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Income Generation, Food and Nutrient Security for Improved Livelihoods and Health Outcomes among People Living with HIV (PLWHIV)

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INCOME GENERATION, FOOD AND NUTRIENT SECURITY FOR IMPROVED LIVELIHOODS AND HEALTH OUTCOMES AMONG PEOPLE LIVING WITH HIV (PLWHIV)

Abstract

The HIV/AIDS epidemic occur in populations where malnutrition is already endemic. Good nutrition supports overall health and maintains the immune system in people living with HIV (PLWHIV), it also maintains a healthy weight and absorption of HIV medicines. To maintain adult body weight and physical activity, energy requirements increase by 10% in asymptomatic and by approximately 20% to 30% during symptomatic and subsequently during AIDS. There is need to increase energy intake to take care of the increased energy expenditure that accompanies HIV-related infections and help in recovery of weight lost during HIV and related infections. Dietary intake of micronutrients at RDA levels may not be sufficient to correct nutritional deficiencies in HIV-infected individuals, therefore there is need for increased consumption of micronutrients (iron, zinc, and selenium) beyond RDA levels. Financial and technical support could also improve their dietary quality and increase nutrient intakes. This study aims at using evidence-based nutrition intervention among PLWHIV to enhance their livelihoods through increased nutrient intake and income generation. The main objective is to improve the health and livelihoods of PLWHIV by providing additional income and ensure production and consumption of nutritious crackers processed from cassava roots and simisim seeds. A quasi-experimental study design will be employed. 64 PLWHIV aged 19-50 years with moderate malnutrition ((BMI between >16Kg/M^2-<20Kg/M^2) and Haemoglobin (Hb) levels of <7g/dl), who attend clinics at Chulaimbo and Nyahera Sub-County hospitals and willing to participate in the study will be enrolled. 32 participants will be sampled from Chulaimbo hospital into the intervention group and 32 will be sampled from Nyahera hospital into the non-intervention group. Study participant's baseline data of BMI, iron, zinc, and selenium status will be collected. Nutrient content (calorie, iron, zinc, and selenium), Microbial load (total plate count, *E. Coli*, Yeast, and moulds), and organoleptic acceptability of the crackers will be evaluated. Intervention group will be fed on one serving of safe cracker(16g) daily for 22 weeks. The outcome nutritional status will be assessed at the end of intervention to ascertain the effect of on nutritional status. All the study participants will be trained in crop production, food processing techniques, and basic entrepreneurship skills, to positively impact on heath and for income generation. Data will be analyzed using SPSS version 22, Independent sample *t*-test (two-tailed) will be used to compare group means for nutritional status. A two-way analysis of variance with repeated measures will be used to analyze the effect of the factor group (intervention vs, control) on the evolution of each nutrient. Unpaired *t*-test will be used to compare the means of pre-intervention data and post-intervention data and of intervention and non-intervention groups. The study outcome will inform policy with regards to the use of focused evidence-based nutrition intervention as part of HIV/AIDS management.

DR. Habwe Florence Oyiera — 130th October 2023

POST-DOC FELLOW **SIGNATURE** **DATE**

Lucia Kaluki Mutwika	PHD/PH/00080/2019	30th October2023	
PHD STUDENT	**REG. NO**	**SIGNATURE**	**DATE**

Study Protocol

i. Briefly describe background and significance of the research study/project.

HIV infection still remain a major public health problem despite the comprehensive efforts towards ending the epidemic (He, 2021). It is most prevalent in Sub-Saharan Africa and a major concern of health in Kenya where it is usually aggravated by undernutrition and opportunistic infections, inflicting a devastating situation to the affected individuals, families, communities and country (Anabwani & Navario, 2005). Undernutrition and HIV infection are strongly linked and is one of the most common issue among people living with HIV (PLWHIV) and a main contributor to increased morbidity and premature death (Alebel *et al*., 2021; Omwanda *et al.*, 2020). Despite the great efforts and achievements in care. HIV infection and related morbidity and mortality rates in developing countries such as Kenya still remain unacceptably high (Ivers *et al.*, 2009).

HIV infection increases the nutrient requirements and at the same time impairs the nutrient intake and uptake resulting to weakened immune system that increases the risk of opportunist infections thus accelerating the progression of HIV to AIDS (Pribram, 2011). HIV seropositive individual have increased energy requirements due to increased catabolism and are prone to enteropathy. Adequate intake of trace elements such as iron, zinc and selenium play a major role in strengthening and maintaining a healthy immune system (Khalili *et al*., 2008). The energy requirements of asymptomatic HIV infected adults increases by 10% to maintain healthy weight gain and the daily physical activity and by approximately 20 – 30% during symptomatic stage (WHO, 2003). Iron is an essential trace element required in the body for physiological, immune function and all cellular pathways in cell life (Camaschella, 2017). Iron deficiency anaemia increases the risk of infections (Jonker & van Hensbroek, 2014). Anemia among PLWHIV infection is usually associated with HIV infection progression and increased risk for all-cause mortality among this population with the magnitude being more in severe anemia cases (Ajibola I Abioye *et al.*, 2020; Cao *et al*., 2022). Zinc play a key role in muscle mass preservation in the body, its deficiency results to immunological impairments in PLWHIV infection and it leads to

reduced number of circulating T-lymphocytes thus lowering the immune response (Ivers *et al.*, 2009; Osuna-Padilla *et al.*, 2020; Kupka & Fawzi, 2002). Selenium helps in modulating the HIV infection associated chronic tissue inflammation (Ivers *et al.*, 2009; Osuna-Padilla *et al.*, 2020). Selenium deficiency affect the functioning and expression of the selenoproteins resulting to degeneration of tissues and organs (EFSA, 2017). Nutritional status of people living with HIV infection is an important determinant of the HIV infection progression and outcomes (Hendricks *et al.*, 2008). Strong immune system enhances recovery of any opportunist disease within a very short period of time (Ahmad & Ghosh, 2020). Mortality rates are high in people with low immunity, poor nutritional status due to associated comorbidities (Ahmad & Ghosh, 2020) thus the need to ensure adequate caloric and micronutrient intake among PLWHV which can possibly be done through food interventions.

Though the government of Kenya has made an advancement on antiretroviral treatment for people living with HIV infection, many deaths are related to undernutrition (Shifera *et al.*, 2022). Undernutrition remains a major challenge affecting about 1800 million PLWHIV infection globally (Ivers et al., 2009). About 650,000 people living with HIV infection die annually due to AIDS opportunistic illnesses, with around 40.1 million being estimated to have died due to AIDS related illnesses since the pandemic started (UNAIDS, 2022). Undernutrition and HIV progression lead to poor nutritional status (Audain *et al*., 2015). Though there is limited data on micronutrient deficiencies among PLWHIV in Kenya, Globally anaemia affects about 2 billion people , with iron deficiency anaemia being the leading cause (≥ 60%) of anaemia worldwide (Kassebaum & Collaborators, 2016). Zinc deficiency affects >20% of the population in low and middle-income countries, with the risk increased in south Asia and Su-Saharan Africa were it affects >25% of the population (Gupta *et al*., 2020). Selenium deficiency affect 28% of the African population (Ligowe *et al.*, 2020) and 52% of the East African countries population with Kenya having the highest risk of 26%-75% due to inadequate dietary intake (Ngigi, 2019).

Counties closer to Lake Victoria are still having highest HIV infection prevalence in Kenya, with Kisumu being the second County in Kenya with highest number of new HIV infection at 17.5% which is much higher than national statistics of 4.3% and also second leading with 130,036 people

living with HIV infection accounting for 9% of the total HIV population (NSDCC, 2022). Chulaimbo Sub County Hospital attends to 5,800 people living with HIV infection (KHIS, 2023).

Good nutrition is an important component of comprehensive care for PLWHIV infection as it delays the HIV infection progression (Omwanda *et al.*, 2020). Improving the immune function through diversified, modified, and nutritious diet enhances recovery from infections. A nutrient rich diet that has biologically active ingredients and also rich in antioxidant could serve as an effective nutritional approach to restore the body's immune response (Ahmad & Ghosh, 2020).

Sesame seed commonly known as simsim in East Africa is an erect annual herb that is a member of Pedaliaceae family. Simsim contains 45% - 65% of oil and is also a rich source of protein, calcium, iron, zinc, selenium and phytonutrients. (Tanwar & Goyal, 2021). Simsim seed is highly nutritious and can be used as a nutraceutical to improve immune status and also combat malnutrition issues among adults living with HIV infection as well as serve as a global food security due to its potential for development of different value-added simsim food products (Tanwar & Goyal, 2021). Cassava (*Manihot esculenta Crantz*) is a tuberous root which is used as staple food for about a half a billion people in the world. It is a tropical crop grown mainly in Asia, South America and Africa, and is second most important food root crop in Kenya (Klein, 2016). Cassava roots are energy dense thus rich source of calories though low in proteins and minerals such as iron, zinc and selenium (Montagnac *et al.*, 2009). The Kenyan government has been promoting cassava use to improve food security and cassava has been identified a high value traditional crop (Mulu-Mutuku *et al*., 2013).

There is a need to strengthen community based nutrition programmes in addition to nutrition education in order to improve the availability and accessibility of affordable and sustainable healthy diets to help maintain good health and nutrition for PLWHIV infection (Omwanda *et al.*, 2020). The developed food product should comply with the accepted international food safety standards in terms of hygiene especially microbial contaminants (Kruger *et al.*, 2020). The sensory evaluation of food product is very important and should correspond to the food preferences of the consumer (Abolaji *et al.*, 2019). Therefore, this study aims at processing crackers form cassava roots and simsim seeds, and evaluating its effect towards improving the nutritional status of HIV

seropositive adults enrolled for care at Chulaimbo and Nyahera Sub County Hospitals, Kisumu County, Kenya.

ii. Specify question(s) (aims) of proposed research study/project.

Study Objectives

1. To evaluate the microbial load (total plate count, *E. Coli*, Yeast, and moulds) of crackers processed from cassava roots and simsim seeds.
2. To assess acceptability of crackers processed from cassava roots and simsim seeds among HIV seropositive adults enrolled at Chulaimbo Nyahera Sub-County hospitals, Kisumu County, Kenya.
3. To assess the nutrient content (calorie, iron, zinc, and selenium) of crackers processed from cassava roots and simsim seeds.
4. To assess the nutritional status (BMI, iron, zinc, and selenium) of HIV seropositive adults enrolled at Chulaimbo and Nyahera Sub-County hospitals, Kisumu County, Kenya.
5. To assess the effect of consuming crackers on the nutritional status (BMI, iron, zinc, and selenium) among HIV seropositive adults enrolled at Chulaimbo Sub-County hospital, Kisumu County, Kenya.
7. To train the study participants (capacity building) in crop production (home gardens), food processing (crackers) and basic entrepreneurial skills for product marketing and income generation**.**

The PhD student in the project will share some of the objectives stated above. The student's thesis objectives are as follows:

1. To assess the nutritional status of HIV seropositive adults enrolled at Chulaimbo and Nyahera Sub-County hospitals, Kisumu County, Kenya.
2. To analyse iron, zinc, selenium and calorie levels in crackers processed from cassava roots and simsim seeds.
3. To evaluate the microbial load (total plate count, *E. Coli*, Yeast, and moulds) of crackers processed from cassava roots and simsim seeds.
4. To assess acceptability of the processed crackers among HIV seropositive adults enrolled at Chulaimbo and Nyahera Sub-County hospitals, Kisumu County, Kenya.

5. To evaluate the effect of consuming crackers processed from cassava roots and simsim seeds on the nutritional status of HIV seropositive adults enrolled at Chulaimbo Sub-County hospital, Kisumu County, Kenya.

iii. Describe study/project design, population, and study/project procedures.

Study Site

This study will be conducted at Chulaimbo and Nyahera Sub County Hospitals in Kisumu West Sub County, Kisumu County, Kenya. Chulaimbo hospital is located between Maseno and Daraja Mbili, along Kisumu-Busia highway while Nyahera hospital is about 60km apart, located between Daraja Mbili and Nyang'ori long Daraja Mbili-Nyang'ori road. Other study activities will be carried out at Maseno University, Kenya. Laboratory analysis of food and blood samples will be done at the University of Nairobi, department of food science, nutrition, and technology laboratory.

Study Population

The study population will comprise 64 HIV seropositive adults aged 19 – 50 years enrolled for care at Chulaimbo and Nyahera Sub-County hospitals, Kisumu County, Kenya. The total HIV seropositive clients enrolled at Chulaimbo hospital population is 5800 while Nyahera's population is 1379, and the target population will be 5660 that is the number of HIV seropositive adults (KHIS, 2023). Sample size of 64 participants as calculated bellow will be enrolled into the study. Chulaimbo and Nyahera hospitals were purposively chosen for the study because they are public hospitals that attend to people of low socio-economic status. The hospitals are also close to Maseno University where most study activities like food product processing and training of study participants will take place. Otherwise, the study could equally be carried out in any other health facility that gives HIV services and care to HIV seropositive population.

The two hospitals were identified for sampling of the intervention (Chulaimbo) and control (Nyahera) which are about 60km apart to avoid contamination of the non-intervention group with the intervention cracker. This was also to eliminate the possibility of sampling more than one participant in the same household or same homestead who might be in the different groups.

Study Design

A quasi-experimental study design will be employed. 64 PLWHIV aged 19-50 years with moderate malnutrition ((BMI between >16Kg/M^2-< 18.5 Kg/M^2) and Haemoglobin (Hb) levels of

> 8g/dl – 12 g/g/dL), who attend clinics at Chulaimbo and Nyahera Sub-County hospitals and willing to participate in the study will be enrolled. 32 participants will be sampled from Chulaimbo hospital into the intervention group and 32 will be sampled from Nyahera hospital into the non-intervention group. Study participant's baseline data of BMI, iron, zinc, and selenium status will be collected. Nutrient content (calorie, iron, zinc, and selenium), Microbial load (total plate count, *E. Coli*, Yeast, and moulds), and organoleptic acceptability of the crackers will be evaluated. Intervention group will be fed one servings of safe 230g cracker daily for 16 weeks. The outcome nutritional status will be assessed at the end of intervention to ascertain the effect of on nutritional status. All the study participants will be trained in crop production, food processing techniques, and basic entrepreneurship skills, to positively impact on heath and for income generation**.**

Sample Size Determination

The appropriate sample size calculation for both intervention group and control group was based on statistical formula tailored for quasi-experimental studies. The sample size was determined using the formula for sample size calculation, as outlined by Bolarinwa (2020):

$$n = \frac{(Z\alpha + Z\beta)\, 2\sigma 2}{\delta 2}$$

n - Sample size for each group.
σ - Standard deviation for previous studies at 1.2 (Lubeka et al., 2020).
δ – effect size which is the anticipated difference in mean outcomes between intervention group and control group, 0.32 from previous studies (Lubeka et al., 2020).
$Z\alpha$ – (1.96) the standard normal distribution at 5% level of significance.
$Z\beta$ – 0.842 at 80% power of the study.
Using this values, the calculation yields;

$$n = \frac{(1.96+0.842)2\ (1.2)2}{0.322}$$

$$= \frac{(7.85)\ (1.44)}{0.4}$$

$$=28.26$$

= 29 participants per group
This indicates 29 participants per group. To account for potential non-respondents, a 10% will be added, resulting to a total of 32 participants per group and giving a total of 64 study participants (Sharma et al., 2020). According to Cohen's guidelines, a minimum sample size of 30 participants per group is sufficient to achieve adequate statistical power of 0.8 at a significance level of 0.05, in a quasi-experimental design, with reliable estimates of population and for hypothesis testing (Brysbaert, 2019; Gülkesen et al., 2022; Lubeka et al., 2020).

Sampling Procedure

Chulaimbo and Nyahera Sub-County hospitals were purposively chosen because they are public health facilities close to Maseno University where most study activities will be undertaken. Besides the distance, they are within Kisumu County which is the second leading in HIV prevalence in Kenya. Kisumu West Sub County is a resource limited setting and the second leading with the highest number of HIV seropositive clients in Kisumu County after Kisumu central Sub County which is in urban setting. Chulaimbo Sub County hospital houses the HIV compressive care center run by USAID 'Nuru ya Mtoto' a Non-governmental organization. Nyahera and Chulaimbo serve high number of HIV seropositive clients monthly.

64 moderately malnourished HIV seropositive adults with the following indicators and willing to participate in the intervention study will be purposively selected 32 in intervention group from Chulaimbo hospital and 32 in control group from Nyahera hospital.

a) BMI of > 16Kg/M^2 - < 18.5 Kg/M^2,
b) Hemoglobin levels of > 8 g/dl – 12 g/dL

Moderately malnourished HIV seropositive adults will be identified daily by the researcher assisted by research assistants in the baseline survey until the required number is attained. Participants will be fed on one serving of crackers daily (230 g) for a period of 16-weeks after which their nutritional status will be assessed.

Conceptual Framework

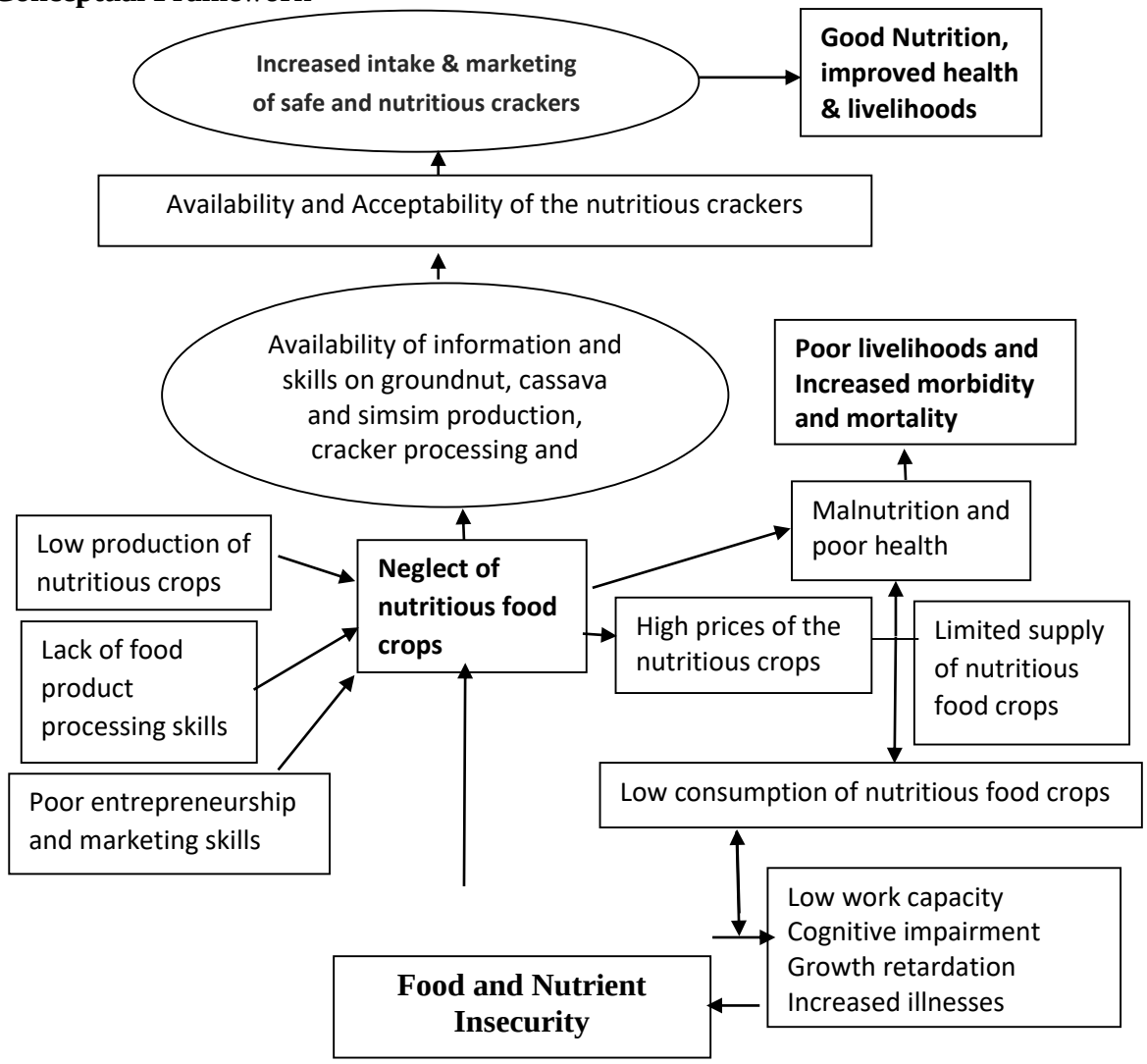


Fig. 1.1: Conceptual framework of the interaction between food and nutrient insecurity with crop With the availability, acceptability, consumption and marketing of nutritious food crops and food products with Nutrition and Health status.

Source: Adopted and modified from WHO, (1995). Global Burden of Disease (1990).

Materials & Methods

A. Blood Sample Collection and Analysis

A total volume of 6ml of a venous blood sample will be drawn from each participant in the morning by a phlebotomist using standard aseptic procedures, 3mls volume will be collected at the beginning of the study for baseline data and 3mls volume at the end of the study to assess effect of intervention. The blood sample will be collected into a trace element free vacutainer tube containing lithium heparin an anticoagulant to prevent clotting of the blood sample. The collected blood sample will be stored in a cooler box with ice pack at 4^{o}C and immediately transported to University of Nairobi, department of food science, nutrition, and technology laboratory for analyses. Hemoglobin levels will be determined in the collected whole blood sample using HemoCue machine. The reminder of the whole blood will be centrifuged within two hours after collection at 2500rpm for 10 minutes using trace element free techniques to separate and obtain the serum. The serum will be transferred to a trace element free tubes, labeled and frozen at -20^{o}C awaiting analysis of iron, zinc and selenium (Houghton *et al.*, 2019). Iron, zinc, and selenium levels will be determined using atomic absorption spectroscopy (AAS). The frozen sample will be left to thaw for 20 minutes. Iron will be determined by analyzing the ferritin level, aliquot of 100µl will be used to determine ferritin levels using enzyme immunoassay method in AAS. Aliquot of 250µl will be used to determine zinc and selenium levels. Flame AAS will be used to determine zinc level and selenium levels using microwave digestion with hydride generation methods in AAS (Chege *et al.,* 2013; Houghton *et al.,* 2019). All analyses will be done in triplicates both at baseline and after 16 weeks of intervention.

B. Food Sample Collection and Analysis

Cassava roots and simsim seeds will be purchased from Jubilee local market in Kisumu town (coordinates 0.1014095, 34.75911372). All the chemical reagents will be purchased from Sigma-Aldrich. The crackers will be processed at Maseno University department of nutrition and health foods laboratory and nutrient content analyzed at The University of Nairobi, department of food science, nutrition, and technology laboratory.

1. Cassava Roots

Dry cassava roots will be sorted by hand picking to remove dirt and foreign matter, a portion will be packaged for nutrient analysis of the dried raw sample. The sorted cassava roots will be washed under running water double glass distilled and deionized water to remove dirt then it will be dried under sun in open air. The dried cassava roots will be milled into flour and sieved by 0.5 mm size sieve (Habwe, 2015). The milled flours will be packaged in plastic container, sealed, and stored at room temperature in a cool dry room awaiting blending with simsim seed for preparation of crackers.

2. Simsim Seeds

Simsim seeds will be sorted by hand picking to remove dirt and foreign materials then a portion will be packaged for nutrient analysis of the dried raw sample. The sorted simsim seeds will be washed under running water double glass distilled and deionized water to remove dirt then it will be dried under sun in open air. The dried simsim seeds will be grilled until golden brown to improve colour and flavour then will be milled into flour and sieved by 0.5mm size sieve (Habwe, 2015). The milled flour will be packaged in plastic container, sealed, and stored at room temperature in a cool dry room awaiting blending with cassava flour and groundnuts powder for preparation of crackers.

4. Formulation of Cracker Flour

To prepare crackers, wheat flour will be replaced with cassava root flour and simsim seeds powder. The formulation of cassava root flour and simsim seeds powder blends will be based on the micronutrient content for individual flour with reference to nutrient recommended levels for adults (Habwe, 2015). The Recommended Dietary Allowance (RDA) for iron is 16mg for women during premenopausal stage and 11mg postmenopausal while for men is 11mg a day, Zinc 11mg a day for men and 8mg for women and selenium is 70µg per day for both men and women (EFSA, 2017).

5. Cassava-Sesame Crackers

100g of cassava flour and simsim seed powder blends will be mixed at ratio of 1:1 (50g cassava root flour and 50g of simsim seed flour (to prepare 5 servings, each serving weighing 16g). The flour will be whisked together with 1g of baking powder. 10g of margarine and 10g of sugar will be beaten together till light the added slowly to the flour mixture, 14mls of water will be added to the mixture slowly while stirring until dough is formed. The prepared dough will be rolled on a

floured board to a thickness of 1/8 to ¼ then cut into desired shapes which will be placed on a greased baking sheet and baked at a preheated oven to 175^{o}C for 10 minutes (Habwe *et al.*, 2017). The prepared crackers will be packaged in a food grade plastic container and labelled with computer generated random numbers awaiting analysis and distribution to HIV seropositive adults for Intervention.

6. Sample Analysis

The sample nutrient analysis will be done at the University of Nairobi department of food science, nutrition, and technology laboratory. This will be done for raw food materials and crackers processed from cassava root flour and simsim powder. All determinations will be carried out in three replicates.

7. Energy Content Determination

Proximate analysis will be done then total energy content of the sample will be determined by multiplying the gram of total lipids, proteins, and carbohydrates by Atwater factors of caloric values per gram. Protein content crackers will be determined using the Enhanced duma method, total lipids by Soxhlet Esther extraction method, moisture and Ash content will be determined by use of dry ashing method. The total energy value of the cracker will be calculated by using the Atwater factors by multiplying the total grams of carbohydrates, proteins and lipids by 4, 4, and 9 respectively (AOAC, 2000a).

8. Moisture Content Determination

3g of the sample will be weighed into a dried dish of known weight and oven dried at 105^{o}C for 5 hours then allowed to cool for 30 minutes. The cooled sample will be weighed and moisture content will be calculate as follows (Thangaraj, 2019).;

$\%W = (A - B) \times 100$

%W – Percentage of water

A - Weight of the sample before drying

B – Weight of the sample after drying

9. Ash Content Determination

3g of the sample will be weighed in a silica tared dish and will be incinerated for 3 hours at 450^{o}C in a muffle furnace then allowed to cool for 30 minutes. The cooled sample will be weighed and ash content will be calculate as follows (Thangaraj, 2019).

% of ash content= (A/B) x100

A - Weight of the sample before drying

B – Weight of the sample after drying

10. Lipid Content Determination

3g of the sample will be weighed in a thimble then covered with a piece of cotton wool, then the thimble will be inserted in to a Soxhlet liquid extractor. 300ml of petroleum ether will be weighed in round bottom flask 500ml clean beaker of known weight. The solvent in the flask will be heated for until it boils then the heat source will be adjusted to allow the solvent to drip from the condenser into the sample chamber and extraction will be continued for 6 hours then removed from the heat source and the extractor will be detached from the condenser. The flask will be replaced from the heat source and redistilled for solvent recovery. The flask will be placed in an oven at 102^{o}C and the content will be dried for 1 to 2 hours till the constant weight is reached then it will be allowed to cool The cooled sample will be weighed and lipid content will be calculate as follows (Thangaraj, 2019).

% lipid content= $(W_1 – W_2)/S$ x100

W_1 - Weight of the empty flask (g)

B – Weight of flask and extracted fat (g)

S – Weight of the sample

11. Crude Protein Content Determination

Material and reagents.

a. Kjeldahl flasks/tubes
b. Digestion and distillation units.
c. Conical flasks,400ml
d. Burette,50ml
e. Conc. H_2SO_4
f. Kjeldahl catalyst tablets

g. 40% NaOH solution (to be prepared by the technologists)
h. 0.1N HCl solution (to be prepared by the technologists)
i. 0.1N NaOH solution (to be prepared by the technologists)
j. Phenolphthalein indicator.
k. Methyl orange indicator
l. Nessler`s reagent/ not a must
m. Pipette, 25ml.
n. Test tubes

Procedure

· Weigh accurately about 0.5g of sample in a nitrogen-free filter paper.

· Fold carefully the filter paper and place it in a Kjeldahl flask.

· Add 1 Kjeldahl catalyst tablet and 10ml of conc.H_2SO_4

· Heat slowly and carefully the mixture in a fume cupboard, then increase the heat and boil until a clear solution is obtained. Continue boiling for a further hour. (Take 3-4 hours depending on sample type)

· After cooling, add distilled water to have liquid to $^1/_4$ of the flask.

· Add some drops of phenolphthalein

· Place a 300ml conical flask containing 25ml of 0.1 NHCl solution and some drops of methyl orange indicator under the outlet of the distillation unit.

· Connect the Kjeldahl flask to the distillation unit.

· Add into the Kjeldahl flask enough 40% NaOH solution to change the colour of the solution.

· Distil until a drop of distillate does not react with Nessler`s reagent placed in a test tube.

· Back titrate the distillate with 0.1N NaOH solutions to change the colour of the solution.

· Calculate the crude protein content of the sample.

NB: (Carry out a blank determination for correction of the acid titre)

12. Carbohydrate Content Determination

The total carbohydrate content will be determined by calculating the difference in 100- (percentage of proteins+ percentage of total lipids + percentage of total Ash + percentage of water).
100 - (Weight in grams [protein + fat + water + ash] in 100g of food.

13. Determination of Iron, Zinc and Selenium Levels

Atomic absorption spectroscopy (AAS) will be used to determine iron, zinc, and selenium levels. The content levels will be calculated and expressed in mg/100g. Standard solution for analysis will be prepared as follows; 25g of each sample will be weighed into crucible dish, 25ml of 20% sulphuric acid will be added to the sample then dried in an oven at 110ºC, and then the dish will be transferred to a furnace set at 250ºC and temperature will slowly be raised to 500ºC then heated for 6 hours. The sample will be cooled, and it will be digested with 10ml of HNO_3 then made up to 100ml with water in a volumetric flask and heated up to 100°C till white fume is seen. The sample will be cooled and solution made up to 100ml which will be used for determination of iron, zinc and selenium levels (AOAC, 2000b).

Micro Biological Load Determination

14. Total Plate Count

Total plate count will be determined using conventional method. 1g of each sample will be dissolved in 10ml of distilled water. Serial dilution will be made, 1ml of the prepared solution will be pipetted to 9mls of distilled water in a test tube the 1ml of this solution will be transferred to petri dish then 15ml of melted plate count. Agar will be added and will be solidified at 37ºC for 24 hours then colonies will be counted using a colony counter (Laryea *et al*., 2018).

15. **Escherichia Coli (*E. Coli*)**

Escherichia coli will be determined using conventional method. 1g of each sample will be weighed then added to 10ml of Butterfield's Phosphate Buffer diluents and will be homogenized at high speed to dissolve the sample. Serial dilution will be made, 1ml of the prepared solution will be pipetted to 9mls of distilled water in a test tube the 1ml of this solution will be transferred to petri dish then 15ml melted medium rapidly poured into it. The petri dish will then be inverted and incubated for 24 hours at 44ºC for enumeration of *E.Coli*. The colonies will be counted using colony counter (Laryea *et al*., 2018). The medium will be prepared by dissolving 37g of Rapid

E.coli 2Agar in 1litrs of distilled water. The homogenous mixture will be heated gently till powder is completely dissolved then it will be autoclaved at 122^{o}C for 15 minutes (Laryea *et al.*, 2018).

16. Yeast and Molds

Yeast and molds will be determined using conventional method. 25g of each sample will be dissolved in 225ml of distilled water then serial dilutions will be prepared by pipetting 1ml to 9ml of distilled water in a test tube. 1ml of each dilution will be transferred to a petri dish. 15ml of melted potato dextrose will be added then solution will be solidified at 28^{o}C for 72 hours. The colonies formed will be counted using a colony counter (Laryea *et al*., 2018).

17. Sensory Evaluation

The sensory characteristics of the crackers will be assessed in two phases, first phase will be done at Maseno University foods laboratory. Enrolled HIV seropositive participants will be trained on how to evaluate the sensory attributes of the crackers. Studies have shown that a panel of 20 is adequate for sensory evaluation (Abolaji *et al*., 2019; Akinsola *et al*., 2017; Ijarotimi & Keshinro, 2012). The participants will be requested to score the crackers for sensory attributes that will include appearance, taste, texture and general acceptability. The participants will evaluate the using a 5-point hedonic scale ranging from 1 “dislike very much” the lowest scale; 2 “dislike”; 3 “neither like or dislike”; 4 “like”; and 5 “like very much” being the highest scale (Sharif *et al.*, 2017) All the participants will be briefed before the sensory evaluation then each will be allowed to sit in an enclosed cubical designed for sensory (Akinsola *et al*., 2017), sufficient sample will be used for testing acceptability adequately to avoid carrying over of effects. Water will be provided to rinse the mouth in between the sample testing to reduce the residue material from the previous sample. The cracker will be served in a clear glass container coded with a 3-digit random numbers to minimize bias in judgement (Sharif *et al.*, 2017). Second phase the participants will take home the recommended daily intake of crackers during each visit to the facility for consumption at home. On the subsequent visits the participants will be requested to score the crackers on a 5-point hedonic scale.

Training of Research Assistants

Research assistants will be trained for one week on the study standard operating procedures. They will be trained on food processing, data collection, communication skills, food safety and hygiene. The phlebotomist will be trained on blood sample collection standards.

iv. If a control or comparison group shall be used, a justification should be provided.

A control group comprising of 32 HIV seropositive adults will be used to ascertain the effect of nutrient consumption through food intervention on nutritional status of HIV seropositive adults aged 19-50 years. A control group will be necessary to help ascertain whether it is beneficial to consume the modified crackers in relation to nutritional status (BMI, Iron, Zinc and Selenium).

v. Indicate number and age range of research study/project participants/groups whose records, data or specimens shall be used in the research study/project.

- A food cracker prototype made from cassava roots and simsim seeds will be analyzed for nutrient content (calorie, iron, zinc & selenium), Microbial load (total plate count, Escherichia Coli (*E.Coli*), yeast and molds, safety status), and acceptability. Nutrient content of raw materials (cassava flour, and simsim seeds) will also be analyzed.
- 64 HIV seropositive adults aged 19-50 years of both gender, 32 will be in the Intervention group (IG) and the other 32 will be in the Non-intervention group (NG). All the 64 study participants will have their BMI, Iron, Zinc and Selenium nutritional status assessed at the beginning of the study and at the end of 22 weeks of intervention.

vi. Define inclusion criteria for each group of research study/project participants.

- HIV seropositive adults aged 19 – 50 years enrolled at Nyahera and Chulaimbo hospitals, willing to consent to participate in the study.
- Those exhibiting moderate acute malnutrition with the following indicators.

a) BMI of > 16Kg/M^2 - < 18.5 Kg/M^2,
b) Hemoglobin levels of > 8 g/dl,

vii. Define the exclusion criteria, if any, for each group.

- HIV seropositive adults aged 19-50 years who meet the inclusion criteria but are either pregnant, breastfeeding or are mentally challenged.
- Those not willing to participate/not signed consent form.

The participants not willing to participate or not meeting the inclusion criteria but are malnourished will be taken through nutritional education and counselling to advise them on the need to improve nutrient intake through diet diversity and consumption of neglected but nutritious food products. This will be done face to face on contact with the researcher and research assistants.

viii. If gender, race, or ethnicity is to be used as variables in selecting individuals' records, data, or specimens for use in the research study/project, rationale should be explained.

Individuals of both sexes with moderate malnutrition indicators will be purposively selected to participate in this study. Neither race nor ethnicity will be used as a variable in selecting study participants, only those willing to participate will be enrolled.

ix. Specify period in which these records, data or specimens shall be collected and/or stored and timeframe for entire study/project.

- The entire project will run for a period of 18 months.

x. Possible Study Biases

- Possibility of the participants in the intervention group forgetting to consume the intervention product on some days or all days. This will be addressed by daily short phone message reminders or phone calls where necessary to the study intervention group.

Activity / Year	**2023**			**2024**								**Output**
	May-Oct	**Nov**	**Dec**	**Jan**	**Feb**	**March**	**April**	**May**	**June-July**	**Aug- Sept**	**Oct**	
Writing for Approval												Seek approval and permission from

												1. The Maseno University Ethics Review Committee 2. National Commission for Science, Technology, and Innovation (NACOSTI).
Project Planning. Seek permission from Chulaimbo hospital, HIV comprehensive care centre by USAID Nuru ya mtoto, and The University of Nairobi department of food science, nutrition, and technology laboratory												Recruitment & training of research assistants. Purchase of research equipment and materials. Permission to access protected data from Nyahera and Chulaimbo hospitals. Permission to access protected data from Nyahera and Chulaimbo hospitals. Permission to carry out laboratory analysis at The University of Nairobi.
Food sample preparation												Food samples available well packaged in readiness for the study
Food sample nutrient content analysis												Data on calorie, iron, zinc, and selenium content in the processed food sample available
Baseline survey of the BMI, iron, zinc, and selenium												Baseline data on BMI, iron, zinc, and selenium status of study participants available.

status of study participants. Sampling of study participants and signing of consent.												Signed consent by study participants available
Study participants feeding on the prepared cracker. Capacity build in crop production, food product processing and entrepreneu rial skills												Short phone messages or calls Pictures of training sessions
Assessment of study participants ' BMI status and blood nutrient content at the end of feeding period.												Data on BMI, iron, zinc, and selenium status available
Data entry and analysis Manuscript write-up												Comprehensive analysed data and manuscript submitted for publication
Report writing and Submission												Well bound report available

x. Describe the plan for monitoring conduct of the study/project to ensure participants safety, confidentiality, and data integrity.

- Research assistants will be trained in data collection procedures, data handling and confidentiality.
- Food samples for intervention will be prepared following food preparation and safety standards.
- Food samples will be analyzed for food safely microbial load (Total Plate Count, *Escherichia Coli*, Yeast and Molds) before beefing fed to the participants.
- Call or send short messages to study participants daily to remind them to eat the cracker.
- Engage the research assistants in calling the participants and sending reminder short messages to remind them about consumption and for monitoring consumption of crackers by participants.
- Confidentiality and anonymity will be observed throughout the study, no name will appear on the consent form and data collection instrument, only the age, sex and phone number will appear but documents with this information will be kept confidential under lock and key.
- The information obtained from study participants will strictly be used for the purpose of this study and will not to be used to compromise their privacy and confidentiality.
- Confidentiality and anonymity will be observed throughout the study.

xi. Describe data management and analysis plan.

- Data will be checked for completeness and cleaned.
- Data will be coded and entered in the analytical software SPSS version 22. Data on Nutrient content in food samples, blood samples and BMI status will be analyzed statistically using paired sample *t-test* to compare the two groups.

xii. Describe any study/project limitations and the expected results.

Study Limitations

- Long time is needed to plant and harvest cassava (minimum 6 months), due to this, instead of planting the crops to be used for food product processing, the food crops will be procured form the market.
- Possibility of having participants in both intervention and control groups residing in the same household or same homestead or in the neighborhood. This is eliminated by sampling control and intervention group from different hospitals which are about 60km apart.

- Lack of transport and time by study participants to take part in capacity building activities. The project will facilitate transport for participants and per-diem token to the facilitators to ensure compliance with the project expectations.
- The likelihood to forget to consume the product while at home, to handle this, there will be phone calls and/or short message reminders to the participants daily reminding them to consume the food product.

Expected results.

- Calorie, iron, zinc, and selenium content of processed cracker.
- BMI and micronutrient (iron, zinc, and selenium) status of study participants before and after exposure to consuming crackers for 22 weeks.
- Food safety microbial load (Total Plate Count, *Escherichia Coli* (*E. Coli*), Yeast and Molds in the crackers.
- Sensory data on cracker acceptability levels of appearance, taste, texture, and general acceptability among study participants.
- Study participants trained in crop production, food processing and entrepreneurial skills.
- A published manuscript.
- A project report.

xiii. Provide an itemized budget necessary to conduct the research study/project and a budget justification.

Budget

Item description	**Unit**	**Quantity**	**Unit price US$**	**Cost (US$)**
A. STATIONERY				
Pencils	Pieces	10	0.45	4.5
Ball point	Pieces	10	0.25	2.5
Eraser	Pieces	10	0.2	2
Field notebook	Pieces	10	3	30
Packaging material	Pieces	10,000	0.1	1000
Disposable serving plates (white)	Pieces	100	0.5	5

Blood sample collection and storage materials & equipment				500
SUB-TOTAL				**1544**
RAW MATERIALS				
Procurement of Cassava roots	Kg	50	2	100
Procurement of Simsim	Kg	50	3	150
sugar	kg	10	3	30
Margarine	kg	10	5	50
Baking powder	kg	1	5	5
SUB-TOTAL				**335**
SAMPLE LABORATORY ANALYSIS				
Carbohydrate in cracker	parameter	3	10	30
Moisture in cracker	parameter	3	10	30
Ash in cracker	parameter	3	10	30
Lipid in cracker	parameter	3	10	30
Protein in cracker	parameter	3	10	30
Iron in cracker & 50 blood samples	parameter	3+2(50x3)=303	10	3030
Zinc in cracker & 50 blood samples	parameter	3+2(50x3)=303	10	3030
Selenium in cracker & 50 blood samples	parameter	3+2(50x3)=303	10	3030
Plate count in cracker	parameter	3	10	30
Escherichia Coli **(***E-Coli)* in cracker	parameter	3	10	30
Yeast and Molds in cracker	parameter	3	10	30
SUB-TOTAL				**9,330**
COMPUTER SERVICES				
Printing (consents, sensory)	Consent Pages	100	0.1	10
	Sensory pages	50	0.1	5
Photocopying	Copies	50 of 5 pages	2.25	562.5
Report Binding	Copies	5	5	25
SUB-TOTAL				**602.5**
PER DIEM AND ALLOWANCES FOR RESEARCH ASSISSTANTS & STUDY PARTICIPANTS				
Training 5 research assistants (2 in food processing, 1 in data collection & 2 in drawing and storage of blood samples)	Assistant per diem during training	5	50	250
	Assistants per diem during participation in the study	5 x 5 = 25 months	50	1250

Training of study participants in food processing (within month one)	Participants transport to Chulaimbo hospital	50	10	500
	Facilitators per diem	2	50	100
	Transport of participants to Maseno	2 buses	100	200
Training of study participants in food crop production (within month two)	Participants transport to Chulaimbo hospital	50	10	500
	Facilitators per diem	2	50	100
	Transport of participants to Maseno	1 bus	50	50
Training of study participants in entrepreneurship (within month three)	Participants transport to Chulaimbo hospital	50	10	500
	Facilitators per diem	2	50	100
	Transport of participants to Maseno	1 bus	50	50
Airtime	Days	84	1	84
Transporting samples to the lab in Nairobi	One way air ticket to Nrb	2 people	150	300
	Bus ticket from Nrb	2 people	20	40
Manuscript publication				400
SUB-TOTAL				**4424**
Contingency 10% of total funds (10,000 US$) to the University				1100
Grand Total				**17,335.5**

Budget Justification

- Study activities to be carried out within 12 months.

- Training of study participants to be done monthly for 3 months, and they will be transported to Maseno University for the trainings from Chulaimbo hospital where they will assemble.
- 5 research assistants will be employed to assist in food processing (2), blood sample collection, storage, and transportation (2), and data collection & compilation (1).
- Each parameter will be analyzed three times and the average recorded as the true value.
- Blood and food Samples will be transported to and analyzed at the University of Nairobi department of food science, nutrition, and technology laboratory.
- Participants will be contacted daily via mobile calls or SMS to remind them to eat the food.

Xiv. Provide copies of research study/project data collection forms to be used or a complete list of data variables that shall be collected.

Data Variables that shall be collected

1: Demographic information of study participants including BMI, iron, zinc, and selenium status.

2: Nutrient content of food product (Cracker) (moisture, ash, calorie (energy, protein, lipids), iron, zinc, and selenium) contents

3: Cracker's safety levels data (Total Plate Count, *Escherichia Coli,* Yeast and molds)

4: Cracker's acceptability data collection liker scale (taste, smell, colour, general acceptability)

5: BMI, iron, zinc, and selenium status of study participants after food intervention

6: Pictures of capacity building in crop production, food product processing and entrepreneurial skills

d. Ethical Considerations

i. A clear description of risks, if any, to research study/project participants/groups should be provided.

- No foreseen or anticipated risks.

ii. If a proposed research study/project cannot practically be carried out without access to protected health information, this must be clearly explained.

- This study will need access to protected health information about HIV seropositive clients who visit Nyahera and Chulaimbo health facilities in Kisumu County. Permission will be sought from Nyahera and Chulaimbo health facilities to allow the study to access the protected information.

iii. Identify all sources of data, records, or specimens to be used in the proposed research study/project.

- Food materials will be purchased from Jubilee local market in Kisumu town (coordinates 0.1014095, 34.75911372).
- Records of possible study participants will be obtained from Nyahera and Chulaimbo health facilities in Kisumu County.
- Blood samples will be drawn from HIV seropositive patients who attend Nyahera and Chulaimbo government health facilities.
- Food nutrient content and blood sample analyses will be done at the Nairobi University, department of food science, nutrition, and technology. Hb levels will be assessed on site at Nyahera and Chulaimbo health facilities for the two study groups.

iv. Indicate measures in place to maintain confidentiality of research study/project data and protect the privacy of research study/project participants/groups.

- Participation in the study will be voluntary and participants will be made aware that they will be free to decide to withdraw their participation before or during the study without any consequences.
- Research assistants will be trained in data collection procedures, data handling and confidentiality.
- Confidentiality and anonymity will be observed throughout the study, no name will appear on the consent form and data collection instrument, only the age, sex and phone number will appear but documents with this information will be kept confidential under lock and key.

v. Provide a description of where research study/project data/specimens being collected shall be stored.

- Food samples will be packaged and stored at Maseno University in the department of Nutrition and Health foods laboratory under safe and hygienic conditions at room temperature.

- Blood samples will be collected into trace element free vacutainer tube containing lithium heparin an anticoagulant to prevent clothing of blood samples.
- The collected blood samples will immediately be stored in a cooler box with ice pack at 4^{o}C and transported to the laboratory for analyses.

vi. Specify duration of storage and measures to be taken to ensure security of data/specimens; identify custodian and who shall have access to data/specimens.

- The food samples will be stored at room temperature for a duration of three months the period under which consumption will be taking place by study participants.
- Blood samples will be transported to the laboratory for analyses the same day the samples are drawn from study participants.

vii. Describe the plan for controlling access to stored research study/project data and/or specimens.

- Research specimen will only be handled by the researcher and research assistants.
- The researcher will collect and enter data into SPSS version 22 secured with a password for protection.

viii. If persons not listed as applicant or research study/project team member shall receive or view research study/project data with identifiers, a justification for this must be provided.

- None of the above exists.

ix. All members of research study/project team with access to confidential records, data or specimens collected must sign appropriate institutional agreement(s).

- This will be ensured by keeping the data and records under lock and key.

x. Describe potential benefits of research study/project to community and/or society.

If the study findings reveal the possibility of improved nutritional status of HIV seropositive study participants, this will be a step in the right direction towards ensuring good health among HIV patients who continuously struggle with health challenges.

Capacity building will provide important information and skills to study participants in areas of crop production, food processing and entrepreneurship. This will go a long way in improving livelihoods through reduction of micronutrient malnutrition issues, hunger elimination and income generation among study participants. This can be upscaled to other populations affected by micronutrient malnutrition issues and thus help break the cycle of malnutrition in the community.

xi. Consent issues: Describe how assent/consent will be obtained.

- Permission to carry out this study will be sought from the Maseno University Ethics Review Committee (MUERC) and National Commission for Science, Technology, and Innovation (NACOSTI).
- The researcher will seek voluntary participation of all the participants to participate in the study and obtain their consent by signing a consent form to affirm their willingness to participate.
- The researcher will explain to the participants that the study has no health-related risk, though there will be minimum invasion during blood sample withdraw.
- The information obtained from them will strictly be used for the purpose of the study and will not to be used to compromise the right of privacy and confidentiality.
- The researcher will clarify to the participants that is an academic study, there will be no rewards for participating, requires voluntary participation and they are free to discontinue from the study at any time and stage.
- Confidentiality and anonymity will be assured and observed throughout the study.

APPENDICES

APPENDIX I: INFORMED CONSENT

Study Title

Income Generation, Food and Nutrient Security for Improved Livelihoods and Health Outcomes among People Living with HIV (PLWHIV)

Statement of the Researcher

I Habwe Florence Oyiera, a lecturer at the Department of Nutrition and Health, Maseno University, I hereby request you to participate in this study. The purpose of this form is to information you about this study and allow you to either voluntarily accept to participate by signing or reject to participate. You are free to ask any question about your participation, risks and benefits involved. If you agree to participate in the study after reading all that it entails, you will be given informed consent to sign and a copy for your records.

Purpose of the Study

The purpose of this study is to evaluate the effect of consuming crackers made from groundnuts, cassava roots and simsim seeds on nutritional status of HIV seropositive adults enrolled at Chulaimbo Sub-County Hospital, Kenya. The crackers are expected to enhance consumption of important micronutrients including iron, zinc, and selenium; and enhance energy consumption

through carbohydrates. Micronutrient deficiencies and underweight are major health concerns among PLWHIV and are the leading cause of morbidity and mortality. The study participants will also be equipped with skills in cracker processing, home gardening and crop production, and basic entrepreneurial skills. The skills are meant to ensure continued and increased consumption of the cracker for sustainability. The skills will also ensure promotion and marketing of these crackers to the general population for income generation purposes. If well undertaken, this could lead to improved nutritional status, livelihoods, poverty reduction and increased income generation among the vulnerable population.

Study Procedure

The study will be carried out at Maseno University, Nyahera and Chulaimbo Sub-County hospitals, Kisumu County, Kenya. If you agree to participate in this study, we will measure your weight, height, we will take 6mls of your blood sample for laboratory analysis to check the levels of iron, zinc, and selenium. The sensory evaluation will take place at an open space within the compound of Chulaimbo hospital and will take approximately 30 minutes. Water will be provided to rinse the mouth in between the sample testing to reduce the residue material from the previous sample and avoid carrying over of effects. Drawing of blood samples (3mls at the beginning and 3mls at the end of intervention) will take at most 10 minutes, and this will take place at Chulaimbo and Nyahera hospitals. Training in cracker processing, food crop production, and entrepreneurship will be carried out at Maseno University.

Risks/ Discomforts

The study will not have any physical risk to you though there will be minimum invasion during blood sample withdraw, which will cause a little discomfort. All the information you share will only be used for this study only and will be kept confidential and not revealed to any other entity.

Benefits

Through your voluntary participation in this study, you will receive knowledge and skills in how to process crackers form cassava and simsim, you will be trained in crop production and entrepreneurship. The study findings will provide a chance for the most vulnerable people like PLWHIV and the whole community access to food products that could contribute to improve nutritional status, reduce poverty through income generation, and in the long run, improve the livelihoods of the population.

Willingness to Participate

Your participation in this study is voluntary and you may decide to withdraw your participation before or during the study without any consequences. If you decide to participate, kindly sign this form. You can use thumbprint if you do not know how to write. You will be assigned a unique identification number. The information you give will be kept strictly confidential and used ONLY for this study.

If you have any questions or if you need any additional information concerning this research, you may contact the researcher Habwe Florence Oyiera (Phone - 0713741144), or Maseno University Ethical Review Committee (MUERC), Maseno University, Kenya.

Name of the researcher: Habwe Florence Oyiera **Signature**..... **Date**.....

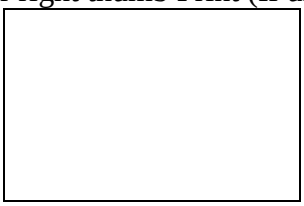
Statement of the participants

The purpose of this study has been well explained to me and I have totally understood. I consent to participate in this study. I was given the opportunity to ask questions. I have understood that my identity will be kept confidential and the information I give will only be used strictly for this research.

..... ……. .....
.....

Participant code Signature Date

Or right thumb Print (If unable to write)



Date..... (To be filled by interviewer.....

....
.....

Name of witness (If necessary) **Signature** **Date**