# A Review on Microbiological Safety of Ready-To-Eat Salads

<sup>1</sup>Garba, M., <sup>1</sup>Dandago, M. A., <sup>2</sup>Igwe, E. C. and <sup>3</sup>Salami, K. D

<sup>1</sup>Department of Food Science and Technology, Kano University of Science and Technology Wudil, Kano State, Nigeria

<sup>2</sup>Department of Food Science and Technology, Nnamdi Azikwe University Awka, Anambra State, Nigeria

<sup>3</sup>Department Forestry and Wildlife Management, Federal University Dutse, Jigawa State Nigeria

Email: maimunagarba2@gmail.com

## Abstract

Eating of road side ready-to-eat (RTE) salads that is made with green leafy vegetables (GLVs) commonly lettuce (Lactuca sativa L.), cabbage (Brassica oleracea var. capitata) or Cress (Lepidium sativum Linn.) as the key constituents coupled with adding tomatoes, sliced onions, cucumber, groundnut cake and so on has been reported to cause severe safety threats owing to its being extremely disposed to microbiologically inclined contamination, thereby leading to a public health issue. Pathogenic microorganisms pollute salads as a result of mishandling of raw vegetables, during preparation of salads and also associated environment. Soils typically harbor abundant microorganisms, some of which are human pathogens such as B. cereus, Clostridium botulinum, C. perfringens, Listeria monocytogenes and Aeromonas. Birds are carriers of pathogens such as E. coli O157:H7, Salmonella and Campylobacter. Reptiles and arthropods are usually in cultivation fields, and therefore have unrestricted access to produce and many bacterial species have evolved to exploit these animals as hosts or vectors. Insect deterioration creates openings that aid the ingress of pathogens into inner plant tissues, thereby enhancing colonization of spoilage and pathogenic bacteria on produce. Not only animals, similarly people working with the vegetables produce are known to be a source and direct contact vector of microorganisms. Hence this type of food is regarded as potential vehicles of food borne illnesses resulting in large and serious national and international outbreaks. Thus, the essence of this study is to review the safety of the RTE vegetable salads, assessing the contamination level of the most prevalent pathogenic microorganisms. RTE salads can be contaminated with different types of foodborne pathogens from farm-to-fork that makes them unsafe for human consumption. Regulatory bodies should design periodic workshop training for RTE food hawkers to help fix the problems and enhance the effectiveness of RTE vegetables salads preparation. Public awareness on the serious health risk associated with mishandled or poorly prepared minimally processed RTE foods most especially vegetable salads should be considered.

Keywords: vegetables, pathogens, source of contamination

#### INTRODUCTION

Garden-fresh fruits and vegetables play a key in healthy diet, it is well recognized in nutrition research as it ensures an adequate intake of vitamins, minerals, fibres (Hannah *et al.*, 2016) and antioxidants (Giusti *et al.*, 2010). Eating of vegetables have established much attention as diet high in vegetables are associated with a lower risk of cancer, coronary heart disease, stroke and other chronic diseases ( Dehghan *et al.*, 2011; Vereecken *et al.*, 2015). Vegetables and fruits are extremely important in human nutrition as sources of nutrients and non-nutritive food constituents as well as for the reduction in disease risks (Boeing *et al.*, 2012). The World Health Organization (WHO) and various governments have developed food guidelines and recommended a minimum of five servings of fruit and vegetables per day, frequent intake is assumed to be an indicator of healthy eating habits (Dehghan *et al.*, 2011).

New produce is popular worldwide because it is recognized as vital source of nutrients and fiber for humans (Gil *et al.*, 2017). RTE leafy salad is considered as foods that can be eat immediately at the point of sale without further preparation. RTE-salads constitute a suitable and appropriate meal for today's lifestyles because they need no cooking or further preparation. RTE vegetables are low-calorie food, they are rich in fiber and provide a great variety of vitamins, minerals, and other phytochemicals (Caponigro *et al.*, 2010; Tatsika *et al.*, 2019). Consumption of RTE salads have increased worldwide (Castro-rosas *et al.*, 2012). Similarly, their consumption is encouraged in many countries by government health agencies to protect people against a range of illnesses such as cancer and cardiovascular diseases (Cerna-cortes *et al.*, 2015).

Street foods are unprotected to abundant microorganisms during preparation, transport, preservation and distribution of foods as well as the personal hygiene of food vendors. At the same time, outbreaks of foodborne illnesses associated with the consumption of fresh produce have increased (Oluwafemi *et al.*, 2013) Subsequently, microbial adulteration can occur during any of the steps in the farm-to-consumer continuum (production, harvest, fresh-cut processing, wholesale storage, transportation or retailing and handling) and this contamination can arise from environmental, animal or human sources (María *et al.*, 2017). Raw vegetables regularly get contaminated with pathogenic microorganisms during harvesting, post harvesting handling, processing, and distribution processes. Commonly, handling practices by street vendors and the environment of presenting cut salads, added a possibility of foodborne disease occurrences (Sadiq *et al.*, 2015).

Fresh plant-origin products may be a vehicle for the transmission of bacterial pathogens inducing human diseases, for example *Escherichia coli, Listeria monocytogenes* and *Salmonella* (Giusti *et al.,* 2010; Campos *et al.,* 2015; Faour-klingbeil *et al.,* 2015; Pluta *et al.,* 2017), *Shigella spp., Staphylococcus aureus, Aeromonas hydrophila* and the spore-formers *Bacillus cereus, Clostridium botulinum* and *C. perfringens* (Faour-klingbeil *et al.,* 2015). The RTE salads are often prepared by hand and this direct contact may lead to an increased incidence of contamination with potential foodborne pathogens, such as *Staphylococcus* (Christison *et al.,* 2008; Gutierrez *et al.,* 2008). The existence and occurrence of pathogenic micro-organisms in RTE salads have been acknowledged in various studies.

Many epidemic incidence occurs due to consumption of raw vegetables contaminated by pathogenic micro-organisms, such as *Salmonella spp*, *Escherichia coli* O157 (Manhique *et al.*, 2020), and *Listeria monoctogenes* (Giusti *et al.*, 2010). Notably, the use of waste water for irrigation and poor food hygiene practices are often associated with a higher risk of food-related diarrheal cases (Amissah-reynolds *et al.*, 2020).

## Vegetables

Vegetables are eatable parts of a plant; they are those herbaceous plants whose parts are eaten as supporting food and they may be aromatic, bitter or tasteless. The consumption of leafy vegetable is part of Africa's cultural custom and they play vital roles in the customs, traditions and food culture of the African household (Hannah et al., 2016). Regular daily consumption of vegetables can assist in prevention of some diseases such as cardiovascular diseases and certain cancers (Mercanoglu and Halkman, 2011). Angelino et al., (2019) stated that the health-promoting properties of fruits and vegetables depend on numerous points, including (i) they are rich in vitamins, minerals and antioxidants; (ii) they are generally low in energy density; (iii) they are a valuable source of dietary fibre, (iv) their consumption is often associated with lower intake of unhealthy foods. Grosch, (2014) stated that vegetables play a vital role in the development of the body given their high nutritional value, are a great resource to help keeping your body healthy and help to prevent diseases such as eye diseases, cancer and cardiovascular diseases. Food insecurity, malnutrition and life-style diseases such as obesity, high blood pressure, carcinogenesis, and diabetes are among the most important global issues that have increased demand for healthy foods, especially fruits and vegetables (Mostafidi et al., 2020).

## **Classification of Vegetables**

Vegetables have been broadly characterized as (1) green leafy vegetables; (2) stem vegetables; (3) flower and immature inflorescence vegetables; (4) fruit vegetables; (5) tuber vegetables; (6) root vegetables; (7) bulb vegetables; (8) herbs and spices; (9) edible fungi; (10) fresh-cut vegetables; and (11) fermented vegetables (Grosch, 2014). From botanical point of view, vegetables can be divided into algae (seaweed), mushrooms, root vegetables (carrots), tubers (potatoes), bulbs and steam or stalk (kohlrabi, parsley), leafy (spinach), inflorescence (broccoli), seed (green peas) and fruit (tomato) vegetables (Toivonen and Brummell, 2008).

#### Green leafy vegetables (GLVs)

Leafy vegetables are crops nurtured in various part of the world, grown using various agricultural practices, and under different climatic conditions to fulfil the demand both of domestic and export market (Gil et al., 2015). GLVs that are cultivated for the consumption of their edible leaves; they include, among others, spinach, turnip, cabbage, parsley and lettuce (Jongman and Korsten, 2017). Leafy vegetables hold an important place in wellbalanced diets as they are valuable sources of nutrition in rural areas (Joshna and Lakshmi, 2013). GLVs are very nutrient-dense and incredibly healthy, they are very beneficial in providing weight loss and maintenance (Settaluri et al., 2015). GLVs occupy an important place among the food crops as these provide adequate amounts of many vitamins like betacarotene, ascorbic acid, riboflavin, folic acid and minerals such as calcium, iron and phosphorous (Kumar et al., 2020; Joshna and Lakshmi, 2013: Garg et al., 2014; Randhawa et al., 2015), carbohydrate, proteins, fibers and antioxidant properties (Garg et al., 2014; Randhawa et al., 2015). There has been an international rise in their production and consumption, aiming at promoting better human (Oliveira et al., 2019). They are cheap and readily affordable to many low-income communities in rural, peri-urban and urban areas and not only rich in macro- and micro-nutrients but also possess bioactive compounds with antioxidant potential. Therefore, they can be crucial for the food and nutrition security of poor families, especially during the drought periods (Gogo et al., 2016). GLVs have been identified as good sources of natural antioxidants such as tocopherols, vitamin C and polyphenols which are responsible for maintaining good health and protect against coronary heart diseases and cancer (Joshna and Lakshmi, 2013). They also contain an enormous range of bioactive health-promoting compounds such as antioxidants and

antimicrobial agents, which provide health benefits beyond basic nutrition (Randhawa *et al.*, 2015).

## Pathogenic microorganisms associated with RTE vegetables

Leafy vegetables such as cabbage and lettuce have been classified by the World Health Organization as a priority focus area relating to the safety of fresh produce from a global perspective. This is mainly due to the increased consumption of these raw vegetables and the frequency of disease outbreaks associated with GLVs (Jongman and Korsten, 2017). Vegetable salads are globally produced and commercialized all year and have been accompanied by new food safety threats since they are eaten raw and usually without proper cleansing practices. Thus, these products are recognized as possible vehicles of foodborne diseases, which are highlighted by large and serious national and international outbreaks (Ahmad *et al.*, 2018).

Leafy vegetables are commonly colonized by diverse microbiota and can become contaminated with human pathogens and parasites while growing in the field or during harvesting, postharvest handling, processing, and distribution (Gil *et al.*, 2015). The factors that influence the survival and/or growth of the micro-organisms on the fresh produce may include the type of organism, the commodity and environmental conditions in the field and thereafter, including storage conditions (Qadri *et al.*, 2015). The Centre for Disease Control and Prevention has described microbial contamination of ready-to-eat foods as a public health concern and usually in the developed countries due to the presence of bacteria that are of public health concern (Abakari *et al.*, 2018). Occurrences of foodborne disease have shown links between pathogens and RTE vegetable salads which carry the potential risk of microbiological contamination (Park *et al.*, 2012; Ahmad *et al.*, 2018). Outbreaks related to fresh produce include cases of *Escherichia coli* O157:H7 (lettuce), *Salmonella Typhimurium* (tomatoes, lettuce) and hepatitis A (spring onion) in Lancaster, UK (Heaton and Jones, 2008).

*Listeria monocytogenes, Salmonella,* and *Escherichia coli* O157:H7 are among the most important pathogens of concern to produce food safety ( Park *et al.,* 2012; Mogren *et al.,* 2018) and *Yersinia spp.* (Mogren *et al.,* 2018). These are all facultative anaerobic bacteria and the majority are motile. They can grow across a wide pH range, from strongly acidic (pH 2 and 3.5 for Cryptosporidium and STEC, respectively) to alkaline (pH 9–10). They have an optimum temperature for growth of between 21 and 37°C, although some are capable of growth at much lower temperatures, close to 0°C (*Y. enterocolitica* and *L. monocytogenes*). Some are widespread in the environment (*L. monocytogenes*) and most are found in the gut and faeces of warm-blooded animals (Mogren *et al.,* 2018). Outbreaks of *salmonellosis* and *listeriosis* due to consumption of vegetables have been reported. Also, *E. coli* O157:H7 and *Salmonella* lead to outbreaks associated with the consumption of pre-cooked leafy green salad vegetables (Qadri *et al.,* 2015).

RTE salads were implicated in a 2013 outbreak of *E. coli* O157:H7 in the United States of America that resulted in the hospitalization of 33 persons and no fatalities (Jongman and Korsten, 2017). *Salmonella spp.* has been detected regularly in surveys conducted on fresh vegetables at fresh-cut processing companies retail establishments in different countries worldwide (Gil *et al.*, 2017). *Salmonella* can pollute fresh produce both during the production through water, soil, insects or other animals contaminated with fecal matter, and during the preparation, through cross contamination (equipment, surfaces, food handlers) (de Oliveira *et al.*, 2019). A range of fresh vegetable products have been implicated in *Salmonella* infection, most usually lettuce, sprouted seeds and tomatoes (Heaton and Jones, 2008). *Listeria* 

*monocytogenes* are ubiquitous in the environment and can be isolated from soil, water, vegetation, the faeces of livestock and vegetation irrigated with contaminated water.

*Listeria* in the environment has the potential of contaminating fresh produce and lead to enteric infection. *L. monocytogenes* serotype isolated from salad vegetables has been shown to be serogroup 1 (Heaton and Jones, 2008). These surveys show variation in prevalence on different types of produce and between countries. *L. monocytogenes* from beansprout (85%), leafy vegetables (22%) and cucumber (80%) in Malaysia. In comparison, only 67% of cucumbers sampled in Pakistan yielded *L. monocytogenes* only from potatoes (50%) and field cress (18%) purchased at farmers' markets. *Escherichia coli* and *Enterobacteriaceae* were isolated from 35 samples of RTE lettuce salads in Mozambique by (Manhique *et al.,* 2020). The incidence of *Bacillus cereus* and *Clostridium perfringens* in 35 salad samples were determined in Johannesburg city and South Africa (Kubheka *et al.,* 2001).

Abakari *et al.*, (2018) observed 30 salads samples vended in the central business district of Tamale, Ghana. *Escherichia coli* was detected in 96.7% of salad samples with levels ranging from 0 to 7.56 log10 cfu/g. *Bacillus cereus* were present in 93.3% of ready-to-eat vegetable salads with counts ranging from 0 to 7.44 log10 cfu/g. Further, *Salmonella spp.* and *Shigella spp.* were present in 73.3% and 76.7% of salads, respectively. *Salmonella spp.* and *Shigella spp.* counts ranged from 0 to 4.54 log10 cfu/g and 0 to 5.54 log10 cfu/g, respectively. Ajayeoba *et al.*, (2015) investigated total of 555 composite samples of vegetables Cucumber (*Cucumis sativas*), Cabbage (*Brassica oleracea*), Carrot (*Daucus carota*) Tomato (*Solanum lycopersicum*) and Lettuce (*Lactuca sativa*) from 30 traditional markets in six states in Southwestern Nigeria in his study on incidence and distribution of *Listeria monocytogenes* in ready- to- eat vegetables in South- Western Nigeria. Lagos state had the highest incidence of *L. monocytogenes* contamination (55%) followed by Ondo (48.89%), Oyo (48.75%), Ogun (44.09%), Osun (34.38%), and Ekiti (33.33%) stated, respectively. Foodborne disease outbreaks of pathogens in developing countries are poorly reported or are not reported at all due to the sub-standard surveillance system in these countries (Jongman and Korsten, 2017).

**Table 1:** Typical Bacteria and Fungi frequently isolated from fresh produce including RTE salads

Bacteria	Fungi
Aeromonas spp., Bacillus cereus, Brucella spp.,	Alternaria spp., Aspergillus niger and Fusarium
Campylobacter spp., Clostridium spp., Enterobacter spp.,	spp.
Escherichia coli, Listeria monocytogenes, Pseudomonas	
spp., Salmonella spp., Shigella spp., Staphylococcus spp.,	
Vibrio spp. and Yersinia spp.	

Source: (Balali *et al.*, 2020)

#### Sources and factors affecting pre-harvest contamination of fresh RTE vegetables

#### Irrigation water

Common challenge of primary production across farming systems is the contamination of fresh produce with waterborne pathogens, arising from contaminated sources of water for irrigation (Jongman and Korsten, 2017). Being a common and essential requirement for crop production, water must be supplied to plants when necessary, and irrigation water sources are used to supplement limited rainfall in many areas (Oluwaseun *et al.*, 2018). In the farm, application of unsafe water adulterated with faecal matter and access of livestock or wild animals in the field lead to the microbial contamination when used to vegetables on the farm (Ahmad *et al.*, 2018). Pathogens causing diseases in humans such as *Yersinia enterocolitica*,

*Escherichia coli, Salmonella* spp. and *Shigella* spp. as well as opportunistic pathogens have been detected in irrigation water (Jongman and Korsten, 2017). Animal droppings and other inputs such as sewage overflow brings entero-pathogens directly to watercourses from which irrigation water may be extracted. The potential for contamination through irrigation water is increased in the developing world, as untreated waste water is used for irrigation of 10% of crops (Heaton and Jones, 2008). Study showed that pathogens such as *E. coli* O157:H7 can inhabit leaf surfaces or can penetrate into the soil when polluted water is used for irrigation (Jongman and Korsten, 2017). Application of non-mineral fertilizer, such as animal manures and slurries, abattoir wastes and sewage sludge introduce pathogens directly to the field, and run-off can contaminate irrigation water (Heaton and Jones, 2008; Cerna-cortes *et al.*, 2015).

#### Soil environment

Soils normally haven an ample consortium of microorganisms, some of which are human pathogens such as B. cereus, Clostridium botulinum, C. perfringens, Listeria monocytogenes and Aeromonas (Olaimat and Holley, 2012; Oluwaseun et al., 2018). They may, therefore, serve as a medium of plant contamination through seeds, roots or surfaces. Some of the primary sources of pathogens in soil include the use of contaminated irrigation water and manure, animal grazing, municipal solid wastes and other effluents. Soil type, soil moisture, pH, temperature, nutrient availability, agronomic practices, as well as soil biological interaction factors affecting the survival and resistance of pathogens in soil (Cerna-cortes et al., 2015; Oluwaseun et al., 2018). It has been reported that E. coli O157:H7 and Salmonella may survive in soil from 7 to 25 weeks depending on the soil type, moisture level, temperature and source of contamination (Olaimat and Holley, 2012). Pathogens may be naturally present in soil or may become incorporated in the soil matrix from organic wastes added as fertilizer. Pathogens within soil may contaminate crops directly when heavy rain or water gun irrigation causes leaf splash. The ability of the pathogen to survive in the environment will impact on the likelihood of crop contamination and pathogen viability at harvest and through to consumption. Initially, the pathogen must survive in the propagation environment until crops are planted out (Heaton and Jones, 2008).

#### Animals and insects

Apart from farm animals, whose functions as reservoirs of enteric pathogens has been kown, wild animals such as birds, reptiles, rodents, amphibians and insects like flies and beetles can also serve as vehicles of pathogens to contaminate cultivation media and produce. Birds have been determined to be carriers of pathogens such as *E. coli* O157:H7, *Salmonella* and *Campylobacter* (Gil *et al.*, 2015; Oluwaseun *et al.*, 2018). Insects are typically ubiquitous in cultivation fields, and hence, have unrestricted access to produce and many bacterial species have evolved to exploit insects as hosts or vectors. Insect deterioration create openings that aid the ingress of pathogenic bacteria on produce. Reptiles including snakes, lizards, chameleons, turtles, as well as other ophidians, saurians and chelonians have been found harboring enteric bacteria like *Salmonella* (Oluwaseun *et al.*, 2018). Contamination of leafy vegetables with pathogenic organisms of human health significance can occur directly or indirectly via animals (Gil *et al.*, 2015; Cerna-cortes *et al.*, 2015).

#### **Human Activity**

Human being working with produce is known to be a source and direct contact vector of microorganisms of significant public health concern. Hygienic practices, from land preparation, planting, weeding, and pruning, to harvest, influence whether produce becomes contaminated from direct human transfer. Personnel who come directly in contact

with fresh produce should be properly trained and advised to follow hygiene and health requirements as preventive measures (Gil *et al.*, 2015).

#### Sources and factors affecting post-harvest contamination of fresh RTE vegetables

Gil *et al.* (2017) specified that processing operations for fresh RTE vegetables involve the use of many unit operations, which can provide opportunities for cross-contamination whereby a small lot of contaminated product may be responsible for the contamination of a large lot. During fresh-cut processing, there are several risk factors that may affect the microbial safety of fresh RTE vegetables. Unhygienic postharvest handling conditions could also lead to the contamination of RTE vegetables (Ahmad *et al.*, 2018). The most relevant factors that affect the microbial safety of these commodities during fresh-cut processing include:

## **Primary Preparation**

Primary preparation includes cleaning, trimming, and coring of raw material. Mechanical or machine harvest has become increasingly prevalent and provides opportunities for increased surface contact exposure (Fallon *et al.*, 2011; Gil *et al.*, 2015). Cross contamination of lettuce can occur through contact with workers' hands (or gloves), knives and automated equipment (conveyor belt). There is a potential to increase the risk of microbial contamination due to unsanitary handling conditions (Gil *et al.*, 2015). The physical environment surrounding the produce surface in the field is considered to be inhospitable for the growth and survival of bacteria due to lack of nutrients and free moisture, temperature and humidity fluctuations (Qadri *et al.*, 2015).

## Temperature

The exact conditions of transportation, processing and retail sale in areas that may have an impact on the safety of fresh RTE vegetables are included. Many publications have described the relevance of low temperature as a strategy to avoid/reduce bacterial growth of foodborne pathogens in vegetables (Stringer *et al.*, 2012). However, maintaining a consistently low temperature throughout the processing fresh RTE salad is challenging. Temperature abuse has been pointed as a major contributing factor to foodborne outbreaks (Gil *et al.*, 2017).

## Processing

Processing of fresh produce consists of a succession of different operations where contamination and cross-contamination could occur (Gil *et al.*, 2017). The unit operations of vegetable salads are also responsible for the contamination of these products which may affect the quality and lead to safety threats (Ahmad *et al.*, 2018). The damage of the tissues during processing operations such as cutting, shredding and slicing not only makes the fresh-cut produce more susceptible to microbial attack compared to intact produce, but also causes damage to vegetable tissues and cellular structure, leading to leakage of nutrients and cellular fluids. Therefore, processing may increase microbial spoilage of fresh-cut produce due to transfer of microflora from surface to the vegetable, which acts as a complete medium for growth (Qadri *et al.*, 2015).

## Equipment

Exteriors of processing equipment and utensils have been recognized as sources of microbial contamination and recontamination. Poorly cleaned and maintained equipment can harbour microorganisms including pathogens, and provide a reservoir of contamination (Gil *et al.*, 2017). The use of contaminated utensils, packaging materials and inappropriate conditions during storage might be the cause of contamination (Ahmad *et al.*, 2018). Vegetable tissue is spoiled when knives are not well kept (Gil *et al.*, 2015). Cut surfaces exude nutrients, which

can be utilized for growth and the pathogens. *E. coli* O157:H7 and *Listeria monocytogenes* have been shown to attach to the cut surfaces of lettuce leaves and penetrate the internal tissue (Heaton and Jones, 2008).

#### Washing water

Washing is a very critical part of any produce preparation process mainly if a raw, processed fresh produce is sold as "ready-to-eat" (Gil *et al.*, 2015). Washing is a key intervention step in the processing of RTE salad to remove dirt, foreign materials, tissue fluids from cut surfaces, and microorganisms. As RTE vegetables do not undergo intensive inactivation or preservation treatments during processing, washing is the only processing step to reduce the microbial load on fresh produce. However, water use during processing of fresh RTE vegetables has been identified as a potential source for cross-contamination by enteric bacteria and viruses during washing (Gil *et al.*, 2017). Contamination due to wash water may result in increase in microbial load on the surface of produce and hence in the final products. Transfer of pathogens from wash water to vegetables becomes an important safety concern and needs to be avoided under all circumstances (Qadri *et al.*, 2015).

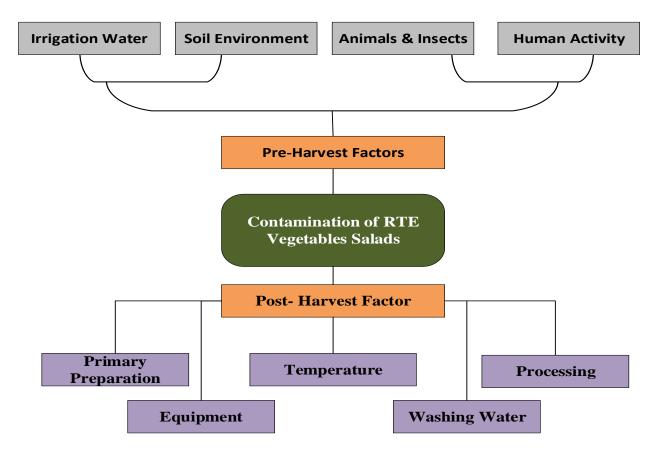


Figure 1: Flowchart for contamination of RTE salads

## CONCLUSION

Leaf salads are essential part of a nourishing and healthy universal. This food is popular due to their suitability and acceptance by consumers; they also convince food security for rural and urban population and also serve as a source of income for hawkers. In contrast the safety of RTE salads have been an issue of public health concern mainly because of the conditions under which they are grown, harvested, prepared and consumed. They can be contaminated with different types of foodborne pathogens from farm-to-fork that makes them unsafe for human consumption. Therefore, regulatory bodies should design a periodic workshop training for RTE foods hawkers to help fix the problems and enhance the effectiveness of RTE vegetables salads preparation, the government agencies should update the list of the food enterprises to be visited such a way that the visitation covers the RTE vegetable salads hawkers, public awareness on the serious health risk associated with mishandled or poorly prepared minimally processed RTE foods most especially vegetable salads should be considered, more researches should be done to adequately address the rampant emergence of foodborne illnesses with regard to RTE vegetable salads.

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