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HAZARD ANALYSIS AND CRITICAL CONTROL POINT PLAN FOR HAZARDS IN UGANDAN AMARANTH VEGETABLE VALUE CHAIN

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ABSTRACT

Currently, there is a high demand for amaranth due to its ability to withstand harsh climatic conditions, making it an ideal crop in the changing climate. There is also increased awareness and education on its nutritional and overall health benefits, and the availability of improved recipes. However, the presence of hazards can hinder the commercialisation of amaranth, which is in most cases traded informally. Food safety issues along the amaranth value chain should, therefore, be addressed to cope with both production and safety demands. The objective of this study, therefore, was to develop a Hazard Analysis and Critical Control Point (HACCP) plan for hazards in the amaranth value chain in Uganda. The seven principles outlined by Codex Alimentarius were followed to develop the HACCP plan. A tree diagram was further used to identify each potential hazard at each processing stage and Critical Control Points (CCPs) along the chain. For the CCPs identified, reliable control mechanisms and corrective actions were established to fulfil the requirements set by the critical limits to guarantee the safety of the products. Verification and records systems were proposed to determine the effectiveness and traceability of the HACCP plan. For each of the identified CCPs, samples were collected purposively and analysed for chemical and microbial contaminants. From the analysis, fifteen processing stages, starting from the land section to cooking and serving, were identified. Out of these, eight stages were defined as CCPs. These were site selection, land and seedbed preparation, irrigation, market display/humidity control, washing before preparation, chopping, cooking, and holding time and serving. At CCP 1, soils were contaminated with lead and cadmium, mercury and aflatoxins but at considerably low levels. At CCP 2, organic fertilisers were only contaminated with *E. coli*. At CCP3, *E. coli* was present in irrigation water. Heavy metals were also present in the irrigation water but were below the critical limits. At CCP4, *E. coli* was absent in water and display surfaces. *E. coli* was, however, present on raw amaranth. *S. aureus* was detected on vendors' hands. At CCP5, water was not contaminated with *E. coli*. At CCP6, only personnel hands were infected with *S. aureus* and *Enterobacteriaceae*. No contamination was detected in CCP7 and CCP8. Strict control of *E. coli* in manure and water and *S. aureus* and *Enterobacteriaceae* on personnel hands is required to ensure the amaranth value chain attains good food safety output.

Key words: Amaranth, food safety, prerequisite programs, HACCP plan, hazards, Uganda

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INTRODUCTION

Due to the rise of overnutrition and its health consequences in developing countries, there has been an increased public health awareness on proper nutrition. These efforts have led to more advocacy for diversified diets with highly nutritious vegetables [1]. The focus on plants has stimulated an interest in indigenous crops that have the potential to improve the nutritional status and livelihoods of millions of people living in middle and low-income countries [2]. Amaranth (*Amaranthus* spp.) is among the leafy vegetables that have been identified to offer great potential for both vegetable growers and consumers.

However, despite its nutritional value, health benefits, agronomic advantages, and potential for income generation, amaranth as a vegetable has not been fully exploited in East Africa. Lack of adequate data on its yield potential, nutrition composition, supply chain, and preparation for consumption has led to vegetable amaranth being demoted to minor crops thus not gaining much interest among researchers [3]. Moreover, the introduction of exotic vegetables such as kale (*Acephala* group) and spinach (*Spinacia oleraceae*) into the cropping system has negatively impacted the domestication, cultivation, and consumption of vegetable amaranth [4]. However, with the onset of climate change, vegetable amaranth is now seen as an ideal crop due to its hardiness [5]. As a result, there is an increased awareness of the plant, education on its nutritional and overall health benefits, and the availability of improved recipes for home preparation [1].

The increasing demand for green leafy vegetables - including amaranth - among both rural and urban populations has stimulated their production, with farmers having to increase their production to cope with the demand. This commercial pressure can hurt food safety as the demand for higher yields over-rides the need for safe food. Foods of non-animal origin that form a significant component of the human diet can pose food safety risks [6]. For example, leafy vegetables, which are regularly colonised by diverse microbiota, can become contaminated with human pathogens and parasites while growing in the field, or during harvesting, in post-harvest handling, processing, and distribution [7]. The recommendation is to implement a food safety management system (FSMS) in the fresh vegetable supply chain to minimise potential risks from food-borne pathogens [8], more so in developing countries where the vegetable supply chain is still regarded as rudimentary and informal. Prerequisite programmes, such as Good Agricultural Practices (GAP), Good Manufacturing Practices (GMP), Good Hygiene Practices (GHP) and Sanitary Standard Operation Procedures (SOPs) are therefore required to improve food safety and form a foundation for setting up an advanced FSMS such as Hazard Analysis and Critical Control Points (HACCP) [9].

Application of the FSMS is widespread in most foods and food products, but little consideration has been given to vegetable amaranth in East Africa. There is a need to guarantee safety along the supply chain of this vegetable due to several safety concerns. *Escherichia coli* (*E. coli*) and *Salmonella* spp. have previously been isolated from vegetable amaranth, where the prevalence of the latter was 11% [10]. In another study, *Streptococcus feacalis*, *Staphylococcus* spp., *E. coli*, and *Bacillus subtilis* were isolated from vegetable amaranth in both rural and urban locations [11]. Also, the growth of amaranth using contaminated irrigation water can result in an accumulation of cadmium, iron, and copper while an increase in some soils can cause accumulation of copper and lead in the crop [12]. Given the demand for vegetable amaranth in Uganda, a possible presence of hazards in its value chain may result in significant public health implications.

This study was carried out in the context of a value addition project aiming to link vegetable amaranth farmers to remunerative markets including local food processing companies. Access to prime markets depends on the producers' capacity to ensure continued supply of high-quality agricultural produce that meets set safety standards [13]. The implementation of HACCP, which is one of the widely recognised FSMS globally, can ensure food safety along the vegetable amaranth value chain. The objective of this study was to develop a HACCP plan for the vegetable amaranth value chain in Uganda. The entire production process of the vegetable amaranth was evaluated, the potential biological, chemical and physical hazards were assessed at every step of the production process, and the critical control points (CCPs) selected. The HACCP plan generated will be used by stakeholders to address the hazards along the amaranth value chain in Uganda.

METHODS

Study area

The 'farm-to-fork' HACCP analysis on the vegetable amaranth value chain commenced from producing households located in Kabubu Gayaza in Wakiso District in Central Uganda to consumers in Kyebando in Kampala, Uganda.

The HACCP plan

Principles of HACCP

In line with the Codex Alimentarius [14], we followed the seven principles outlined below in developing a HACCP plan for the vegetable amaranth value chain in Uganda.

- *Principle 1: Conduct a hazard analysis*
  The purpose of this principle is to develop a list of the potential biological, chemical or physical hazards that likely occur if not adequately controlled and assessing their severity and likelihood of occurrence at each step of the commodity system.
- *Principle 2: Determine the critical control points*
  A critical control point (CCP) is a point, step or procedure at which control actions or measures can be applied, and a food safety hazard can be prevented, eliminated or reduced to acceptable levels.
- *Principle 3: Establish critical limits for each control point*
  A critical limit (CL) is the maximum or minimum value to which a biological, chemical or physical parameter must be controlled at a CCP to prevent, eliminate, or reduce to an acceptable level the occurrence of a food safety hazard. Each control measure associated with a CCP must have an associated critical limit that separates the acceptable from the unacceptable control parameter.
- *Principle 4: Establish a monitoring system*
  This principle aims to establish monitoring procedures for each CL at each CCP. Monitoring helps to assess whether a given parameter is under control, and within the critical limit(s) specified in Principle 3. Monitoring procedures should describe how the measurement of the parameter is taken when the measurement

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is made, who is responsible for measuring the parameter, and how frequently the measurement is taken during production.

- *Principle 5: Establish a procedure for corrective action, when monitoring at a CCP indicates a deviation from an established critical limit*
  Corrective actions are the procedures that are followed when a deviation in a critical limit occurs. The corrective action is thus designed to prevent potentially hazardous food from entering the food chain and stipulates the steps that are needed to correct the process. This principle usually includes identification of the problem(s) and the indicating steps taken to assure that the problem(s) will not occur again.
- *Principle 6: Establish record-keeping procedures*
  A vital component of the HACCP plan is recording information that can be used to prove that a food was produced safely. Ideally, records should include information on the HACCP team and contact person(s), the product description, verified flow diagrams, the hazard analysis, the CCP's identified, the relevant critical limits and their monitoring system, what corrective actions are taken in case there is a deviation and the verification procedures to bring the system back under control.
- *Principle 7: Establish verification procedures*
  The goal of this principle is to establish verification procedures to demonstrate that the HACCP plan is functioning as planned. It is vital to ensure that the HACCP system is audited after a predetermined period to guarantee compliance. Both internal and external audits are necessary for the validation and effectiveness of a HACCP system.

Development of a HACCP plan

Twelve tasks, discussed in subsequent subsections, are required to develop a sound HACCP plan. These tasks were designed to ensure that the seven HACCP principles [14] were applied correctly, and are as follows:

Task 1*: Formation of the HACCP team*

The HACCP team was composed of experts and individuals consisting of: a HACCP specialist, a microbiologist, a representative of the vegetable amaranth farmers, a quality controller from the amaranth processing industry, a public health specialist, a food scientist, and a quality and safety specialist from the Uganda Bureau of Standards.

Tasks 2 and 3: *Product description and intended use*

The product description and intended use are documented in Table 1.

Tasks 4 and 5: *Development and Verification of the vegetable amaranth flow diagram*

The vegetable amaranth flow diagram (from 'farm-to-fork') was established by observation and using information provided by the HACCP team members, the farmers, the processors, and other representatives along the vegetable amaranth value chain. It was verified by interviews with major amaranth producers, sellers at trading centres, proprietors of amaranth processing industries, market and street food vendors. The verified flow diagram (VFD) of the vegetable amaranth value chain is shown in Figure 1.

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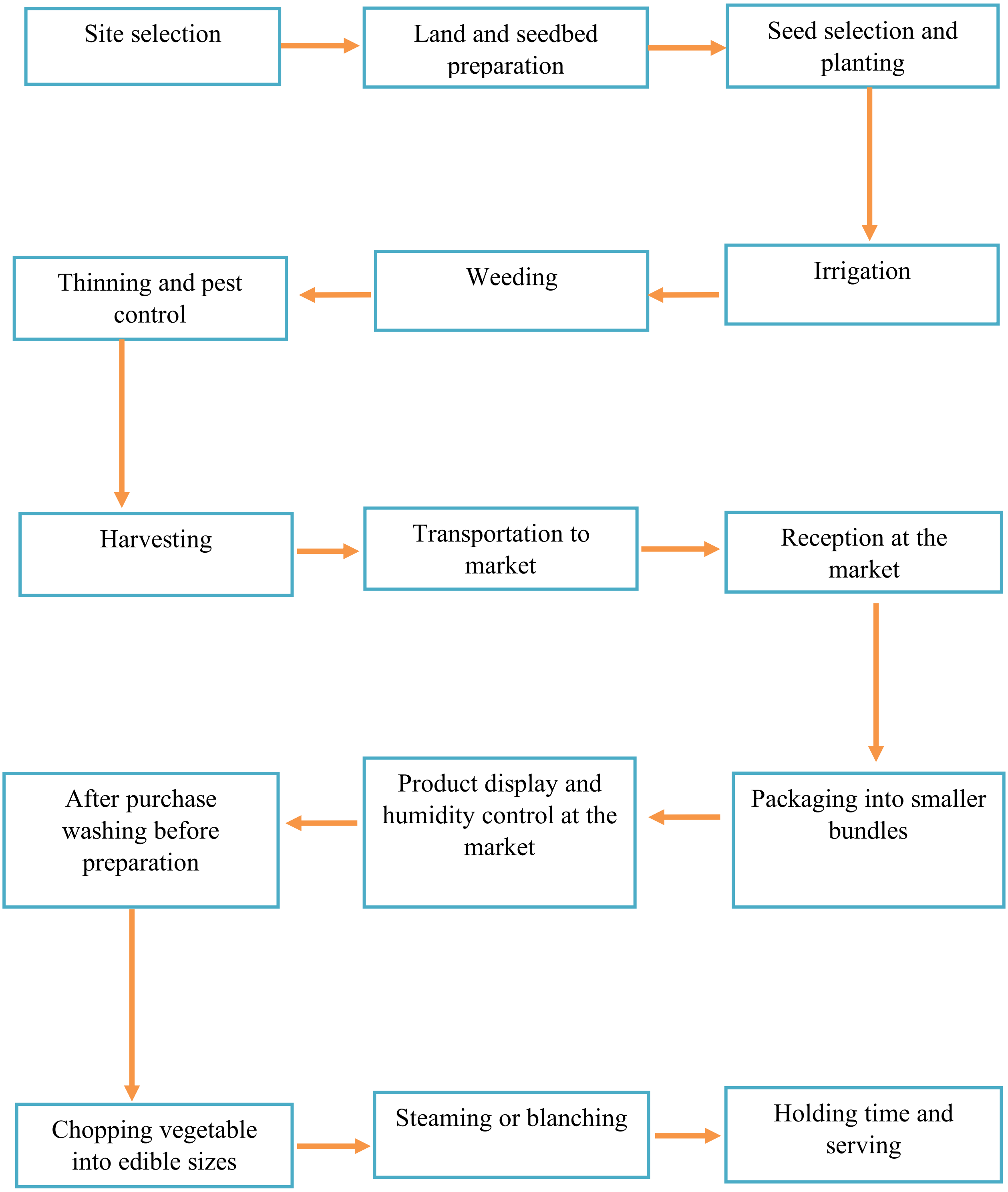

Figure 1: Verified flow diagram (VFD) of the vegetable amaranth value chain that includes 15 steps from 'farm to fork'

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Task 6: ***Hazard analysis and identification of possible control measures***
Task 6 of the HACCP plan included the listing of all potential hazards according to the VFD, conducting a hazard analysis and identifying control measures. A decision tree diagram with a risk likelihood and risk severity matrix was used by the HACCP team to identify potential hazards at each step of the VFD and to label a step as a CCP or not (Figure 2).

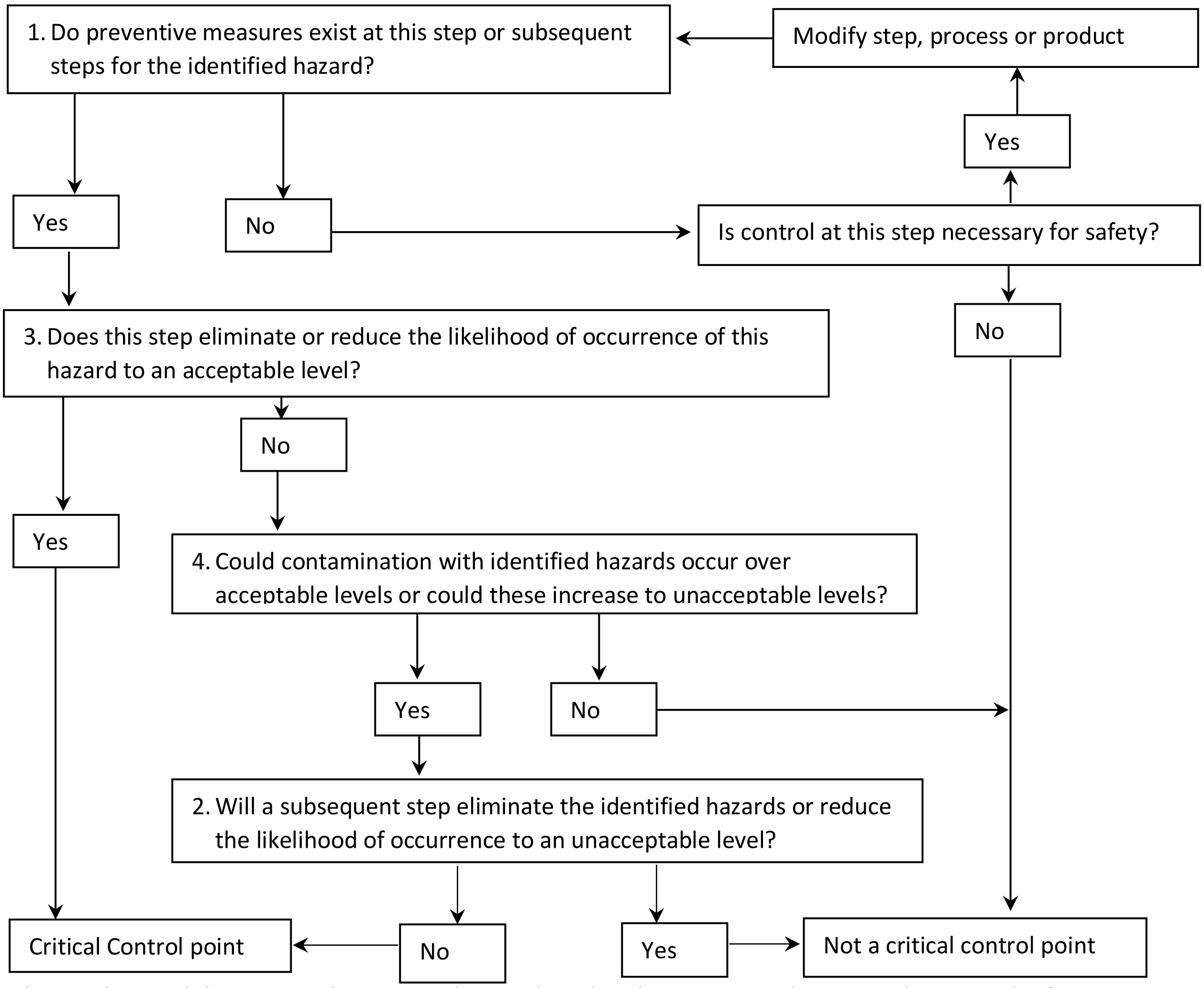

Figure 2: Decision tree diagram with a risk likelihood and risk severity matrix for CCP [14]

For the CCPs identified, critical limits for the relevant parameters were established to signify when a CCP is "in" or "out" of control. Reliable control mechanisms and immediate corrective actions were assigned to the identified CCP to ensure that in the case of a deviation, the system is brought back into control to guarantee the safety of the products. A verification system and record-keeping procedures were also proposed to ensure the effectiveness and tractability of the HACCP plan developed.

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A sampling of vegetable amaranth along its value chain and lab analysis
During the growing of vegetable amaranth, fertiliser application and irrigation are necessary to ensure high yields and year-round production. These farming practices pose a risk for microbial and heavy metal contamination. On the other hand, the high moisture content and water activity of vegetable amaranth pose a high risk for microbial contamination. Also, product preparation involves a lot of manual handling, which represents a threat of possible cross-contamination. It was, therefore, necessary to purposively sample vegetable amaranth along its value chain to assess for possible contamination.

Soil and manure were obtained from two different farms. At the farm level, water was collected from underground wells and ponds. The samples collected from markets and food establishments included; water, hand swabs, swabs of food preparation surfaces, raw and cooked amaranth. Water was analysed for *E. coli*, *Vibrio cholerae*, and *Salmonella* spp. Hand swabs were examined for *S. aureus* and *Enterobacteriaceae*. Raw amaranth, serving utensils, food contact surfaces, and knives were analysed for *E. coli* and *Salmonella*. Cooked amaranth was analysed for *E. coli*, *Salmonella* spp., *S. aureus*, and *Vibrio cholerae*.

S. aureus, *Enterobacteriaceae* and *Vibrio cholerae* were analysed using Baird-Parker agar (supplemented with 20% sterile egg-yolk tellurite emulsion), violet red bile dextrose agar and thiosulfate citrate bile sucrose agar (Merck, Germany), respectively incubated aerobically for 24 hours at 37 °C. *Salmonella* spp. were analysed using a chromogenic rapid method using Salmosyst® Broth Base, Salmosyst® Selective Supplement and Rambach® Agar (Merck, Germany) [15]. *E. coli* was analysed using a chromogenic rapid method using Chromocult® Coliform Agar and *E. Coli* Selective Supplement (Merck, Germany) [16]. Aflatoxins were tested using the modified immunoaffinity method described previously [17]. Heavy metals in soil and water samples were determined using atomic absorption spectrophotometer (Shimadzu Model 6800 with graphite furnace Model GFA 7000, Hydride unit was used for determination of mercury) as previously described [18].

RESULTS AND DISCUSSION

a) Identification of hazards
The amaranth value chain is susceptible to many risks. A list of all potential biological, chemical, or physical hazards that may be introduced, increased, or controlled from 'farm to fork' was, therefore, developed. These included mycotoxins, *Salmonella* spp., *E. coli*, *S. aureus*, insects, heavy metals, chemical residues such as pesticides, and heavy metals.

b) Identification of steps along the verified flow diagram where contamination is most likely to occur
Step 1: ***Site selection***
Heavy metals are ubiquitous in the environment, because of both natural and anthropogenic activities. Humans are exposed to them through various pathways [19]. Consuming food crops grown in contaminated soils results in ingestion of a significant

amount of the metals [20]. Thus, contamination with heavy metals and chemical residues was likely at this step.

Steps 2: ***Land and seedbed preparation***
During land preparation, the farmers use organic fertilisers. Some of these especially manure, harbour significant numbers of micro-organisms such as *Salmonella* spp. and *E. coli*. It is thought that the application of such fertilisers may act as a pathway for microbial hazards into humans, especially in vegetables and fruits [21]. These find their way through water that is splashed during rain downpours or irrigation. Contamination at this step was therefore likely.

Step 3: ***Seed selection and planting***
At this step, the seeds may be exposed to mycotoxins present in the soil. However, compared to cereals, seeds of amaranth are not as susceptible to colonisation with fungi. Contamination at this step was, therefore, considered low.

Step 4: ***Irrigation***
In the field, irrigation water is a likely source of contamination for amaranth vegetables. Water from untreated sources is particularly a significant source of pollution. Long-term wastewater irrigation may lead to the accumulation of heavy metals in agricultural soils and plants, which makes this one of the most severe environmental concern [22]. Also, such water contains high levels of coliforms, which increases levels of contamination on vegetables especially during rainy seasons [23]. Therefore, contamination at this level was highly likely.

Steps 5 to 10: ***Weeding, thinning and pest control, harvesting, transportation to, and reception at the market, and bundling for retail***
Farmers choose to apply chemicals to control weeds, pests, and diseases. Application of these chemicals, especially concoctions of pesticides, may have undesirable and harmful effects on humans [24]. However, humans are not affected by low doses of such chemicals apart from cases of long-term exposure. During harvesting, the vegetables usually are placed directly on the ground. This practice exposes them to microbial and physical contaminants that are present in the soil. Putting plants on the ground that have compost or have been under irrigation five months before harvest increases the risk of contamination with E. coli O157: H7 [25]. Contamination with *S. aureus* can also originate from the hands of the harvesters. Physical hazards including twigs and weeds can also be introduced at this stage.

Moreover, at this stage, there is increased contact between the vegetables and the handlers, especially when tying them into bundles. Inappropriate loading can also expose vegetables to soil from the roots of other vegetables. The vegetables are also exposed to these hazards when taken to the market, because of handling practices. Although risks are present in these steps, chances of contamination are low.

Step 11: ***Display in the market***
At the market, the vegetables are displayed in stalls for sale. At this step, placing the produce on dirty counters or counters cleaned with dirty water is a source of

contamination. Micro-organisms are known to not only contaminate surfaces, but they also survive on these surfaces. Their ability to survive allows pathogenic microbes to multiply to significant numbers, which increases the risk of contamination. Contamination at this step was therefore concluded to be very likely.

Step 12: ***Washing before preparation***
Untreated water is a source of both chemical and microbial hazards. These hazards include heavy metals, *E. coli*, and *Enteroccoci* spp. Contamination at this step was, therefore, concluded to be likely.

Step 13: ***Chopping leaves***
Cutting knives can be contaminated with micro-organisms such as *E*. coli. Infrequent washing of the knives increases the microbial load, which in turn increases the risk of cross-contamination. In instances where washing is frequent, use of unsafe water further increases the risk of contamination. Contact with dirty hands also increases the risk of contamination with *S. aureus* and their toxins, which are not easily destroyed by heat. Contamination at this step was, therefore, very likely.

Step 14: ***Cooking – steaming or blanching***
Time and temperature combined are essential in the control of microbial hazards. If the combination is not right, then micro-organisms and toxins that are present in a product cannot be destroyed. In addition to microbial contamination, the use of polyethene as a cover for the cooking pots may cause monomer migration into the vegetables. Given that this is the last possible control point of such hazards, this step becomes a critical control point.

Step 15: ***Holding time and serving***
Even with adequate cooking, recontamination is possible when dirty serving utensils are used. Recontamination may also occur when the food is not covered during holding. Also, long holding times at room temperature may lead to microbial re-growth where bacteria release toxins into the cooked vegetables that may not affect how the food looks, smells, or tastes but can still cause food poisoning. It was, therefore, concluded that contamination at this step was likely.

c) Possible control measures for identified hazards
The most effective ways to control contaminants when selecting a planting site is to ensure that the area chosen is not waterlogged. In waterlogged soils, some metal oxides become associated with iron and manganese and become soluble [26]. This enhanced solubility may increase uptake of the heavy metals by the crop. Other measures to avoid heavy metals contamination include crop rotation. In the case of mycotoxins, it is essential to limit intercropping with crops such as cereals that are susceptible to mycotoxins.

During land preparation, organic fertilisers can be disinfected to eliminate the threat of microbes. When using herbicides, manufactures directions for use must be strictly followed to prevent residue accumulation in the soils. During planting, only recommended herbicides may be used. Also, intercropping with mycotoxin-susceptible

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crops should be limited. Crop rotation should also be practised to enable recovery of soils from contamination. During irrigation, using potable water can reduce both microbial and chemical contamination. While controlling pests, weeds, and diseases, it is essential to follow the manufacturer's instructions when applying various chemicals. Also, these chemicals should be used at the right time to allow for a safe withdrawal period before harvesting.

Harvesting of the vegetables should be done when it is dry to reduce contamination with soil. Contact between the amaranth leaves and ground should be avoided. Instead of uprooting the whole crop, the leaves should be clipped off with clean scissors. This harvesting method will reduce the amount of soil contamination on the harvested vegetable. During transportation, handlers should avoid stepping on or sitting on the vegetables. Once in the market, the vegetables should not be placed on the bare ground. Instead, they should be placed on a raised platform. Clean potable water should be used to clean contact surfaces, knives, and vegetables. Water should not be reused to avoid build-up of micro-organisms in the water. The farm workers and market retailers should maintain high levels of personal hygiene when handling the vegetables at any of the listed steps. These include hand washing, covering of any open wounds, and wearing clean gloves when working. In case the vegetables were harvested with the roots still intact, these roots should be cut off. During bundling for retail, the vegetables should be packed in clean, well-aerated bags.

During cooking, the vegetables should be blanched or steamed at the appropriate temperature to destroy as many of the present micro-organisms as possible. The cooks should also maintain high levels of hygiene and sanitation. After cooking, the vegetables should be served immediately in clean utensils. If cooked vegetables are not be consumed quickly, they should be stored under cold temperatures. Good personal hygiene must also be observed when serving cooked vegetables.

Tasks 7 to 10: Development of a HACCP Plan the vegetable amaranth value chain
A worksheet summarising the HACCP plan for the vegetable amaranth for human consumption is given in Table 2. The development of the HAACP plan at each step along the VFD (see Figure 1) is also provided below. National and regional standards were consulted to advise on the critical limits recommended herein.

Step 1: Site Selection – Critical Control Point (CCP) 1
This step was identified as a CCP with site selection, crop rotation and limited intercropping as the control measure. This CCP will reduce the exposure of the amaranth crop to heavy metals, chemical residues, and mycotoxins to acceptable levels, hence reducing the levels of any of these contaminants in harvested vegetable amaranth. An appropriate critical limit could include avoiding pieces of land that show traces of heavy metals and chemical residues. Trained agronomists and soil scientists can best monitor this CCP.

Step 2: Land and seedbed preparation – CCP2
Disinfecting organic fertilisers and correct use of herbicides can reduce contaminants at this step. Contaminated manure can taint amaranth with Salmonella spp. and *E. coli*

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while misuse of chemical residues can result in harmful chemical residues in the crop. This step was identified as a CCP with possible control measures that include disinfection of organic fertilisers and adherence to instructions provided by manufacturers of herbicides. The critical limit at this step is set at the absence of both *E. coli* and *Salmonella* spp. in 25g of sampled fertiliser.

Step 3: Seed selection and planting – Good Agricultural Practice (GAP)

During cultivation, the crops can be contaminated with herbicides used in land preparation or mycotoxins from other plants used in intercropping. However, this can be controlled through the use of the recommended herbicides and proper crop rotation. Limiting intercropping can also be used as a control measure.

Step 4: Irrigation – CCP3

This step was identified as a CCP. Irrigation water can be a source of both microbial and chemical hazards. The control measure in this step is the use of potable water. E. coli should be absent in water used for irrigation. Acceptable range of heavy metals in irrigation water is: lead = 65µg/l; Nickel = 1400µg/l; Copper = 17µg/l; Zinc = 2000µg/l.

Steps 5 and 6: Weeding, thinning, & pest control – GAP

Weeding, thinning and pest control are all essential steps in crop management. Without these farming practices, amaranth yields can be meagre. However, these activities represent opportunities where misuse of chemicals can result in harmful chemical residues in vegetable amaranth. Therefore, it is imperative that the manufacturers' instructions and specifications are adhered to strictly. Also, the time of chemical application is of the essence. The correct use and timing of these chemicals can reduce incidences of chemical residues on the harvested crop sent to the market.

Step 7: Harvesting – GAP

At this step, placing the harvested vegetables on the bare soil exposes them to microbial and chemical contaminants. Good agricultural practices such as harvesting in the evening when there is no dew or sun reduce incidences of contamination and wilting. The harvested vegetables should be placed on a clean tarpaulin. Also, clipping of the roots with clean scissors can be a useful control measure to avoid soil contamination.

Step 8: Transportation to market – Good Hygiene Practices (GHP)

Good handling practices are required at this step to reduce contamination that can arise from stepping or sitting on the vegetables, or bringing leaves into contact with vegetable roots. Sprinkling with unsafe water should also be avoided.

Step 9: Reception at the market – GHP

At this step, the vegetables are exposed to soil contaminants if placed on the bare ground. Instead, bundles of vegetable should be placed on raised platforms. In case the plants still have their roots intact, the roots should be carefully chopped off. To prevent wilting of the produce, the vegetables can be sprinkled with potable water.

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Step 10: Bundling for retail – GHP

At this step, the vegetables are packaged for sale by separating them into smaller retail bundles. If these bundles are piled high causing the produce at the bottom of the pile to be compressed, then a micro-environment of high humidity and temperature build-up that favours microbial growth can be created within the vegetable pile. To avoid this possible contamination, the vegetables should be lightly packed in clean, well-aerated bags.

Step 11: Product display and humidity control at the market – CCP4

At this step, the vegetables are displayed in the open for the consumers to select for purchase. Sellers do sprinkle the vegetables with water to prevent wilting and in some cases chop the vegetables in anticipation of consumers or on request. These actions can be a source of contamination. To control this possible contamination, potable water should be used to keep the vegetables looking fresh. Also, the surfaces on which the vegetables are displayed should be cleaned and disinfected with food grade chemicals. The knives used for chopping the vegetables should also be washed and sanitised. The critical limits for this step include an absence of *E. coli* in water and the absence of *E. coli* and *Salmonella* spp. on knives and cutting boards or surfaces.

Step 12: Washing after purchase and before preparation – CCP5

Washing vegetables is a crucial step carried out at home, in a hotel kitchen or at an eatery. At this step, water is the primary source of contamination. For this reason, it is advisable to use potable water to wash the vegetables thoroughly. The washing water should not be reused. Preferably, running water can be used. The water should be free of *E. coli*. This step is a CCP because if microbes are introduced by use of contaminated water and the cooking method used to prepare the vegetables is not sufficient to destroy the bacteria significantly, and then food poisoning may occur.

Step 13: Chopping vegetables – CCP6

This step was identified as a CCP. Contamination is most likely to result from contaminated cutting utensils and unclean hands. Hands of personnel, cutting surfaces, and knives should be cleaned using running water to wash away microbes. The personnel can also wear clean gloves that are cleaned at a regular interval. Kitchen staff should wear hairnets and beard-nets to prevent physical contamination with hair. As critical limits, *S. aureus* should be absent on staff hands. *Salmonella* spp. and *E. coli* should be absent on meal preparation surfaces and knives. This step is a CCP because, during cooking, the preparation method used may not be sufficient to destroy the microbes to a significantly low number.

Step 14: Cooking – CCP7

Cooking was identified as a CCP. As control measures, appropriate time-temperature combinations should be used during steaming or blanching. *Salmonella* spp. and *E. coli* should be absent in cooked vegetables. *E. coli* should also be absent in water used for cooking. Other critical limits such as cooking time and temperature can be set. To limit microbial contamination in the vegetables, they should be cooked for at least 10 minutes at 90-100 °C.

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Step 15: Holding time and serving – CCP8
Food holding time and serving were identified as a CCP. Serving the vegetables in dirty utensils can result in cross contamination. Also, holding the vegetables at room or warm temperatures for an extended period - more than 2 hours - can result in microbial growth. As a control, the vegetables should be served in clean utensils. If not served immediately, they should be stored at a cold temperature to reduce the rate of microbial growth. Food handlers should also maintain a high level of personal hygiene. At this step, *Salmonella* spp. and *E. coli* should be absent in serving utensils. *S. aureus* should not be present on food handlers' hands. Other control limits such as a storage temperature of <4°C should be maintained.

Task 11: ***Establish verification procedures***
Validation procedures will be established for each of the CCPs, and the quantitative results will provide overall verification on representative samples of the soil, batches of harvested vegetable amaranth and swabs of food handlers' hands. The HACCP plan will be audited quarterly and amended as necessary.

Task 12: ***Establish documentation and record keeping***
The HACCP plan will be fully documented, with appropriate record keeping at each step.

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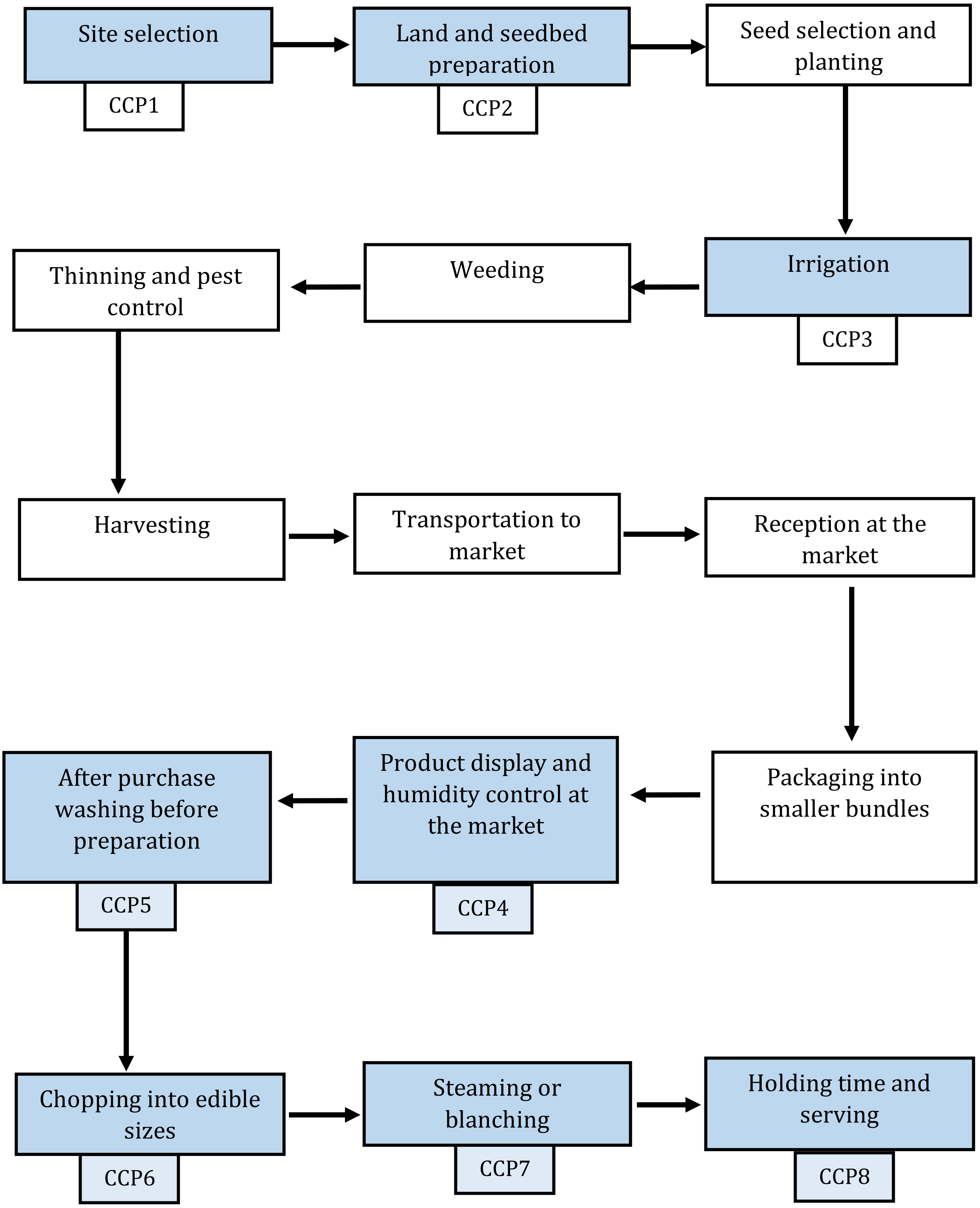

Figure 3: Modified flow diagram of the vegetable amaranth value chain with identified CCPs that includes 15 steps from 'farm to fork with eight identified CCPs highlighted

Chemical and microbiological contaminants at amaranth value chain CCPs

Table 3 shows the results of the analysis for chemical and microbial contaminants at each of the CCPs along the amaranth value chain. At CCP 1, the soil was contaminated with lead and cadmium. However, the two were not beyond the levels considered safe for human health. These levels are 400 mg kg-1 and 2.5 mg kg-1for lead and cadmium respectively [10]. These low levels of contamination may be expected because the soils in rural areas are not as highly polluted when compared to those in urban areas. Also, the soils had low traces of aflatoxin B_1 and B_2. At the current agricultural practices, hazards at CCP1 are within the desired levels.

At CCP 2, organic fertilisers were contaminated with *E. coli*. Further analysis showed that *E. coli* 0157: H7 was absent. *Salmonella* spp. was also absent. Although both *E. coli* and Salmonella are present in animal excreta [27], their ability to contaminate vegetables is mainly dependent on their survival capabilities in both the soil and manure [28]. Therefore, current conditions are not conducive for the survival of both E. coli 0157:H7 and Salmonella. However, GAP such as thermal treatment of manure may be necessary to reduce contamination with other species of *E. coli*.

At CCP3, *E. coli* was present in irrigation water, but *E. coli* 0157:H7 was absent. The presence of *E. coli* is an indication that the water may be contaminated with other gastro-intestinal pathogens, which indicates the need for strict control of the water supplied to irrigate the farms. The water can be treated with recommended chemicals such as chlorine or *Moringa oreifera* as a coagulant. Heavy metals were also present in the irrigation water but were below the critical limits. Therefore, at this CCP, microbial contamination requires strict control.

At CCP4, *E. coli* were absent in water used by vendors to keep vegetables fresh and on work surfaces. This reduced risk may be because the vegetable sellers buy their water from government-licensed suppliers who supply treated water. *E. coli* was, however, present on raw amaranth. This hazard can be attributed to the presence of *E. coli* at CCP1. Fresh produce can be contaminated by pathogens that are transferred to the edible portion of the crop (leaves) through soil splash created by rain-sized water droplets [29].

Nevertheless, although *E. coli* 0157:H7 and *Salmonella* spp. were absent on the amaranth, *S. aureus* was detected on the hands of vendors. The contamination with *S. aureus* is a significant source of food microbial contamination [30] and shows a possible risk of contamination of amaranth that may result from poor hygiene practices. Food handlers also presented a potential contamination risk at CCP 6 because their hands were contaminated with *S. aureus* and *Enterobacteriaceae*. No contamination was detected at CCP5, CCP7, and CCP8.

CONCLUSION

Eight CCPs were identified in the vegetable amaranth value chain in Uganda. These are site selection, land and seedbed preparation, irrigation, product display and humidity control at the market, washing after purchase before preparation, chopping, cooking, and holding time and serving. The high number of CCPs emphasises the risk posed by

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inadequate control measures along this value chain. Other steps were identified as prerequisite programs. Hazards identified at the CCPs include heavy metals, mycotoxins, and microorganisms. Underlying conditions that maintain food hygiene practices and good agriculture practices may be effective control measures against the hazards identified. Training of employees and stakeholders in the eight CCPs (see Figure 3) is also vital to ensure that the risks are below the critical limits. Although chemical and microbial contaminants were detected in some of the CCPs, there are indications that the current practices employed along the value chain are sufficient to lower or prevent contamination in the amaranth vegetables value chain. However, strict control of *E. coli* at CCP2 and CCP4, *S. aureus* at CCP4 and CCP6 and *Enterobacteriaceae* at CCP6 are required to ensure the amaranth vegetable value chain attains adequate food safety. A real-life video of the vegetable amaranth value chain from producers in Kabubu Gayaza in Wakiso District in Central Uganda to consumers in Kyebando in Kampala can be found at https://youtu.be/Xbkf3hr5z7o.

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Table 1: Description of vegetable amaranth available in the local market

Name of product	Vegetable amaranth for human consumption
Description	Amaranth leaves, with a moisture content of between 80 - 85 percent; They are highly perishable and susceptible to mechanical damage such as bruising. Vegetables should be stored in a well-ventilated environment.
Customer specification	For household consumption; Green light-coloured and non-wilted leaves are preferred.
Conditions of storage	Bulked in cold storage or a cool, dry, dark place. Well-ventilated polyethene bags can also be used to store the vegetable.
Shelf life	One day in a cool, dry, dark place. Two days in an airy area; or cold storage such as a refrigerator.
Intended use	Cooked before consumption; steamed, stir-fried or blanched. Can be mixed in other sauces such as groundnut sauces.
Packaging	Ventilated polyethene bags. Ventilated plastic containers.
Target consumer	Local market: rural and urban.

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Table 2: Hazard description and HACCP plan for dry beans

	Hazard description and HACCP plan		
CCP	Critical limits	Monitoring	Corrective actions
CCP1: **Site Selection**	No waterlogged sites. Minimal traces of heavy metals.	Check farmer records to establish if the site has been rotated and trash from the previous crop has been well managed. Evaluate the history of the previous crop, waterlogging history, metal deposits, and waste disposal methods. Observe the site to identify any dumping activity.	Train farmers on the importance of waste management, proper crop rotation, and the dangers of using waterlogged sites. Reject sites that are heavily contaminated with heavy metals.
CCP 2: **Land and seedbed preparation**	*Salmonella* spp. and *E. coli* must not be detected in any 25 g of fertiliser.	Analyze *Salmonella* spp. and *E. coli* in fertilizers. Check directions for proper herbicide and fertiliser Application.	Reject fertilisers that are highly contaminated with Pathogens. Train farmers in proper application of agricultural inputs.
CCP 3: **Irrigation**	The absence of E. coli in irrigated water. An acceptable range of heavy metals in irrigation water is: Pb=65µg/l; Ni=1400µg/l; Cu=17µg/l; Zn=2000µg/l	Investigate the source of irrigation water. Check records on the water quality used for irrigation.	Reject unsafe irrigation water. Train farmers in water collection techniques and on disadvantages of using contaminated water.
CCP 4: **Market display/ humidity control**	The absence of dirt in water and on working surfaces.	Check records on training in proper handling practices. Monitor the quality of potable water and check for the visual presence of soil. Check the cleanliness of working surfaces.	Train traders and food handlers in proper water, sanitation, and health practices. Discard soiled water.
CCP 5: **Washing before preparation**	There should be no visual stones, soil, foreign material, wood splinters, or glass chips. Microbial quality of washed amaranth leaves should be within the set limits.	Check records for training in Good Manufacturing Practices (GMPs) and Good Hygiene Practices (GHPs) for eateries.	Train personnel on basic GMPs and GHPs. Discard contaminated amaranth.
CCP 6: **Chopping**	There should be no visible foreign matter	Monitor hygiene of the personnel and check for the	Reject amaranth leaves if poor hygiene of sellers is

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	such as stones, nails, hair. Working surfaces must be clean, and the preparation area must be well lit.	presence of open wounds, long, or dirty nails. Check records of training on GMPs and GHPs. Examine the appearance of amaranth leaves. Check for the cleanliness of work surfaces and lighting in preparation areas.	evident and working areas/surfaces are dirty. Train the personnel in GHPs and GMPs.
CCP 7: **Cooking**	Cooking temperature should be between 90˚C to 100˚C for about 10 minutes.	Check for the efficiency of boilers or heating sources. Monitor cooking time. Check records for training on GMPs at eateries.	Reheat if the cooking time and temperature have not been reached.
CCP 8: **Holding time and serving**	Holding and serving temperature should be at least 55˚C.	Check and monitor holding and storage temperatures. Check the hygiene of the serving personnel. The hygiene of the serving equipment should be checked and frequently monitored.	If the amaranth leaves are just warm or cold, ensure that they are re-cooked to destroy the growing microbes.

Table 3: A summary of Variables and their respective measurements at each of the CCP

CCP	Sample	Variable	Level
1: Site selection	Soil	Aflatoxin b1	<0.05 mg kg^{-1}
	Soil	Aflatoxin b2	<0.03 mg kg^{-1}
	Soil	Lead	12.13 ±0.14 mg kg^{-1}
	Soil	Cadmium	0.10 ±0.03 mg kg^{-1}
	Soil	Mercury	<0.005 mg kg^{-1}
2: Land and seedbed preparation	Organic fertiliser	*E. coli*	Present
	Organic fertiliser	*E. coli* 0157	ND
	Organic fertilizer	*Salmonella*	ND
3: Irrigation	Water	*E. coli*	Present
	Water	*E. coli* 0157:H7	ND
	Water	*Vibrio cholerae*	ND
	Water	Lead	12.31 ±1.17 µg l^{-1}
	Water	Nickel	66.43 ±3.04 µg l^{-1}
	Water	Copper	3.95 ±0.19 µg l^{-1}
	Water	Zinc	705.22 ±7.23 µg l^{-1}
4: Market Display	Water	*E. coli*	ND
	Display surfaces	*E. coli*	ND
	Display surfaces	*Salmonella*	ND
	Raw amaranth	*E. coli*	Present
	Raw amaranth	*E. coli* 0157:H7	ND
	Raw amaranth	*Salmonella*	ND
	Hands	*S. aureus*	1.32 ±0.19 log cfu cm^{-2}
	Hands	*Enterobacteriaceae*	1.08 ±0.04 log cfu cm^{-2}
5: Wash water	Water	*E. coli*	ND
	Water	*Vibrio cholerae*	ND
6: Chopping	Hands	*S. aureus*	1.21 ±0.14 log cfu cm^{-2}
	Hands	*Enterobacteriaceae*	1.90 ±0.07 log cfu cm^{-2}
	Knives	*E. coli*	ND
	Knives	*Salmonella*	ND
	Chopping surface	*E. coli*	ND
	Chopping surface	*Salmonella*	ND
	Raw vegetables	*E. coli*	ND
	Raw vegetables	*Salmonella*	ND

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CCP	Sample	Variable	Level
7: Cooking	Cooked amaranth	*E. coli*	ND
	Cooked amaranth	*Salmonella*	ND
	Cooked amaranth	*S. aureus*	ND
	Cooked amaranth	*Vibrio cholerae*	ND
	Water	*E. coli*	ND
	Water	*Salmonella*	ND
8: Serving	Serving spoon	*E. coli*	ND
	Serving spoon	*Salmonella*	ND
	Plate	*E. coli*	ND
	Plate	*Salmonella*	ND

The acceptable range of heavy metals in irrigation water is: Lead =65µg/l; Nickel =1400µg/l Copper =17µg/l; Zinc =2000µg/1. All the metal averages from the lab results in CCP 3, irrigation was below the given acceptable range

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REFERENCES

1. **Afari-Sefa V, Chagomoka T, Karanja DK, Njeru E, Samali S, Katunzi A, Mtwaenzi H and L Kimenye** Private contracting versus community seed production systems: Experiences from farmer-led seed enterprise development of indigenous vegetables in Tanzania. *Acta Horticulturae*, 2013; **1007**: 671–680.

2. **Stone A, Massey A, Theobald M, Styslinger M, Kane D, Kandy D, Tung A, Adekoya A, Madan J and E Davert** Africa's indigenous vegetables. In The State of the World 2011: Innovations That Nourish the Planet. Washington, DC: World Watch Institute, 2011.

3. **Berinyuy J and D Fontem** Evaluating post-harvest opportunities and constraints. *African Journal of Food Agriculture Nutrition and Development*, 2011; **11(2)**: 4647–4663.

4. **Chelang'a PK, Obare GA and SC Kimenju** Analysis of urban consumers' willingness to pay a premium for African Leafy Vegetables (ALVs) in Kenya: A case of Eldoret Town. *Food Security*, 2013; **5(4)**: 591–595.

5. **Alemayehu FR, Bendevis MA and SE Jacobsen** The Potential for Utilizing the Seed Crop Amaranth (Amaranthus spp.) in East Africa as an Alternative Crop to Support Food Security and Climate Change Mitigation. *Journal of Agronomy and Crop Science*, 2015; **201(5)**: 321–329.

6. **Vojkovská H, Myšková P, Gelbíčová T, Skočková A, Koláčková I and R Karpíšková** Occurrence and characterization of foodborne pathogens isolated from fruit, vegetables and sprouts retailed in the Czech Republic. *Food Microbiology*, 2017; **63:** 147–152.

7. **Gil MI, Selma MV, Suslow T, Jacxsens L, Uyttendaele M, and A Allende** Pre- and post-harvest preventive measures and intervention strategies to control microbial food safety hazards of fresh leafy vegetables. *Critical Reviews in Food Science and Nutrition*, 2015; **55(4)**:453–468.

8. **Ssemanda JN, Reij M, Bagabe MC, Muvunyi CM, Joosten H and MH Zwietering** Indicator micro-organisms in fresh vegetables from "farm to fork" in Rwanda. *Food Control*, 2017; **75**: 126–133.

9. **Mahajan R, Garg S and PB Sharma** Food safety in India: a case of Deli Processed Food Products Ltd.," *International Journal of Productivity and Quality Management*, 2014; **14 (1)**: 1–20.

10. **Le Quynh Chau H, Trung Thong H, Van Chao N, Hoang Son Hung P, Van Hai V, Van An L, Fujieda A, Ueru T, and M Akamatsu** Microbial and parasitic contamination on fresh vegetables sold in traditional markets in Hue City, Vietnam. *Journal of Food and Nutrition Research*, 2014; **2(12)**: 959–964.

11. **Tsado EK, Adesina O and SB Oyeleke** A survey on the bacterial load of selected fruits and leafy vegetables in Minna Metropolis of Niger State, Nigeria. *Journal of Animal Production Advances*, 2013; **3(1)**: 6–11.

12. **Rahman MM, Azirun SM and AN Boyce** Enhanced accumulation of Copper and Lead in amaranth (*Amaranthus paniculatus*), Indian mustard (*Brassica juncea*) and sunflower (*Helianthus annuus*). *PLoS ONE*, 2013; **8(5)**: e62941.

13. **Wambui JM** Quality improvement of the Kenyan horticultural produce through standards and management. *African Journal of Food Agriculture Nutrition and Development*, 2017; **17(1)**: 1–9.

14. **FAO (Food and Agriculture Organization).** Manual on the application of the HACCP system in mycotoxin prevention and control. Rome, Italy: Food and Agriculture Organization of the United Nations, 2001.

15. **Ossmer R** Salmosyst and Rambach - agar. A rapid alternative for the detection of Salmonella. P. in Congress - Poster - Salmonella and Salmonellosis. Ploufragan/Saint - Brieux - France, 1992.

16. **Kilian M and P Bülo** Rapid diagnosis of Enterobacteriaceae**.** *Acta Pathologica Et Microbiologica Scandinavica. Section B*, 1976; **84**: 245–251**.**

17. **Lewis L, Onsongo M, Njapau H, Schurz-Rogers H, Luber G, Kieszak S, Nyamongo J, Backer L, Dahiye AM, Misore A, DeCock K and C Rubin** Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya. *Environmental Health Perspectives*, 2005; **113**: 1763–7.

18. **Ali MHH and KM Al-Qahtani** Assessment of some heavy metals in vegetables, cereals, and fruits in Saudi Arabian markets. *Egyptian Journal of Aquatic Research*, 2012; **38**: 31–37.

19. **Wilson B and FB Pyatt** Heavy metal dispersion, persistance, and bioccumulation around an ancient copper mine situated in Anglesey, UK. *Ecotoxicology and Environmental Safety*, 2007; **66(2)**: 224–231.

20. **Khan S, Cao Q, Zheng YM, Huang YZ and YG Zhu** Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environmental Pollution*, 2008; **152(3)**: 686–692.

21. **Heaton JC and K Jones** Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: A review. *Journal of Applied Microbiology*, 2008; **104(3)**: 613–626.

22. **Chauhan G and PUK Chauhan** Risk assessment of heavy metal toxicity through contaminated vegetables from waste. *International Journal of Advanced Technology in Engineering and Science*, 2014; **51(2)**: 444–460.

23. **Rai PK and BD Tripathi** Microbial contamination in vegetables due to irrigation with partially treated municipal wastewater in a tropical city. *International Journal of Environmental Health Research*, 2007; **17(5)**: 389–395.

24. **Hernández AF, Parrón T, Tsatsakis AM, Requena M, Alarcón R and O López-Guarnido** Toxic effects of pesticide mixtures at a molecular level: Their relevance to human health. *Toxicology*, 2013; **307**: 136–145.

25. **Islam M, Morgan J, Doyle MP, Phatak SC, Millner P and X Jiang** Persistence of *Salmonella enterica* serovar *typhimurium* on lettuce and parsley and in soils on which they were grown in fields treated with contaminated manure composts or irrigation water. *Foodborne pathogens and disease*, 2004; **1(1)**: 27–35.

26. **Bhattacharyya P, Chakraborty A, Chakrabarti K, Tripathy S and MA Powell** Copper and zinc uptake by rice and accumulation in soil amended with municipal solid waste compost. *Environmental Geology*, 2006; **49(7)**: 1064–1070.

27. **Liu C, Hofstra N and E Franz** Impacts of climate change on the microbial safety of pre-harvest leafy green vegetables as indicated by Escherichia coli O157 and Salmonella spp. *International Journal of Food Microbiology*, 2013; **163(2–3)**: 119–128.

28. **Franz E, van Hoek AHAM, Bouw E and HJM Aarts** Variability of Escherichia coli O157 strain survival in manure-amended soil in relation to strain origin, virulence profile, and carbon nutrition profile. *Applied and Environmental Microbiology*, 2011; **77(22)**: 8088–8096.

29. **Monaghan JM and ML Hutchison** Distribution and decline of human pathogenic bacteria in the soil after application in irrigation water and the potential for soil-splash-mediated dispersal onto fresh produce. *Journal of Applied Microbiology*, 2012; **112(5)**: 1007–1019.

30. **Wambui JM, Karuri E, Lamuka P, and J Matofari** Good Hygiene Practices among Meat Handlers in Small and Medium Enterprise Slaughterhouses in Kenya. *Food Control*, 2017; **81**: 34–39.

DOI: 10.18697/ajfand.85.17425