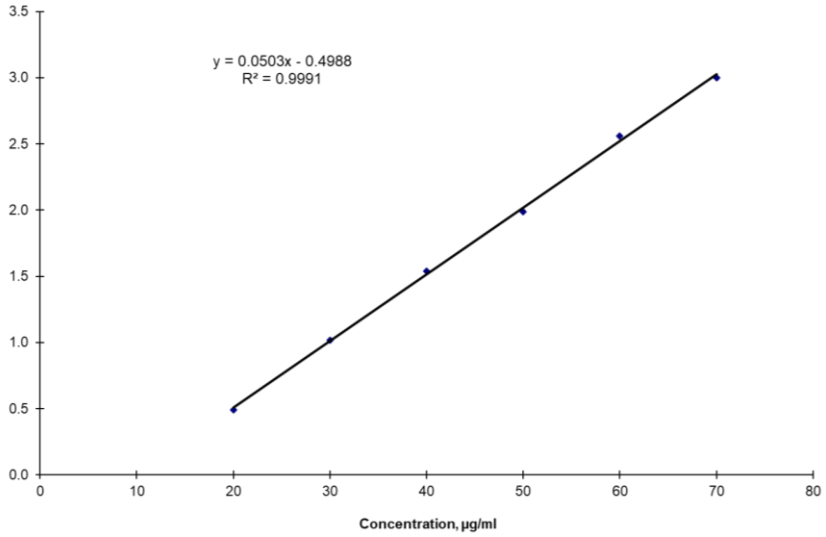
Sample Preparation

The sample solution was prepared by taking 100 mg of VD3 and 10 ml of formic acid in 50ml of the volumetric flask by placing in Sonicator while methanol was used as Blank. After filtration (0.45-µm) process, 2 ml of filtrate solution and 2, 4 dinitrophenyl hydrazine compound was added into 100 ml volumetric flask while volume was made up to the mark and absorbance was measured at 265 nm with the help of UV-vis spectrophotometer. VD3 tablet samples were taken at regular intervals for the accelerated stability study.

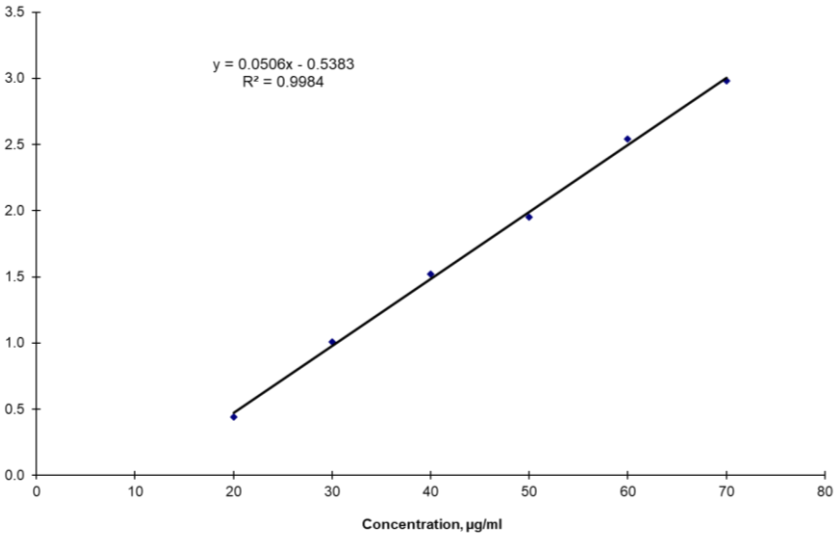
UV-vis spectrophotometer and climatic chamber were used in this procedure. At a given temperature of 40 °C and moisture Content of 75 %, the stability of VD_3 was tested by UV-vis at 1st month of manufacturing of the drug, then after three months, and lastly at six months after manufacturing. Five samples of VD_3 ranging from concentration 20-60 µg/ml were prepared and checked by the developed analytical procedure. These samples were used for preparing the calibration curve and the regression equation was calculated. Six samples were prepared to check the precision of the proposed developed procedure. The ruggedness of the analytical procedure was demonstrated by employing minor changes (solvent composition) in developed analytical procedure. Three brands of VD_3 were analyzed on different UV-vis models, performed by different analysts, on different days and time and results were calculated to demonstrate the robustness.

Preparation of Calibration Curve

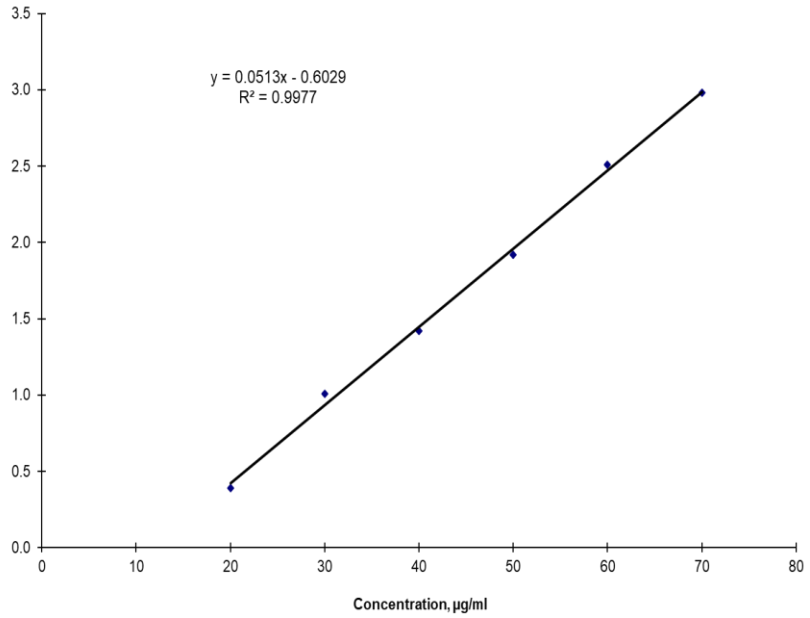
By plotting the absorbance against the concentration, the calibration curve of VD_3 of three different brands were generated. The concentration range of VD_3 for the calibration curves were 20-60 µg/ml.



Calibration Curve of brand 1



Calibration Curve of brand 2



Calibration Curve of Brand 3

Fig. 1: A calibration curve of VD3.

Linearity:

The linearity was estimated by analyzing the three different brands of VD3 at the different concentrations of VD3 in tablet dosage form. The Beer–Lambert's concentration range was found to be 20-60 µg/ml. The linearity of the relationship between absorbances and conc. was determined by plotting the calibration curve for VD3 as shown in the above Figure. Five different conc. of each brand was taken and measure at their absorbance at the respective wavelengths. Then mean, standard deviation, correlation coefficient, and slope were calculated.

Brand 1		**Brand 2**		**Brand 3**	
Conc.	**Abs**	**Conc.**	**Abs**	**Conc.**	**Abs**
20	0.950	20	0.952	20	0.911
30	1.425	30	1.421	30	1.418
40	1.900	40	1.807	40	1.904
50	2.375	50	2.370	50	2.369
60	2.850	60	2.850	60	2.811
Mean	1.900	Mean	1.880	Mean	1.88
SD	0.75	SD	0.75	SD	0.75
R^2	0.9991	R^2	0.9984	R^2	0.9977

Conc. (µg /ml), D (Day) Abs (Absorbance), Standard Deviation (SD), Correlation Co-efficient R^2

Precision:

The Inter-day precision was identified for a solution of conc. (40 µg/ml) of three brands of VD3 tablet and were analyzed for the three times on a different day. (Day 1, Day 2, Day 3). Average, SD, %RSD were calculated and the % RSD was not more than 2 %.

Results of Method Precision (Inter-day) of Different Brands of VD3:

Brand 1				Brand 2				Brand 3			
Conc.	**Day1**	**Day 2**	**Day 3**	**Conc.**	**Day 1**	**Day 2**	**Day 3**	**Conc**	**Day 1**	**Day 2**	**Day 3**
40	1.89	1.90	1.91	40	1.90	1.91	1.91	40	1.89	1.9	1.88
Avg.	1.90			Avg.	1.90			Avg.	1.89		
SD	0.01			SD	0.005			SD	0.01		
%RSD	0.52			%RSD	0.26			%RSD	0.52		

Conc. (µg /ml), D (Day) Abs (Absorbance), Average (avg.), Standard Deviation (SD)

In the intra-day variation, the study was determined for a solution of conc.40 µg/ml of three brands of VD3 tablet and was investigated thrice on the same day at different timings. Mean, Standard deviation, & % RSD were calculated as shown in the below tab

Result of Method Precision (Intra-day) of Different Brands of VD3:

Brand 1				Brand 2				Brand 3			
Conc.	**Abs 1**	**Abs 2**	**Abs 3**	**Conc.**	**Abs 1**	**Abs 2**	**Abs 3**	**Conc.**	**Abs 1**	**Abs 2**	**Abs 3**
40	1.91	1.88	1.90	40	1.92	1.90	1.91	40	1.89	1.92	1.89
Avg.	1.89			Avg.	1.91			Avg.	1.90		
SD	0.01			SD	0.01			SD	0.01		
% RSD	0.52			%RSD	0.52			% RSD	0.52		

Conc. (µg /ml), Abs (Absorbance), Average (avg.)

Range

Brands	**Range (µg/ml)**	**Regression equation**	**R^2**
Brand 1	20-60	0.0503×-0.4988	0.9991
Brand 2	20-60	0.0506×-0.5383	0.9984
Brand 3	20-60	0.0513×-0.6029	0.9977

Accuracy (% Recovery):

A standard addition procedure was used for the determination of accuracy study. The 40 µg/ml sample solution of each brand of VD3 was spiked with an extra 50, 100 and 150 % of standard concentration of VD3. Absorbance was measured at 265 nm and the concentration of the drug was determined. The experiment for each brand was performed three times. Amount recovered, % recovery, average recovery, and % RSD were calculated as shown in the below Table.

Brand	**Initial Amount (µg)**	**Level (%)**	**Amount Added (µg)**	**Amount recovered (µg)**	**% Recovery**	**Average Recovery**	**%RSD**
Brand 1	40	50	20	19.95	99.75	99.21	0.85
		100	40	39.87	99.67		
		150	60	58.94	98.23		
Brand 2	40	50	20	19.98	99.90	99.60	0.51
		100	40	39.95	99.89		
		150	60	58.81	99.01		
Brand 3	40	50	20	19.99	99.95	99.94	0.02
		100	40	39.97	99.92		
		150	60	59.89	99.96		

Amount

Optical Characteristics of VD3

Beer's law limit (µg/ ml)	20-60
Correlation Coefficient	0.9991

Regression equation	0.0503×-0.4988

Robustness:

The robustness of the method was determined by carrying out the analysis by taking the standard concentration from the three brands of VD3. The assay was performed by different analysts, on different instruments, and on different day/time.

Statistical Validation for Robustness studies of VD3

Sr. No	Parameter	Brand 1	Brand 2	Brand 3
1	System	Shimadzu-1600	Hitachi-UV	Shimadzu-1800
2	Sample	Brand 1	Brand 2	Brand 3
3	Date	7/12/2020	8/12/2020	9/12/2020
4	Day	Monday	Tuesday	Wednesday
5	Time	11:00 AM	12:00 PM	1:00 PM
6	Analyst	X	Y	Z
7	Sample	40 µg/ml	40 µg/ml	40 µg/ml
8	Absorbace	1.901	1.892	1.894
9	Assay	101.11%	99.84%	99.57%

Ruggedness:

The Ruggedness was carried out to calculate the effect of small changes in the spectrophotometric conditions for the determination of VD3 in different brands. By changing the composition of solvent, taking the sample concentration 40 µg/ml, the absorbance, standard deviation, %RSD, and %assay was calculated.

Statistical Validation for Ruggedness Studies of VD3.

Sr. No	Brands	Wavelength(nm)	Conc. (µg/ml)	Abs.	Avg. Abs.	STD	%RSD	%Assay
1	Brand 1	259	40	1.869	1.853	0.009	0.48	98.56
2		259	40	1.850				
3		259	40	1.855				
1	Brand 2	265	40	1.917	1.900	0.01	0.52	99.63
2		265	40	1.895				
3		265	40	1.913				
1	Brand 3	270	40	1.953	1.970	0.017	0.86	99.39
2		270	40	1.987				
3		270	40	1.976				

Limit of Detection (LOD) and Limit of Quantification (LOQ):

Limit of detection (LOD) is the minor concentration of the sample that can be identified. LOD was determined by equation LOD- 3.3 s/m where S is the standard deviation and m is the slope of the calibration curve. Limit of Quantification (LOQ) is the lowest concentration of sample that can be quantitatively identified by suitable precision and accuracy. LOQ was determined by equation LOQ-10 s/m.

Specificity:

In specificity, by comparing the spectrum of tablet solution with that of the standard solution was determined. Then the spectrum of sample was checked if there is any interference of excipients.

Statistical Analysis

By applying suitable analytical tests, analytical analysis can be done, e.g., p values observed level of significance of rejection of more than 0.25 to the coefficient of the regression and zero-time intercepts for the single batches. Statistical software Minitab 15 (Two-way analysis) was applied to get accurate and precise results of the given method.

RESULT

In this study, three different brands of VD3 tablets i.e., (a) Brand 1, (b) Brand 2, and (c) Brand 3 containing 125, 125, and 400 IU respectively of VD3 were used. The brands of VD3 tablets were found to meet the Pharmacopoeial requirements of method validation as shown in the Table. At a recommended temperature and moisture content, batch 1,2, and 3 of brand 1 of VD3 were tested and their stability study was performed at the initial month, then at the third month, and six months of their manufacturing. Accelerated stability study data shows that assay results of VD3 of brand 1 of batch 1, batch 2 batch 3, were reduced from 100.87 % to 100.02 %,102.89 % to 101.32 %, and 100.54 % to 98.38 %. The p-value is not more than 0.05 according to this standard out of two "p" value,1 "p" value of brand 1 tablet was not lie in range.

At a recommended temperature and moisture content, batch 1, 2, and 3 of brand 2 of VD3 were tested and their stability study was performed at the initial, third, and six months of their manufacturing. Accelerated stability study data shows that assay result of VD3 of brand 2 of batch 1, batch 2, batch 3 was reduced from 105.60% to 98.11%,107.71% to 97.48%, and 110.42% to 104.65% in the first six months. The "p" value is not more than 0.05 according to this standard, out of two "p" values, 1 "p" value of brand 2 tablets was not in range.

Table: Parameters of the analytical analysis of results in the determination of assay content of VD3 tablets during accelerated stability study conditions.

Product Name	Parameters for Accelerated Stability	Batch 1	Batch 2	Batch 3	p-Value	
					Months	Batches
Brand 1	Initial month	100.87 %	102.89 %	100.54 %	0.014	0.003
	Three months	100.43 %	101.61 %	99.31%		
	six months	100.02 %	101.32 %	98.38 %		
Brand 2	Initial month	105.60 %	107.71 %	110.42 %	0.019	0.096
	Three months	102.75 %	105.63 %	105.18 %		
	six months	98.11 %	97.48 %	104.65 %		
Brand 3	Initial month	104.59 %	102.64 %	103.59 %	0.012	0.367
	Three months	102.62 %	98.13 %	98.52 %		
	six months	96.21 %	97.22 %	98.19%		

At a recommended temperature and moisture content, batch 1, 2 and 3 of brand 3 of VD3 were tested and their stability study was performed at the initial month, then at the third month and six months of their manufacturing accelerated stability study data shows that assay result of VD3 of brand 3 of batch 1, batch 2, batch 3 was reduced from 104.59% to 96.21%, 102.64% to 97.22% and 103.59% to 98.19%. The "p" value was not more than 0.05 according to this standard out of two "p" value,1 "p" value of brand 2 tablets was not in range. The method was found linear in a range of 20 to 60 µg ml^{-1} with a good correlation of 0.99 to lower % RSD (below 2 %) of % assay values, observed during replicate analysis of different tablets as part of precision, indicate the suitability of the method. For chamber saturation time, the procedure was found rugged.

DISCUSSION

During accelerated stability study, VD3 tablet dosage form of brand 1 was provided with the temperature of 40 ±2 °C and moisture content of 75 % ±5%. During this heat and humidity, VD3 remains unchanged. At the same time, during the storage of the other two brands of VD3, heat and moisture content were increased from the normal range and its lea breakdown (Iqbal 2011). When VD3 Brand 1 tablet having 100%-100.89 % result was compared to LC, liquid extraction and RP-HPLC methods having recovery 93%-102 %, 98%-100.9 %, and 94.43% respectively, it showed confirmation of previous findings and precision and accuracy of the new method (Tiles 1994 – Staff et al., 2003). Nature of VD3 breakdown is unidentified, but after passing some time the decrease in the test result of VD3 is due to the oxidative breakdown of VD3 during its storage.

Under the selected elaborated conditions i-e at appropriate temperature and humidity, after storage VD3 test was performed for stability purpose. (Hoffmann-La Roche 1989). From our research, it is verified that VD3 does not maintain its stability at high heat and moisture content and starts breakdown and loss efficiency at increased heat and moisture content from the normal level. (Connors *et al*., 1986, Grady *et al*., 1980, Merck 1976). When both heat and moisture contents are increased from their normal range at the same time, VD3 starts to breakdown most rapidly where all the test samples of VD3 are losing their efficacy after few days of their storage during accelerated stability test conditions.

CONCLUSION

Hence a precise, accurate, and simple UV-vis spectrophotometric method was developed for the analysis of VD3. It was concluded that elevated heating and moisture content are two important factors that affect the stability of VD3 tablets of different brands so VD3 found susceptible to both of these factors. Recently formulated procedure for the determination of stability of VD3 tablet under different circumstances/environmental conditions produced high-quality outcomes. We analyzed three tablet dosage forms of VD3 of different manufacturers. The stability of the brand 1 tablet dosage form of VD3 is long-lasting as compared to the other two tablet dosage form brands of VD3. Brand 1 of VD3 is kept at an appropriate temperature and moisture content so it didn't degrade and remained stable during its shelf life. But in case of other two brands of VD3, during their storage temperature and humidity was increased from their normal level so VD3 does not remain stable and loose efficacy before their shelf life.

SUGGESTIONS

Tablet dosage form is one of the complicated preparations that comprises minerals and vitamin rich nutritional compounds. VD3 is sensitive to high temperature and humidity, so while their preparation, deposit and distribution when it come in contact with high temperature and moisture content it started degradation and lost functional activity.

Assessment of stability features of cholecalciferol are exceptionally so hard, due to susceptibility of VD3, and its sensitivity towards high temperature and high moisture content and formulations that contain more than one vitamin.

This may be used to conduct further systematic studies on the evaluation of VD3 stability in tablet mixtures.

1. In the tablet dosage form of VD3 recognition of unfamiliar aerophilic and deterioration product.

2. Stability- Ascertained procedures for test results of VD3 in the existences of deteriorated compounds were generated.
3. For the time, assay of VD3 in tablet dosage form, connection of speed of compound breakdown with UV-vis procedure were studied.

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