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Correlation between Time of Harvesting and Duration before Cooling on the Microbial Quality of French Bean (Phaseolus vulgaris L.)

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Abstract: French bean is a major export crop from Kenya into United Kingdom and Europe. It is usually consumed cooked. Food safety is one of the most important factors that influence success of fresh produce export. Microbial contamination is one of the food safety concerns in fresh produce industry. This study focused on understanding the correlation between harvesting time and duration before cooling on the microbial quality of French beans. To assess the microbial quality of French beans, samples of French beans were harvested at different times of the day, 7am, 9am, 11am , 1pm and 3pm. The harvested beans were then held in the produce shed before start of cooling for various durations; 0 hour, 2 hours, 4 hours, 6 hours and 8 hours. The samples were later graded and packed in modified atmosphere packaging (MAP) bags. The samples were analyzed following standard plate technique to determine the microbial qualities of French bean samples. Total viable counts (TVC) in these samples showed mean values ranging from 0.7 to 3.3×10^5 CFUs g-1 for total *Enterobacteriacea, Listeria monocytogenes*, moulds and *Staphylococcus aureus*. Of the microorganisms isolated, *Enterobacteriacea* (71.6%) was the highest, followed by *Staphylococcus aureus* (20.9%), Moulds (7.2%) and *Listeria monocytogenes* at 0.3%. The harvesting time and duration before cooling had significant effect on population of microorganisms with those harvested early in the morning having the highest population. The high presence of microbial load in samples harvested early morning can be attributed to poor personal hygiene of the food handlers and excessive leaf wetness in the morning. There is need to do further research on the effect of solar radiation on the growth and survival of Listeria monocytogenes, it was evident that the Listeria population decreased significantly with every delay in harvesting, which could also be attributed to dry leaf and pod surfaces. French bean contamination in the field may result from various sources, ranging from soil, irrigation water, contaminated harvesting tools and equipment and contamination from harvesters and food handlers. It is therefore important to know the possible sources of contamination and put in place measure to eliminate or reduce contamination. Hygiene practices like hand washing after using the toilet, before handling produce and avoiding touching of face, skin and nose can be useful in reducing contamination from food handlers. Use of gloves to protect hands during harvesting is necessary to reduce contamination.

Keywords: Harvesting time, Duration before cooling, *Phaseolus vulgaris* L., Microbial quality, Totall Viable Count, *Enterobacteriaceae, Listeria monocytogenes, Staphylococcus aureus,* Food Safety, Hazard Analysis Critical Control Points (HACCP)

1.0 Introduction

French bean (*Phaseolus vulgaris* L.) is cultivated in many parts of the world for its beans which can be harvested and consumed when immature or when shelled and dried (Kasim and Kasim, 2014). Green beans are generally harvested at physiologically immature stage and at this time the growth is rapid and beans exhibit comparatively high respiration rate even at low temperature (Kasim and Kasim, 2014). With conducive conditions, a number of important microbes may contaminate fresh produce thus causing faster deterioration. Physical and physiological changes in viability and quality of the fresh produce are as a result of the wounds associated with production and processing. Potential sources of contamination can be at the preharvest stage, and these include soil, irrigation water, manure, and even domestic animals (Likotrafiti *et al.*, 2013; Buyukunal *et al.*, 2015)

Fresh fruits and vegetables are heavily infested with various types of microorganisms from the soil that are problematic since they are raised in soil thus are subjected to greater risks of contamination. Fresh

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vegetables have fleshy tissues that provide substrates and conditions that are conducive for the growth and survival of various microorganisms (Luo *et al.*, 2010). The internal watery plump tissues are nutrient rich and have neutral pH enhancing the possibilities of microorganisms' exploit of the host by degrading the polymers to release water and other intracellular constituents for use as nutrients for their growth. Fungi produce extracellular pectinases and hemicellulases that are important fungal spoilage (Miedes and Lorences, 2004). The fungi are able to colonize healthy, undamaged plant tissues by gaining entry either through the calyx (flower end) during flower development, along the stem, or through natural openings. However, spoilage microorganisms can be introduced to the crop through the seed, during crop growth in the field, during harvesting and postharvest handling, or during storage and distribution.

Soilborne microbes found on produce are also present at harvesting on handling equipment, in the storage facilities, and on food contact surfaces throughout the distribution chain. Therefore, early intervention measures during plant growth and at harvesting provide drastic reductions in yield loss due to microorganisms' spoilage. Cold storage is an excellent way to preserve fresh vegetables to retain valuable sensory attributes and nutritive properties. However, cold storage only slows but does not stop enzymatic and microbial degradation that causes the development of off odors and flavors, color and texture changes, and nutrient loss during long-term storage. Microbiological quality of any produce is important, however, assuring that a product is of the highest microbiological quality is often difficult. Therefore the present study was conducted to investigate the microbial quality of French beans harvested at different time during the day and subjected to different precooling durations.

2.0 Materials and Methods

2.1 Sample Collection

The samples of French bean used in this study were obtained from Naivasha, which is located at Latitude 0° 43′ 0″ S, and Longitude 36° 26′ 0″ E, a leading region in horticultural production for export and local markets in Kenya. French bean pods were harvested from a commercial planting at five different times during the day 7am, 9am, 11am, 1pm, and 3 pm. The harvested pods were later subjected to different pre-cooling durations of 0 hours, 2 hours, 4 hours, 6 hours and 8 hours. The beans were harvested in a perforated tray and later moved to a field shed constructed from iron sheet roofing. The harvested beans were later packed into netted bags and weighed.

2.2 Determination of microbial population in French bean samples

The standard plate count technique was used to determine the microbial population in the samples. The fresh French bean samples were cut into 1-2 cm pieces with a sterile scalpel and crushed then 1g of the sample was mixed with 9 ml of distilled water and shaken properly. Using a fresh pipette at each stage, a serial dilution was carried out to 10^4 dilution i.e. 1ml of the solution was drawn out using a pipette and was introduced into a second test-tube containing 9ml of distilled water and this was done serially until the last tube with 10^4 dilution was attained. Sterile petri dishes were set out for the last dilution. Using sterile pipette for each 1ml of each dilution was pipetted into the centre dishes. About 15ml of Potato dextrose agar and nutrient agar was poured into each plate. The medium was allowed to solidify, then inoculated and incubated at 37ºC for 48 hours. The numbers of colonies were counted with the use of a colony counter. The colony count was calculated by multiplying the number of colonies counted by the dilution factor

2.3 Enumeration of Molds

Laboratory analyses of molds were performed in accordance with the ISO 7954:1987 standard (Anonymous, 1987).

2.2. Data analysis

Microbial counts were transformed to logarithms before computing means and population densities were reported as log cfu g^{-1}. Data from microbiological analyses were evaluated by analysis of variance using Genstat Statistical Software, Release 15 and the level of significance for all tests was 0.05 by Tukey's honestly significant difference test ($p \leq 0.05$).

3.0 Results

3.1 Effect of harvest time and duration before cooling on the total viable count of microbes on French bean

The changes in population of total viable counts following different harvesting times and time to cooling are presented in table 1. Total viable counts in these samples showed mean values ranging from 0.7 to 3.3×10^5 CFU g^{-1} for total *Enterbacteriaceae*, *Listeria*, molds, *Staphylococcus*. Significant differences were observed ($p \leq 0.05$) in microbial counts from French bean harvested at different times and stored for various times before cooling (Table 1). French samples harvested early in the morning had the highest total viable counts (CFU g^{-1}) .In general, French bean samples harvested at 7am had the highest population of total viable of all the microorganisms while those harvested at 1pm had the lowest total viable counts when compared with other harvesting times.

Table 1: Total plate count (10^5) of French bean samples harvested at different times.

Harvesting time	Time to cooling					Mean
	0 hours	2 hours	4 hours	6 hours	8 hours	
7am	4.8[a]	0.1[c]	2.3[a]	5.5[a]	4.1[a]	3.3±1.0[a]
9am	2.7[b]	0.3[bc]	0.2[c]	3.0[b]	0.4[c]	1.3±1.0[c]
11am	4.9[a]	4.0[a]	0.1[c]	3.0[b]	0.3[c]	2.4±1.0[a]
1pm	0.0[d]	0.5[b]	0.9[b]	1.9[c]	0.1[c]	0.7±1.0[c]
3pm	1.9[c]	0.1[c]	1.1[b]	0.9[d]	3.2[b]	1.4±1.0[c]
Mean	2.8	1.0	0.9	2.9	1.6	1.8
LSD ($p \leq 0.05$)	0.3	0.3	0.3	0.3	0.3	1.3
CV (%)	11.0	11.0	11.0	11.0	11.0	11.0

Means within column followed by different letters are significantly different based on Fishers Protected LSD test ($p \leq 0.05$).

3.2 Effect of harvesting time and duration before cooling on population of *Enterobacteriaceae* and *Staphylococcus aureus* on French bean

Different harvesting time had significant ($p \leq 0.05$) effect on the population of *Enterobacteriaceae* and *Staphylococcus aureus* isolated from Fresh bean samples (Table 2). Samples harvested early in the morning had the highest population of both *Enterobacteriaceae* and *Staphylococcus aureus*. French bean samples harvested at 9.00 am and 1.00 pm had the lowest population of *Enterobacteriacea* and *Staphylococcus*, however, the population increased after the second, fourth and sixth hour of holding before cooling but then reduced after the eighth hour of holding before cooling. In general, for *Staphylococcus* the population was highest following 7am harvesting followed by those samples harvested late in the evening. For *Enterobacteriaceae*, the population was highest following 7am harvesting followed by those samples harvested at 1.00 pm.

The changes in population of *Listeria monocytogenes* counts following different harvesting times and duration before cooling are presented in the Table 2. *Listeria monocytogenes* counts in these samples showed mean values ranging from 68 to 453 CFUs g^{-1}. Significant differences were observed ($p \leq 0.05$) in the population of *Listeria monocytogenes* from French bean harvested at different times and stored for various times (Table 3). French bean samples harvested early in the morning had the highest density of *Listeria monocytogenes* counts (CFU g^{-1}). However, the population continued to rise during the holding period before cooling up to 970 CFU g^{-1}. At the same time samples harvested late morning (11am) had the lowest CFU g^{-1} for *Listeria monocytogenes*. In general, samples harvested early in the morning had the highest microbial population compared with other harvesting times.

Table 2: Effect of harvesting time and duration before cooling on the population of different bacterial spp isolated from French beans

Harvesting time	Time to cooling					
Staphylococcus	0 hours	2 hours	4 hours	6 hours	8 hours	Mean
7am	29452.0[a]	3519.0[b]	17656.0[b]	6654.0[c]	20654..0[a]	15587.0[a]
9am	3009.0[d]	1496.0[b]	2976.0[c]	12189.0[b]	2573.0[b]	4449.0[d]
11am	26257.0[b]	17563.0[a]	1749.0[c]	9038.0[c]	3146.0[b]	11551.0[b]
1pm	1051.0[d]	1720.0[b]	15101.0[b]	23150.0[a]	1530.0[b]	8511.0[c]
3pm	7183.0[c]	2237.0[b]	29617.0[a]	6493.0[c]	18500.0[a]	12806.0[a]
Mean	13390.0	5307.0	13420.0	11505.0	9281.0	10581
LSD ($p \leq 0.05$)	2805.4	2805.4	2805.4	2805.4	2805.4	2805.4
CV (%)	16.2	16.2	16.2	16.2	16.2	16.2

Enterobacteriaceae						
7am	123136.0^{b}	445.0^{b}	29102.0^{b}	7102.3^{c}	20803.0^{b}	36118.0^{b}
9am	6871.7^{c}	400.0^{b}	4026.7^{c}	3959.7^{cd}	565.0^{c}	3164.6^{b}
11am	19686.0^{a}	1057.7^{a}	1483.3^{c}	59677.0^{b}	399.0^{c}	16461.0^{c}
1pm	150.0^{d}	1300.3^{a}	51505.0^{a}	112501.0^{a}	420.3^{c}	33175.0^{b}
3pm	11867.0^{b}	360.0^{b}	2470.0^{c}	1490.0^{d}	445467^{a}	92331.0^{a}
Mean	32342	712.6	17717.0	36946	93531	36250
LSD ($p \leq 0.05$)	3213.4	3213.4	3213.4	3213.4	3213.4	3213.4
CV (%)	5.4	5.4	5.4	5.4	5.4	5.4
Listeria						
7am	191.3^{a}	211.7^{a}	391.7^{a}	501.7^{a}	970.7^{a}	453.4^{a}
9am	41.7^{d}	121.0^{c}	73.7^{c}	219.7^{b}	20.0^{d}	95.2^{c}
11am	79.0^{c}	46.0^{e}	71.0^{c}	136.0^{c}	45.7^{c}	75.5^{d}
1pm	50.7^{d}	80.3^{c}	80.3^{b}	110.3^{c}	19.6^{d}	68.3^{d}
3pm	113.0^{b}	195.0^{b}	315.0^{b}	71.0^{d}	151.3^{b}	169.1^{b}
Mean	95.1	130.8	186.3	207.7	241.5	172.3
LSD ($p \leq 0.05$)	5.6	5.6	5.6	5.6	5.6	5.6
CV (%)	2.0	2.0	2.0	2.0	2.0	2.0

Means within column followed by different letters are significantly different based on Fishers Protected LSD test ($p \leq 0.05$).

3.3 Effect of harvesting time and duration before cooling on population of molds on French bean

Harvesting time did not significantly affect the population of molds on the French beans samples ($p \leq 0.05$). French bean samples harvested at 11am in the morning had the least population of molds compared with other treatments but this wasn't any significantly different.

In general, 6 hours delay before start of cooling resulted in the highest molds population, whereas immediate cooling realized the lowest mold population.

Table 3: Effect of harvesting time and duration before cooling on the population of molds isolated from French beans

Harvesting time	Time to cooling					
	0 hours	2 hours	4 hours	6 hours	8 hours	Mean
7am	3450^{a}	1850^{b}	2950^{b}	3352^{c}	5652^{a}	3450^{b}
9am	1669^{a}	4183^{a}	5383^{a}	13583^{a}	3406^{b}	5645^{a}
11am	3157^{a}	2632^{ab}	2677^{b}	3777^{c}	2669^{b}	2982^{b}
1pm	1551^{a}	4351^{a}	2051^{b}	1550^{d}	2600^{b}	2421^{b}
3pm	3950^{a}	3517^{a}	1750^{b}	6450^{b}	3283^{b}	3790^{b}
Mean	2755	3306	2962	5742	3522	3657.6
LSD ($p \leq 0.05$)	2011.2	2011.2	2011.2	2011.2	2011.2	2011.2
CV (%)	33.5	33.5	33.5	33.5	33.5	33.5

Means within column followed by different letters are significantly different based on Fishers Protected LSD test ($p \leq 0.05$).

3.4 Effect of harvesting time on population of molds, *Enterobacteriaceae, Staphylococcus aureus* and *Listeria monocytogenes* on French bean

Different harvesting times had significant ($p \leq 0.05$) effect on the population of various microorganisms isolated from French bean samples (Table 5). On average, the highest population of microorganisms isolated from French bean samples was for *Enterobacteriaceae* followed by *Staphylococcus aureus*. The least population of microorganism isolated from the French bean samples was *Listeria monocytogenes.* There was significant variation with regards to harvesting time except for *Enterobacteriaceae*. The amount of *Enterobacteriaceae* was highest in samples harvested at 3pm. The amount of *Staphylococcus aureus* and *Listeria monocytogenes* was highest in samples harvested at 7am. The population of all the microorganisms was generally high in the samples harvested early in the morning. There was a general reduction in microorganism population in samples harvested between 9am and 11am, however, there was a spike in the microorganism population for samples harvested at 3pm.

Table 5: Effect of harvesting time and duration before cooling on the population of different microorganisms isolated from French beans

	Time of Harvesting					
Microorganisms	7am	9am	11am	1pm	3pm	Mean
Enterobacteriacea	36117.6^{a}	3164.6^{a}	16460.5^{a}	33175.3^{a}	92330.7^{a}	36249.7^{a}
Staphylococcus aureus	15587.0^{ab}	4449.0^{a}	11551.0^{ab}	8511.0^{ab}	12806.0^{ab}	10580.8^{b}
Moulds	3450.0^{b}	5645.0^{a}	2982.0^{b}	2421.0^{b}	3790.0^{b}	3657.6^{b}
Listeria monocytogenes	453.4^{b}	95.2^{b}	75.5^{b}	68.3^{b}	169.1^{b}	172.3^{b}
Mean	13902.0	3338.5	7767.3	11043.9	27273.9	12665.1
LSD ($p \leq 0.05$)	25764.1	3799.3	12046.8	24150.1	69529.2	25948.5

Means within column followed by different letters are significantly different based on Fishers Protected LSD test ($p \leq 0.05$).

4.0 Discussion

The results show that harvesting time and time before cooling (precooling duration) had significant effect on the population of microorganisms isolated form French bean samples. Total viable counts in these samples showed mean values ranging from 0.7 to 3.3×10^{5} CFUs g^{-1} for total *Enterbacteriaceae, Listeria monocytogenes*, *Staphylococcus aureus* and molds. It's been reported that the bacterial population varies depending on seasonal and climatic condition and may range from 10^{4} to 10^{8} per gram. The microorganisms isolated from the French bean samples directly reflects the sanitation of the processes of cultivation, harvesting, storage and processing of the produce (Eni, *et al*., 2010). Fruits and vegetables are widely exposed to microbial contamination through the soil, dust, water, and during harvesting (Eni, *et al*., 2010).

Staphylococcus aureus is commonly found in the environment (soil, water and air). It is known to cause food poisoning. It is also commonly found in the nose and skin of human. It is readily killed during pasteurization or cooking. The main source of contamination is from food handlers from the nose and hands. Despite *Staphylococcus aureus* colonizing a wide range of animals, people are the main reservoir of food contamination (*Montville and Mathews 2008*)

The high population of *Staphylococcus aureus* realized in samples harvested early morning may be attributed to poor personal hygiene of food handlers. Even though hand wash water is normally provided, it is likely that majority of the harvesters do not wash their hands in the morning, perhaps fearing to touch cold water.

Listeria monocytogenes population was highest in samples harvested at 7am (453.4 CFU g^{-1}), decreasing steadily to lowest population in samples harvested at 1pm (68.3 CFU g^{-1}).

Listeria monocytogenes is widespread in the environment and can spread along the chain of produce handling. At field level, *Listeria monocytogenes* is likely to be spread from contaminated irrigation water or soil arising from

animal and human feces. *Listeria monocytogenes* can also be spread from contaminated equipment and surfaces during handling, e.g. contaminated creates and grading tables can be source of contamination.

Listeria monocytogenes is known to be one of the major causes of foodborne illnesses, and may cause serious and sometimes fatal infections in children, frail and elderly people. It can also cause stillbirths in pregnant women. The drop in *Listeria monocytogenes* population from morning towards mid-day and afternoon can be attributed to the effect of solar radiation. There is need for more research on the impact of solar radiation on *Listeria monocytogenes* growth and survival.

The high density of bacterial pathogens isolated from the presents study are similar to those reported by Uzeh *et al*., (2009) and Eni *et al*., (2010) where bacterial population as high as 9.9×10^6 was reported. Within the stores, bacterial contaminants contaminate the produce, and multiply when the environmental conditions are favorable (Abadias *et al*., 2008). The population of microorganisms isolated was in the order *Enterobactereacea, Staphylococcus aereus*, molds, and *Listeria monocytogenes.* These microbes have been reported in vegetables and fruits (Qadri *et al*., 2015). Harvesting time and storage temperature are important factors influencing the quality of fresh produce (Kader, 2002; Luo *et al*., 2010). However, our results show that total viable counts of microbes contaminating French beans were exceptionally high in samples harvested early in the morning but reduced when the samples were harvested as from 9am.

Early morning comes with a lot of dew and leaf wetness, and these factors are predisposing for spread and multiplication of microorganisms. In addition, food handlers' personal hygiene is likely to be at its lowest early in the morning, coming from home and exchanging handshakes greetings may be a contributing factor to high levels of microbial load.

Fresh produce remains metabolically active through to harvest and during this period, physiological changes occur (Barth *et al*., 2009). Infection of fresh produce occur at any time during the crop development stage, for French beans, losses due to post harvest spoilage may be as a result of latent infection in the field by pathogens that become active following infection during harvest, cleaning, storage, and distribution (Barth *et al*., 2009). Presence of the pathogen, in a conducive environment during storage enhances density of these pathogens and therefore spoilage.

The results in the present study show that the population of *Listeria monocytogenes* was least compared with other isolated microbes. Given widespread use of animal feacal wastes in agriculture, the availability even in small quantities is of great concern since *Listeria mononcytogenes* is an important foodborne pathogen causing severe illness with high mortality rate (Directorate, 2002). The occurrence of *Listeria monocytogenes* in fruits and vegetables has been reported by various researchers with different rates of prevalence (Arumugaswamy *et al*., 1994; Breer and Baumgartner, 1992; De Simon *et al*., 1992)

In the present study, mold counts isolated ranged from 2421 to 5645 CFU /g. Molds were also detected by researchers Avecedo *et al*., in levels of 4.5×10^4 CFU/g in salad samples, and by Badosa *et al*., in levels of 7×10^4. The authors reported the presence of *Penicillium, Aspergillus*, and *Fusarium* spp. in salads. Many of the molds belong to genera that are toxigenic, this indicates potential for mycotoxins production on vegetables (Jeddi *et al*., 2014; Onuora *et al*., 2013). The high populations of these microorganisms indicate a possibility of unhygienic production environments and improper handling and processing of the produce (Onuora *et al*., 2013). The colonization of the produce with these microorganisms shows their ability to establish within the produce to initiate decay.

During harvesting, and throughout the handling before storage, it is important to minimize the wounds and bruising to reduce the entry points for these pathogens. Fungi, primarily gain entry into fresh vegetables through the formation of germtube that penetrates the cuticle and epidermis (Barth *et al*., 2009). However, most spoilage microorganisms infect and initiate decay at wounds in the epidermal layer, and through natural openings such as stomata and lenticels. Microbial contamination of produce is one of the major concerns on Food Safety, especially when the product involved is a ready to eat (RTE).

Management of microbial contamination with regards to food safety management calls for robust systems based on Hazard Analysis Critical Control Points (HACCP) principles, which works to identify all the risks and have in place measures to reduce these risks in the supply chain. French bean contamination in the field may come from various sources, including irrigation water, especially when the method of irrigation used delivers water directly onto edible part of the crop. Low-lying crops are known to be more susceptible to bacterial contamination due to ease of splashes from the soil. Where animal manure is used, there is greater risk of bacterial contamination. According to GlobalG.A.P., it is advisable to use organic and animal manure several days before harvest to reduce risk of contamination.

Considering the food safety risks associated with microbial contamination, it is important for farmers to have in place a robust food safety management systems that shall prevent or reduce contamination. This includes and not limited to Good Agricultural Practice (G.A.P), Hazard Analysis Critical Control Points (HACCP), food safety and hygiene trainings, microbial testing and validation and equipment hygiene. Considering the public health issue, fresh vegetables are common sources of various pathogenic microorganisms. It is important to ensure farmers apply good agricultural practices and good manufacturing practices during production. French bean farmers should be informed on the microbial sources of contamination and therefore should be trained on hygienic production of this produce.

5.0 Conclusion

Time of harvesting has an effect on the microbial contamination of French beans. There tends to be higher microbial contamination for French beans harvested early in the morning.

6.0 Recommendations

Good agricultural practice and personal hygiene of food handlers are very important for the assurance of food safety in the fresh produce industry. Considering the risk of contamination coming from soil, water and organic manure, it is very important that the irrigation water quality is regularly monitored for presence of microbial contamination. Where possible, irrigation methods that get irrigation water into direct contact with edible part of the crop should be avoided.

Food handlers must be trained on basic food hygiene principles before starting work. Where possible, hand wash water should be warm, this will encourage food handlers to wash their hands, especially early in the morning when temperatures are very low and weather is chilly.

Where possible, French bean harvesters can be provided with hand gloves to minimize the risk of contamination that is as a result of handlers' personal hygiene.

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