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Hygienic quality of commonly consumed vegetables, and perception about disinfecting agents in Lomé

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Abstract: Frequent cases of poisoning have been reported in Lomé, following the consumption of vegetables. This study was carried out to evaluate the microbial quality of three vegetables commonly consumed: lettuce (*Lactuca sativa*), tomato (*Lycopersicon esculentum*) and jute mallow (*Corchorus olitorius* L.). The microbiological status of 90 samples of vegetables collected in three vegetable production sites were examined on the basis of AFNOR methods and criteria. Our results revealed that all the samples were free of *Salmonella* sp. and *Staphylococcus aureus*. The microbial risk identified concerned the anaerobic sulfite-reducing bacteria, thermotolerant coliforms and moulds. A survey carried about the use of disinfectants in households revealed that 8% of consumers cleaned vegetables with water, 18% use bleach, and 22% use saline water, while 25% use a potassium permanganate solution. The issue of the present study revealed that vegetable consumers in Lomé are still exposed to anaerobic sulfite-reducing bacteria and sensitization should be conducted in the populations to emphasis the use of disinfecting agents.

Keywords: Hygien, garden products, microbial contamination, disinfectant

Introduction

The fast urbanization of sub-Saharan African cities has resulted in an increase in food demand for market gardening productions (Temple *et al*., 2005; David-Benz *et al.*, 2005). Vegetable production offers a significant opportunity by providing employment for the poor people in Lomé (Schilter, 1991; Adebooye and Opabode, 2004). Vegetables and fruits are fully recognised for their benefits towards healthy living, by their protective properties against cancer and other chronic degenerative diseases such as cardiovascular diseases and diabetes (Ragaert *et al*., 2004; Amitabha, 2005; Idogun *et al.*, 2008; Heber, 2004). The daily fruit and vegetable intake of 400 g to 600 g is recommended by the World Health Organization, Food and Agriculture Organization of the United Nations, and the World Cancer Research Fund (Pollard *et al*., 2009).

Attention on vegetables as vital dietary components is significant for sub-Saharan African populations, as leafy vegetables have long been known to be indispensable ingredients in traditional sauces that accompany carbohydrate staples (Smith and Eyzaguirre, 2007; Gueye and Diouf, 2007; Voster *et al*., 2007). However production practices, growth conditions and the location of the edible part during growth can affect their microbial status, which may affect the health of consumers. Possible sources of these pathogens are soil, faeces (manure, both of human and animal origin), water (irrigation, cleaning), ice, animals (including insects and birds), handling of the products, harvesting and processing equipment, and transportation (Aycicek *et al*., 2006; Okonko *et al*., 2008; Amoah, 2005; Beuchat, 2002; Damen *et al*., 2007; FAO, 2007).

Most of raw vegetables are normally consumed without being cooked, so the possibility of food poisoning exists (Aycicek *et al*., 2006). Unfortunately, the increase in consumption has been correlated with an increased frequency of outbreaks of illness associated with raw fruits and vegetables (McMahon and Wilson, 2001; Bhagwat, 2004). Previous investigations have shown that efficacy of washing and sanitizing treatments reduce microbial populations on fresh vegetables (Gil *et al*., 2009; Alvaro *et al*., 2009).

In the developing countries, the diarrheic diseases of food or hydrous origin kill 2.2 millions people annually (FAO, 2007). The incidence of these diseases has increased, despite the introduction of preventive quality systems such as the HACCP concept and the promulgation of regulations in food safety (Nguz *et al*., 2005). Frequent food born diseases have been reported in Lomé, following the consumption of vegetables. The purposes of this study was to evaluate

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the microbial quality of three vegetables among those commonly consumed in Lomé and moreover to find out the disinfecting agents used by households to treat vegetables.

Materials and Methods

Samples collection

The study was conducted on three commonly consumed vegetables in Lomé: *Lactuca sativa* L. (lettuce), Asteraceae, *Lycopersicon esculentum* Mill. fruit (tomatoes), Solanaceae and *Corchorus olitorius* L. (jute mallow), Tiliaceae. A total of 90 samples were collected from gardens on the littoral (Agblogamé, Baguida and harbour area) in eastern Lomé. Each sample was placed separately in a sterile plastic bag and taken immediately to the laboratory. For lettuce, only aerial part was used for bacteriological analyses.

Microbiological analysis

The microbiological procedures used to analyze vegetable were those recommended in the standardized routine methods adopted in the UEMOA countries (West African Economic and Monetary Union). These analyses related the following germs enumaration: total aerobic flora, total coliforms, thermo-tolerants coliforms, anaerobic sulfite-reducing bacteria, *Staphylococcus aureus*, fungi and *Salmonella* spp. French Association for Normalisation criteria concerning frozen vegetables were used to appreciate the conformity of the analysed samples.

For microbiological purposes all media were purchased from Biomerieux (France). Microbial enumeration was performed as follows: 10 grams of each sample were crushed in 90 ml tryptone salt in aseptic conditions. Decimal dilutions up 10^{-1} to 10^{-5} were prepared from these suspensions. One milliliter of each dilution was used for cell enumeration. Total aerobic bacteria were determined with Plate Count Agar after 24 hours incubation at 30°C. Total coliforms and thermotolerant coliforms were enumerated on Violet Red Bile Lactose after 24 hours incubation at 30 and 44°C, respectively. Chapman agar was used for *Staphylococcus aureus* enumeration. This was made by counting coagulase positive colonies after 24 hours incubation at 37°C. The amount of Sulphite Reducing Bacteria was assessed by Most Probably Number with tryptone-sulfite neomycin broth after 20 hours incubation at 44°C. Sabouraud-Chloramphenicol agar was used for the isolation and identification of Yeast and moulds. Plates were incubated for 3 to 5 days at 30°C. Pure isolates of mould were identified by using macroscopic and microscopic characteristics of the colonies and the germs. For *Salmonella* spp., Buffered Peptone Water was used for preenrichment at 37°C for 24 hours; afterwards enrichment at 37°C for 24 hours was made with Rappaport Vassiliadis soya Broth prior to isolation and counting on Hektoen and SS agar at 37°C (24 hours).

Survey on vegetables disinfection in households

The survey was carried out between July and August 2009 in Lomé. Ten districts were covered by the investigation (Agoè, Adawlato, Bè, Gbossimé, Kodjoviakopé, Nyékonakpoè, Hanokopé, Hédzranawoé, Adidogomé, Adakpamé). Information on disinfecting agents used by households was gathered by individual interviews in 30 vegetable sale points (markets, corners of street, vegetable gardens) using questionnaire. Questions were about the treatment of vegetables before their consummation; the disinfecting agents and the applied doses.

Results

Microbiological quality of vegetable samples

Ninety samples of vegetable were analyzed in this study. The following microorganisms were enumerated: total aerobic bacteria, total coliforms and thermotolerant coliforms, *Staphyloccocus aureus*, aerobic sulfite reducing bacteria (ASRB), yeast and moulds and *Salmonella* species. The assessment of microbiological quality according to the criteria used showed the following results: neither *Salmonella* species nor *Staphyloccocus aureus* were isolated from the vegetables examined. Figure 1 displays the percentages of conform vegetables as a function of microorganisms. In accordance with the AFNOR limits (Table 1), the samples were much contaminated by total aerobic microorganisms, total coliforms and thermotolerant coliforms, especially for *Corchorus olitorius* L. whose conformity concerning these germs was null. Referring to ASRB, the hygienic quality was also unsatisfactory for the three vegetables, and the conformity values ranged from 47 to 87%. Referring to Yeast and moulds conformity of the analyzed products were from 87% to 100%. The identified moulds included *Aspergillus flavus*, *Aspergillus nidulens*, *Aspergillus niger*, *Mucor* sp and *Rhizopus*.

Perception of the consumers on the use of disinfecting agents

Table 2 displays the number of respondents in relation with the liquid they use to wash their vegetables. According to the table, 8% of the

Table 1. AFNOR limits for vegetables

Microorganisms	Criteria
Flora Total Aerobic (30ºC)	5.10^5 cfu/g
Total coliforms (30ºC)	1000 cfu/g
Coliform Themo-tolerants (44 ºC)	15 cfu/g
Anaerobic sulfite-reducing bacteria	10 cfu/g
Staphylococcus aureus (37°)	10 cfu/g
Moulds (30ºC)	1000 cfu/g
Yeasts (30ºC)	500 cfu/g
The *Salmonella*	0cfu/25g

Values are the number of colony forming units per gram of sample, in the case Salmonella the criteria does not allow any bacteria in 25 g of sample.

Table 2. Percentage of respondents using a particular agent to wash their vegetables

Disinfecting agents	Respondents (n=384)
Water	8.07
NaOCl solely	17.70
$KMnO_4$ solely	25.00
NaCl	22.14
NaOCl or $KMnO_4$	3.65
NaOCl or NaCl	4.69
$KMnO_4$ or NaCl	7.03
$KMnO_4$ or NaOCl or NaCl	4.69
Vinegar	5.21
Lemon	1.04
Liquid soap	0.78

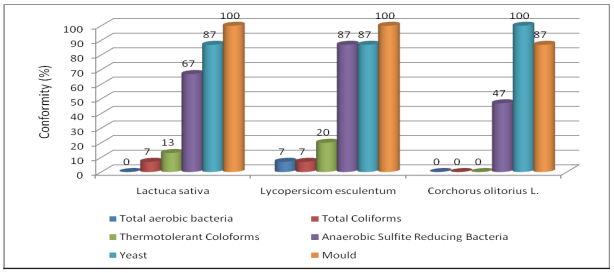

Figure 1. Compliance compared with each germ

surveyed people use solely water for the washing; indeed, more than 92% of the respondents think that it is necessary to disinfect vegetables before their consumption. They use various products such as sodium hypochlorite, potassium permanganate, salt, lemon, vinegar and liquid soap. The results revealed that 25% and 18% of surveyed people treated their vegetables with $KMnO_4$ and NaOCl, respectively. Salt (NaCl) was also used by a significant proportion (22%) as disinfectant, while 21% of the surveyed people used in a non-exclusive way or sometimes a combination of the three products ($KMnO_4$, NaOCl and NaCl). A small proportion of the consumers use vinegar, lemon and liquid soap (5%, 1% and 1%, respectively).

Discussion

The present study aimed to assess the microbiological quality of common vegetables in Lomé. According to our results, all the analyzed vegetable samples were of satisfactory quality referring to *Staphyloccocus aureus* and *Salmonella* sp, but they were contaminated by the other germs. Studies carried out in Spain and Italy respectively by Abadias *et al*. (2008) and Caponigro *et al*. (2010) revealed no case of salmonellosis linked to the consumption of fresh-cut vegetable. The main infectious risks identified in these vegetables produced in Lomé gardens were especially the anaerobic sulfite reducing bacteria which may survive to heart treatment, since some of these germs particularly *Clostridium* are spore producing bacteria. The main species of the genus that are mostly implicated in food born diseases are *Clostridium botulinum* and *Clostridium perfringens*. In the United Kingdom and United States, *C. perfringens* bacteria are the third-most-common cause of food-borne illness, with poorly prepared meat. Their enterotoxin mediating the disease is inactivated at 74°C but can be detected in contaminated food, if not heated properly. Neurotoxin production is the unifying feature of the species *C. botulinum*. Seven types of toxins have been identified and allocated a letter (A-G). Most strains produce one type of neurotoxins but strains producing multiple toxins have been described (Satterfield *et al*., 2010). Botulin toxin produced by *C. botulinum* is often believed to be a potential bio weapon as it is so potent that it takes about 75 ng to kill a person, 500 g of it would be enough to kill half of the entire human population.

In the present study, the Thermotolerant coliforms counted remained of tolerable level fixed by the criteria. Previous study by Sackou *et al*. (2006) reported similar findings, suggesting fecal contamination of lettuces cultivated in the districts of Abidjan. These results also corroborated those found by Amoah (2005) and Kozan *et al*. (2005) whose data emphasized that if washing and disinfecting procedures of the raw eaten vegetables are neglected, they can be important vehicles of transmission human enteric pathogens and helminthes. Presence of moulds on vegetables constitutes also a risk because of the mycotoxins which they can produce and which are harmful to health of consumers. Possible sources of these pathogens result from farming methods used (Aycicek *et al*., 2006; Okonko *et al*., 2008). The sources of microbial contamination may be the gardening sites where the samples were taken. Indeed, on considerable vegetable gardens of Lomé and its suburbs, the owners use water of doubtful origin, and the organic fertilizers such as cattle faeces, domestic waste are incriminated (Schilter, 1991. Previous work carried out by Kokkinakis *et al*. (2007) in Greece and Amoah (2005) in Ghana showed that the microbiological quality of vegetables depends firstly on the quality of irrigation water, soil and organic manure.

Disinfection aims at removing the microorganisms from vegetable. The investigation about using of disinfecting reveals that the majority of subjects (92%) used one or plural disinfecting agents in order to reduce the microbial activity of vegetables. But by

ignorance, an important proportion among this group used some products which have not antimicrobial activity i.e. salt, vinegar, lemon and liquid soap. The most common method used to reduce the microbial activity of fruits and vegetables is the disinfection of washing water by chlorination (Alvaro *et al*., 2009). Previous investigation (CSNEJ, 1988), indicated that the hypochlorite of sodium is bactericidal, fungicidal, sporicide and virucide; and its use improves hygienic quality of the fresh vegetables. Another precaution to limit the risks of infection coming from vegetables would be to wash them with potassium permanganate. However, in the United States (CFSAN, 1998) Seo and Frank (1999) specified that the microorganisms can penetrate the lesions of vegetable's tissues and remain inaccessible to disinfecting. The results of their study on *Lactuca sativa* showed that the effectiveness in baths of chlorine remains limited to the surface; and microorganisms infecting tissues are not completely eliminated. Eight percent the surveyed people don't use any disinfecting agent for vegetables. In Abidjan, this category of consumers reached 44% (Sackou *et al*., 2007). This situation reflects the low level of sensitizing concerning the adequate hygienic practices. Most of infections risks from vegetables can be reduced through disinfection during kitchen processes (Hamilton *et al*., 2006).

Conclusion

This study showed that vegetables produced in Lomé represent a microbiological risk for consumers, and moreover, during the washing, many of those consumers use products which have no antimicrobial activity. It is therefore urgent to sensitize the consumers, especially the restorers about vegetable contamination problems. Further studies could be recommended to evaluate the development of resistance of the concerned microorganisms frequently identified on the vegetable.

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