KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

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EVALUATION OF EXTERNAL AND INTERNAL MICROBIOLOGICAL QUALITY OF FRESH GRAPES FROM SELECTED GHANAIAN MARKETS

By

Florence Akua Amamoo (Mrs)

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By

FLORENCE AKUA AMAMOO (MRS)

(BSc. Laboratory Technology)

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DECLARATION

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ABSTRACT

The aim of this study was evaluation of external and internal microbiological quality of fresh grapes (*vitis vinifera*) on the Ghanaian market. Microbial quality was determined by enumerating aerobic mesophiles and detections for specific microorganisms, such as *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium*. In all, twenty four samples were analysed for both external and internal investigations, and were conducted for four consecutive batches at weekly intervals. Results obtained from microbiological examination showed detections of mesophiles and *Staphylococcus aureus* indicating non- compliance with Ghana Standards Specification for fresh fruits and vegetables, which reports $1x10^3$CFU/g for TVC and $1x10^2$CFU/g for *Staphylococcus aureus.* The results for *Escherichia coli* and *Salmonella tyhpimurium* was

compliance with Ghana Standards Specifications which reports $1x10^2$CFU/g for *E. coli* and complete absence of *S. tyhpimurium*. The study concludes that, the TVC results which indicate that, the grapes contain large bacterial load, might be contributed from the different sources such as from, the pre-warehousing, harvesting and poor handling practice at the post-harvest activities. Also the significant relationship between the presence of *Staphylococcus aureus* load externally on the grapes and the retail source of collection indicates poor hygienic practice, which may be because the handlers do not use gloves, hair net, etc. The absence of *Escherichia coli* and *Salmonella typhimurium* also show that fresh grapes are free from feacal contamination which is very commendable. The study also recommended that, authorities who supervise the growth of food crops safety and public health should endeavor to check, the various stages of fruit processing to ensure they are in conformity with best practices. Also vendors should be educated on personal hygiene and how to relate it to the handling of fruits crops before bringing them to their various places of sales.

TABLE OF CONTENTS

CONTENTS **PAGES**

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Ready-to-eat (RTE) foods have been identified as any edible that is mostly eaten in its raw state, WHO (2004). In addition, Maffei *et al.* (2013) implied that fresh crops are very critical in human diet, which makes it health stimulant. The antioxidant composed in fresh crops in particular, has the effectiveness of shielding the human cells from the occurrence of free radicals, which results in etiopathogenesis of most chronic diseases, Jeong *et al*. (2006) and Carlsen *et al*. (2010).

From Hanson *et al.* (2012), foodborne diseases are paramount in producing considerable disease and mortality annually, while Fratamico *et al.* (2005) further implicated this assertion in their study by stating that, the occurrence rate of foodborne diseases is on the ascendency and that industrialized countries suffer yearly from infected food caused by variety of microorganism. Whereas in the developing countries, are disadvantaged as a result of over population, poverty, poor environmental conditions and insufficient hygiene generally. Cruickshank *et al.* (1990) held the view that beside the advantage of fresh crops, it is viewed as high-risk foods because they do not require any processing before to eating. The intervention of much better diagnostic systems and control measures has promoted the development of confirming ready-to-eat foods as a source of foodborne disease Seow *et al.* (2012).

Poorna and Randhir (2001) also outlined some reasons that place the microbiological quality of ready-to-eat foods at a high risk level as follows; the use of waste water for watering the plants, food handlers not practicing personal hygiene, unhygienic storage and processing areas and the use of dirty containers. Food borne illness from ready to eat food is of increasing worry, since microbial

contamination can occur during any of the steps in the farm, not forgetting both animal and human factors, (European Commission Health and Consumer Protection Directorate- General, 2002; Baker-Reid *et al*. 2009). Bacteria and fungi present on crop plant during its progressive stages, are mainly not dangerous and mostly, act as an actual life protector to any plague by the groups of disease – causing agents answerable for food crops spoilage, (Janisiewicz and Korsten 2002; Andrews and Harris 2000).

Eckert and Ogawa(1988) stated that, spoilage microorganisms present on crop are more likely to occur during the various stages of development to the postharvest handling of the crops, also the soil spoilage microbes present at the time of harvest can show up on harvesting tools, handling materials in the packinghouse, the storage facility in the distribution chain. Consequently, effective steps must be taken early at the growth and harvesting stages through the use of recommended agricultural activities, as it contributes in reducing yield loss due to contamination at the different stages in the farm.

Burnett and Beuchat, (2001)in highlighting the essential role of fresh crops, also maintained that, mostly microbiological risk is associated with, harvesting, distribution, and commercialization. This makes it very prudent to put up steps to mitigate these risks. Foodborne bacterial pathogens usually associated with fresh crops were identified as follows: coliform bacteria, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella sp.*Tambekar and Mundhada (2006)

1.2 Statement of Problem

Poorna and Randhir (2001) postulates that, spoilage microbes are normal means by which human beings become contaminated and that microbiological risk assessment is the only mechanism for evaluating food security as well as safety among water supplies. As a result, to the fact that, fresh crops play a very nutritional role in the human body and it's very assessable from the grocery shops

and from street vendors, which gives the concern to pay particular attention to how these crops are handled among the various vendors as well. According to the (WHO 2013) most countries reported that inadequate inspection personnel, to ensure that, basic standards in food safety practice are adhered to and also registration, education and health examinations were not part of the mechanisms use to monitor the safety of street vended foods. Therefore, the gap the study seeks to fill is to evaluate the microbiological quality of grapes sold both by some selected street vendors and grocery shops in the Greater Accra Metropolis, in line with promoting food safety among ready-to-eat food and also examine the knowledge on food safety practices.

1.3 Research Questions

The study is organized on the following formulated questions:

1. What are the potential external and internal harmful microorganisms associated with fresh grapes on the Ghanaian market?
2. What is the microbiological quality of fresh grapes on the Ghanaian market?
3. What are the measures put in place to reduce the microbiological risk of fresh grapes on the Ghanaian market?

1.4 Research Objective

The main objective of this study is to evaluate the external and internal microbiological quality of fresh grapes on the Ghanaian market.

1.5 Specific Objectives

1. To examine the potential external and internal harmful microorganisms such as *Salmonella typhimurium*, *Staphylococcus aureus*, *Escherichia coli* and Mesophilic bacteria associated with fresh grapes on the Ghanaian market.

2. To evaluate *Salmonella tyhpimurium*, *Staphylococcus aureus*, *Escherichia coli* and Mesophilic bacteria using Triple Sugar Iron Agar, Baird Parker Rabbit Plasma Fibrinogen Agar and Tryptone-bileglucoronic medium respectively on the Ghanaian market.
3. Assess the measures put in place to reduce the microbiological risk of fresh grapes on the Ghanaian market.
4. Suggest ways of improving the microbiological quality of fresh grapes.

1.6 Significance of the Study

To start with, given the nutritional benefits of ready-to-eat food in the human body, it is therefore very imperative that the microbiological qualities of such foods are not undermined. This study is therefore relevant as it seeks to examine the external and internal microbiological quality of fresh grapes on the Ghanaian market, in order to help explore the full nutritional value of the crop.

Secondly, this study will help examine the microbiological risk associated with the grapes on the market and as well make suggestions in minimizing such risks on which can be harmful to the human body, as they also form the basis of infections in the human's body.

Thirdly, the study will also to add up to the store of knowledge with respect to literature on the external and internal microbiological quality of fresh grapes.

1.7 Scope of the Study

The study will be restricted to Greater Accra region, which is made up two (2) Metropolis, nine (9) Municipal Assemblies and five (5) districts. The choice of the Greater Accra as the study area becomes important because its jurisdiction lies in a densely populated part of Accra and as a result issues of sanitation and food safety is important for human survival. From the purpose of the study to evaluate the microbiological quality of ready-to-eat foods, primary data from both street vendors

and grocery shops who sell fresh grapes. Thus, the study will cover both grapes sold in the grocery shop and by street vendors.

1.8 Organization of Chapters

The study is organized in these five chapters.

Chapter One presents the introduction, and address the background of the study, statement of the problem, objectives of the study, research questions, significance of the study, scope of the study, and organization of the study.

Chapter Two is devoted to the review of pertinent literature and presentation of the conceptual framework of the study.

Chapter Three addresses the methodology which describes the profile of the study area, addresses the research design, study population, sampling procedure and sample size, data instrument, data collection and ethical considerations.

Chapter Four presents the data analysis and, finally, Chapter Five presents the summary of findings, conclusions and recommendations.

Chapter five (5) gives a summary of the major findings of the study, conclusions drawn from the study and recommendations.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

The eruption of food poisoning has given reason for the essence of microbiological control in the food industry. Antioxidants present in fresh fruits has the ability to safeguard the body cells from danger of free radicals, the cause and development of nearly all chronic diseases or abnormal conditions. (Bauer *et al*. 2006,Carlsen *et al*. 2010).

In view of the vitality of fresh crops, their safety is of great essence, also microbial pollution can relate to the crop during any of the production stages in the farm to the sales point. These could range from environmental, animal or human sources (European Commission. Health and

Consumer Protection Directorate-General, 2002;Baker-Reid *et al*, 2009). De Roever (1999) implied that, contamination of fresh crops occurs at all stages during pre and post-harvest periods. Miedes and Lorences (2004) further added that, microorganisms that cause damage to their host plants using water and nutrients from these plants for their survival do this by secreting extracellular lytic enzymes that lower polymers such as polysaccharides to release the water and the nutrients. Extracellular enzymes pectinases and hemicellulases are essential components produced by fungi especially for fungal decomposition. Francis *et al*. (1999) outlined microorganisms that serves as the source of food poisoning and can cause human infections are as follows: *Aeromonashydrophila*, *Citrobacterfreundii*, *Enterobacter cloacae* and *Klebsiella* sp. have been noted in lettuce and salad vegetables. Beuchat and Cousin (2001) in addition stated that, lots of food-borne pathogens can yield myco- toxins, which are accountable for human and animal infections.

Lequeu *et al*. (2003) purported that, decomposingmicroorganisms can get into food crop tissues during the development of fruit,Lequeu *et al*. (2003) further stated that, successful confirmation however, demands the contaminate microbe to prevail over any impediments. Mandrell (2006) similarly implied that, an indirect host of epiphytic microorganisms that posse additional competitive challenge to the contaminated organism, which typically colonizes the outermost fruit surface.

2.2 Preharvest and Harvest Factors

If fungicide is applied poorly during preharvest, mostly through inadequate washing, and culling, greater portions of the fruits are destroyed during storage,(Miedes and Lorences, 2004;Van Kan, 2006). *B. cinerea*is is especially sensitive and a plant pathogen that has multiple enzymes cutinases and lipases that have the tendency of reducing nutrients found in pectin, Van Kan (2006).Sapers

et al. (2001) in a related study said that, in maintaining the safety of crop produce, cleansing reagents namely ozone, chlorine dioxide, and peroxyacetic acid can be applied as they have been confirmed for use on fresh produce. Kader (1992) revealed that, some of the simplest ways of reducing the field heat of harvested crop include comprised air refrigeration, vacuum cooling, and deepening in ice.

Kader, (1992) and Sommer *et al*.(1992) also held the view that, though infection and microbiological damage occur at any time during its development. Most importantly, the period of high probability of decomposition, is at the stage where the crop rips. Sommer *et al.* (1992) maintained that, losses due to postharvest spoilage or pathological spoilage are a result of either persistent invasions in the field, that become more active following harvest or of cross contamination during harvest, cleaning, storage, and distribution. Sommer *et al.* (1992) added that, the presence of pathogenic microbes on a prone host fruit or vegetable, in addition to favourable conditions such as high temperature, gives the three elements vital for the infection to appear such as host, environment, and pathogen.

Sugar and Spotts (1995) stated that, aerial fungicide that the farmers spray against fungi during preharvest also reduces postharvest decomposition during storage. Mahovic *et al.* (2005) related that, insect pest management will lower insect infection to crops and also reduce microbial crosscontamination by the insect vector.

2.3 Impact of Microbiological Spoilage

Sapers, *et al*.,(2001) and O'Connor-Shaw *et al*., (1994) stated that, technological innovation has helped reduced crop contamination in the packaging and processing of food crops and as such strict adoption to these technological innovation, will reduce the risk of food crop contamination which is mostly characterized by the following: discoloration on the cut surfaces, for instance cut

lettuce has pink surface, cut potato appear brown, processed pineapple and cabbage also looks brown and gray respectively, there are those that also appear water-soaked or translucent, these are cut watermelon, papaya, honeydew and tomatoes, moisture loss in “baby” carrots and celery sticks, off- aroma is also associated with broccoli florets and diced cabbage packaged with low percentage in oxygen and high percentage in carbondioxide, flavor changes in cut kiwifruit and texture changes are also noticed with processed strawberry, grated celery, kiwifruit and papaya with these indications gives an indication of contamination of the crop.

Sufficiently bacterial counts that were very high during processing on automated operated machines used for cutting and packaging fillers of a lettuce implying that products that are well cleaned still has the tendency of getting contaminated again, haven gone through processes where vegetable and fruit wreckages can accrue on materials such as, cutters and package-filling equipment was revealed by Cantwell and Suslow (2002).

2.4 Overview of Street food

Inadequate knowledge of food handling safety, among street vendors in basic food safety measures confirms the risk consumers are prone to, regarding health and safety, (Rane, 2011). Food contamination can result in increased diarrhea, which has the potency of killing yearly almost 2.2 million people in the world. In addition, foodborne infection can also initiate other critical health challenges suchlike brain and neural disorders, reactive arthritis, kidney and liver failure, cancer and death (WHO, 2013).

2.4.1 Food Safety Knowledge and Attitudes

Food safety is viewed with immense importance because it aids in critical public health function, also the behavior of consumers has an effect on problems bordering on safeness of food which are

the components of importance to those who produce and sell food, public officials and well-being mentors. This delight has been related to deliberations on how food safety should be explained and how consumers see food safety and select food. (WHO, 2000).

Jackson *et al*. (2003) relate some human infections to food-borne diseases and believed that, it was possibly due to the lack of food safety knowledge of the food handlers. Gotsch *et al*. (2012) held the view that, knowledge is "a complex process of remembering, relating, or judging an idea or abstract phenomenon (cognitive abilities)". Other studies have shown that, street food vendors often operate without license, which makes monitoring and training them in food safety practices very difficult and mostly results in the unhygienic conditions they operate in, with the high potency of causing microbial damage to the food crop, (Muinde and Kuri, 2005). The WHO further held the view that, food safety knowledge both on the part food handlers and consumers isan effective method to reduce food-borne illness and economic losses related to foodborne diseases (WHO, 2000).

2.4.2 Food Safety Practices

According to WHO (1989) food vendors are critical in maintaining food safety measures, right from the packaging of the food to the consumer. Specifically, food vendors within sufficient knowledge in food handling practices are more prone to the risk of pathogens. This gives reasons to the fact that, poor food handling practices by street vendors is an impediment to the safety of the foods they sell.

As part of ensuring the safety of crops on the street, street vendors must be encouraged by government to replace the conventional methods with modern plastic or stainless steels. Moreover, government should endeavor to inform vendors to wear gloves and masks during the preparation and processing street foods (WHO, 2015). Many studies have showed that educated street vendors have positive impact on food hygiene practices. Education of street food vendors on food safety

practices is very critical in preserving the safety of food crops which may be the most costeffective way to reduce the presence of foodborne diseases among street vended foods, (INFOSAN, 2010). Despite the critical role education play in food safety practices among street vended foods, the lack of will power to translate the acquired knowledge into practice isa challenging factor in ensuring food safety practices among street vendors. It is also evident that, street vendors with low education usually have poor knowledge of food safety practices and translates it into the bad conditions under which they operate, making education very essential in the fight against microbial damage caused to street vended foods, (Subratty, 2004).

2.4.3 Microbial Quality of Food Crops

The nutritious benefits of food crops in human growth are appreciable, which makes the need to intensify food safety in the sector most especially among developing countries (Von Holy and Makhoane, 2006). Failure to intensify food safety among street vendors, may increase the work load for public health workers, since high levels of coliforms associated with different pathogens such as *Escherichia coli, Salmonella spp*., *Staphylococcus aureus,Bacillus cereus,Clostridium perfringens* and *Vibrio cholerae* (Cho *et al*. 2011; Hanashiro *et al*. 2005; Mankee *et al*. 2005).

2.4.4 Nutritional Benefit of Street Foods

Street vended food crops play a very essential role in solving the problem of urban food insecurity, which makes their safety very important, to human health, (Ohiokpehai, 2003). According to Mpuchane *et al*. (2001), interest should be on the production and processing of street foods which will help retain minimize nutrient losses. Attention on street food vendors is very critical due the harm they tend to pose should there be any form of contamination from their end, in order to extend the shelf-life of their foods (Ohiokpehai, 2003).

CHAPTER THREE

METHODOLOGY

3.0MATERIALS AND METHODS

3.1 Materials

The various media, equipment, and chemicals that were used for the analysis were obtained from the Ghana Standards Authority (GSA, Accra). The analyses were carried out using the protocols employed in the microbiology laboratory of the organization.

3.2GENERAL METHODS

3.2.1Sample Collection

A total of twenty four fresh grape samples were purchased from street vendors and grocery shops in the Accra metropolis. This was done weekly for four consecutive weeks. For each purchase the samples were kept in an ice chest with ice packs and sent to the Microbiology Laboratory for microbial analysis.

The grape samples were obtained from Junction Mall, Accra Mall, Koala Supermarket to represent the grocery shops and the street vendors' samples were obtained from motor way toll booth, Dworwulu traffic light and Legon-Madina road for the microbiological test.

3.2.2Microbiological Analysis

Microbiological analysis of the samples (fresh grapes from the grocery and the street vendors) were done by determining counts of mesophilic bacteria and selected microorganisms, such as *Staphylococcus aureus, Salmonella typhimurium* and *Escherichia coli*.

3.2.3. Sample Preparation for external microbiological quality of the fresh grapes

Thirty grams (30.0g) of the fresh grapes were weighed and transferred into sterile sampling bags labeled with the different sources and 270.0ml of maximum recovery diluents (MRD) was added to obtain 1:10 dilution. Further dilution was done by transferring 1.0ml of the suspension1:10 to a McCartney bottle containing 9.0ml diluent to obtain 1:100 dilution and further serial dilutions done until 1:10000 dilutions obtained.

3.2.4 Sample Preparation for internal microbiological quality of fresh grapes

Thirty grams (30.0g) of the fresh grapes were weighed and transferred into sterile stomacher bags labeled with the different sources and 270.0ml of maximum recovery diluents (MRD) was added, placed in a stomacher and operated for 2 minutes to obtain 1:10 dilution. Further dilution was done by transferring 1.0ml of the suspension1:10 to a McCartney bottle containing 9.0ml diluent to obtain 1:100 dilution and further serial dilutions done until 1:10000 dilutions obtained.

3.2.5 Enumeration of Mesophilic Bacteria

Using sterile pipette 1.0ml each of 1:10 dilutions of the sample were dispensed into two separate sterile Petri dishes.Using the same procedure, the other dilutions were also dispensed in the same manner and 15.0ml of molten Plate count agar cooled in an incubator to 45 ± 1^{o}c was added. The agar and the inoculum were uniformly mixed by gently swirling or tilting each plate taking care

not to generate bubbles the plates were inverted and incubated at 30 ± 1°C for 72hours. Plates containing colonies between 30 and 250 colonies were selected and counted under an illuminated colony counter and recorded (ISO 4833, 2003).

3.2.6 Determination of *E. coli*

Determination of *E. coli* was done using the pour plate technique with TBX Agar. Using sterile pipette, 1.0ml each of the 1:10 dilution of the sample were dispensed into two separate sterile petri dishes. Using the same procedure, the other dilutions were also dispensed in the same manner and 15.0ml of molten TBX agar cooled in a water bath at 44°C to 47°C was added. The agar and the inoculum were uniformly mixed by gently swirling or tilting each plate taking care not to generate bubbles the plates were inverted and incubated at 44±1°C for 24 hours. Plates containing colonies typical of β- glucuronidase-positive *Escherihcia coli* characterized by blue/green coloured colonies not less than 150 were counted under an illuminated colony counter and recorded (ISO 16649-2:2001).

3.2.7 Detection of Staphylococcus aureus

Detection of *Staphylococcus aureus* was done using the pour plate technique with Baird Parker (Rabbit Plasma Fibrinogen as supplement) BPA-RPF Agar. Using a sterile pipette, 1.0ml each of the 1:10 dilution was dispensed into two separate sterile Petri dishes. The same procedure was followed as the other dilutions were also dispensed and 15.0ml of molten BPA-RPF agar cooled in an incubator to 45 ± 1°C was added. The agar and the inoculum were uniformly mixed by gently swirling or tilting each plate taking care not to generate bubbles. The plates were inverted and incubated at 37 ± 1°C for 48 hours. Plates containing colonies of coagulase positive

staphylococci characterized by the formation of gray or black colonies surrounded by an opaque halo of fibrin not less than 150 colonies were counted and recorded (ISO 6888-2, 1999).

3.2.8 Detection of *Salmonella*

Method for detection of *Salmonella spp*. includes four stages of the detection process and depending on the need to obtain confirmations it lasts from 5 to 7 days:

- Pre-enrichment in non-selective liquid medium
- Enrichment in selective liquid media
- Plating and identification on selective media
- Serological and biochemical identification of suspected colonies (confirmation of identity).

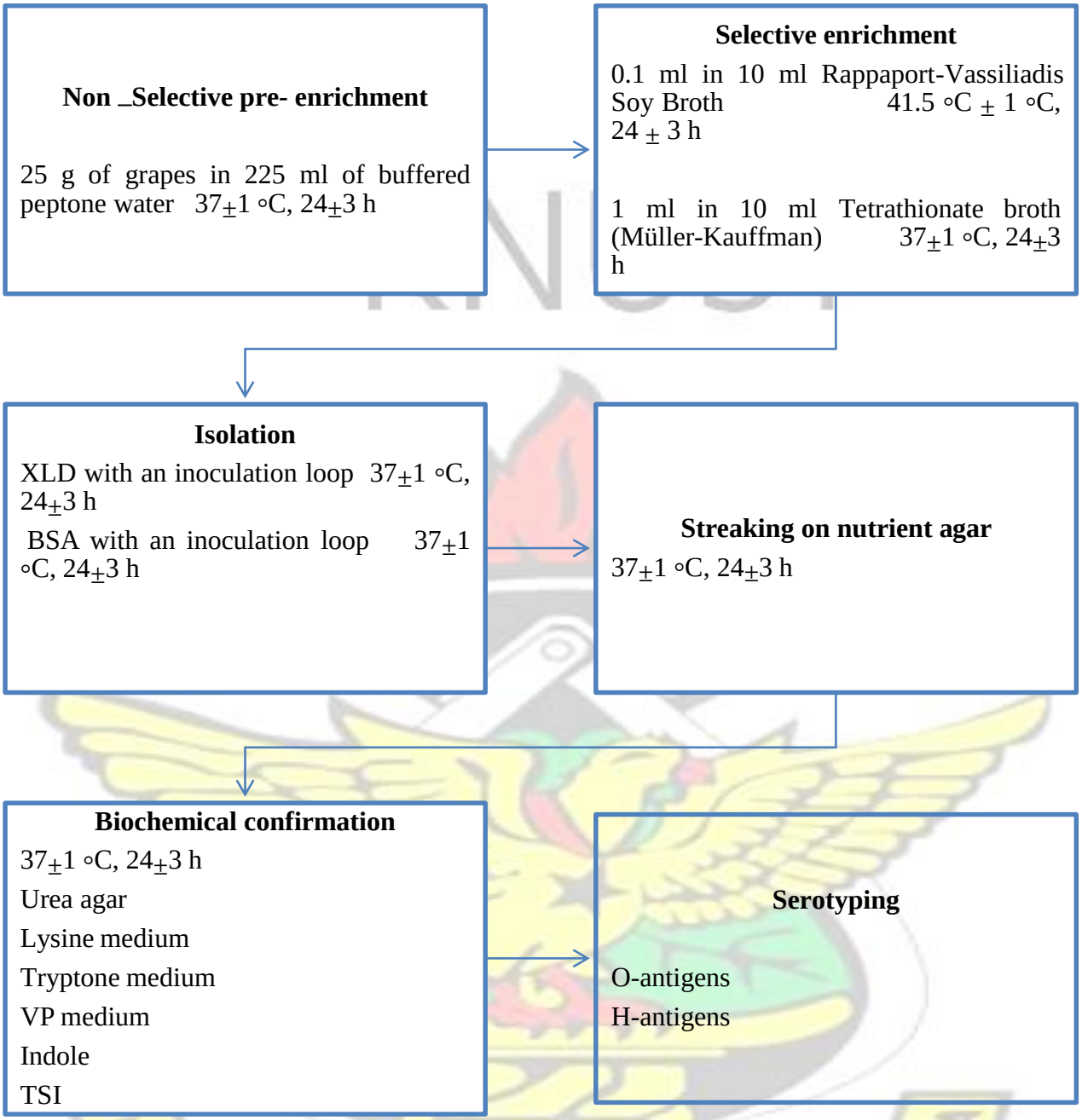

Figure 3.1: Expression of results: results expressed as presence or absence in test portion of 25g of the sample (ISO 6579, 2002).

3.3 Statistical Analysis

The study used the independent sample t test to analyse results from the microbiological test conducted on the two groups of grapes collected from both street vendors and the grocery shops engaged in the study. This was attained by scoring the results from the microbiological test on the scale of 1, 2 and 3 where 1 represents acceptance, 2 exceed limit and 3 represents 'none detected' thus, 'acceptance' indicates microbial load less than the limit, whereas 'exceed limit' results indicate microbial loads greater than the limit, while 'none detected' represents results without the presence of the microorganism tested. After which, a test of significance was conducted on values with respect to the standard value accepted for microbiological test or load from the Ghana Standard Board.

Plate 1: Staphylococcus aureus on Bpa-Rpf

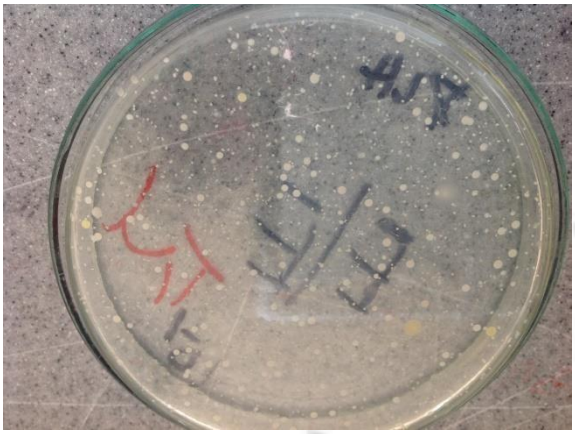

Plate 2: Visible Growth of Mesophilic Bacteria on PCA

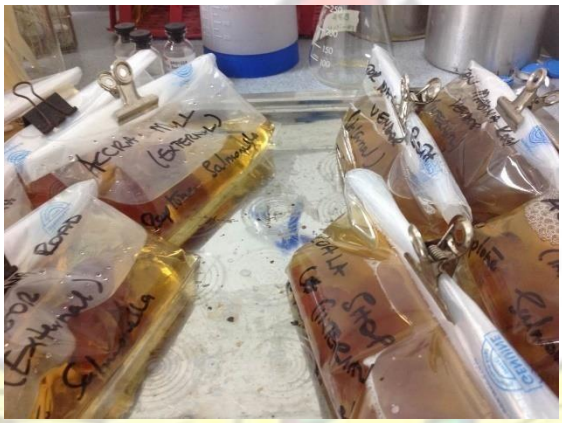

Plate 3: Grape Sample Prepared for Inoculation

Plate 4: Grapes Bought from Grocery Stores and Street Vendors

CHAPTER FOUR

RESULTS

4.1 Internal Microbiological Test

Table 4.1a indicates that, from the grapes collected with respect to the internal microbiological test results for weeks one and two the Total Viable Count (TVC) as presented in the table shows that mesophilic bacteria counts from the Junction Mall (JM), and Koala Supermarket (KS) were within the accepted value of $1x10^3$CFU/g. While the TVC results of the grapes collected from the street vendors Motor Way Vendor (MWV), Dzorwulu Road Vendor (DRV), Legon-Madina Road (LMRV) exceeded the limit count $1x10^3$CFU/g. Results for *Staphylococcus aureus* from the grocery shops Junction Mall, Accra Mall and Koala Supermarket were within the limit $1x10^2$CFU/g, however *Staphylococcus aureus* count for the street vendors Motor Way Vendor, Dzorwulu Road Vendor and Legon Madina Road Vendor exceeded the limit $1x10^2$CFU/g. *Escherichia coli* and *Salmonella* typhimurium were not detected for weeks one to four in both samples collected from the grocery shops and the street vendors. Results for weeks three and four also show that the TVC for Motor Way Vendor, Legon Madina road Vendor exceeded the limit $1x10^3$CFU/g. The TVC results for the grocery shops were within the acceptable limit $1x10^3$CFU/g. *Staphylococcus aureus* count for Motor Way Vendor and Legon Madina road Vendor also exceeded the limit $1x10^2$CFU/g.

Table 4.1a Internal Microbiological Test Results on Fresh Grapes

Week	Parameter (CFU/g)	Sample source					
		MWV	LMRV	DRV	JM	AM	KS

	TVC	$3.4x10^5$	$3.8x10^4$	$4.38x10^4$	$7.6x10^1$	$5.5x10^3$	$1.2x10^3$
1	*S.aureus*	$5.6x10^3$	$4.5x10^2$	$2.0x10^2$	$<10x10^1$	$4.2x10^2$	$1.8x10^2$
	salmonella	ND	ND	ND	ND	ND	ND
	E.coli	ND	ND	ND	ND	ND	ND
	TVC	$2.88x10^6$	$4.25x10^3$	$3.45x10^3$	$1.98x10^2$	$9.58x10^2$	$3.75x10^2$
	S.aureus	$5.65x10^3$	$<10x10^1$	$5.0x10^1$	$<10x10^1$	$<10x10^1$	$<10x10^1$
2	*Salmonella*	ND	ND	ND	ND	ND	ND
	E.coli	ND	ND	ND	ND	ND	ND
3	TVC	$4.4x10^5$	$5.55x10^3$	$3.55x10^2$	$9.0x10^1$	$2.35x10^2$	$2.35x10^2$
	S.aureus	$1.3x10^3$	$8.1x10^3$	$<10x10^1$	$<10x10^1$	$<10x10^1$	$<10x10^1$
	Salmonella	ND	ND	ND	ND	ND	ND
	E.coli	ND	ND	ND	ND	ND	ND
4	TVC	$2.68x10^6$	$5.60x10^3$	$1.45x10^2$	$5.8x10^1$	$2.45x10^2$	$1.35x10^2$
	S.aureus	$1.5x10^3$	$1.8x10^3$	<10x10	$<10x10^1$	$<10x10^1$	$<10x10^1$
	Salmonella	ND	ND	ND	ND	ND	ND
	E.coli	ND	ND	ND	ND	ND	ND

Source: Sampled Grapes (2017); Standard Specification: Total viable count limit ---- $1x10^3$, *Staphylococcus aureus* limit- $1x10^2$, *Escherichia coli limit*- $1x10^2$,*Salmonella typhimurium*- Absent.ND: Not Detected

In the case of the Total Viable Count(TVC) of microbial load on the grapes collected, the study shows that there appears to be a relationship between the internal (TVC) of microbial load on the fresh grapes and the source from which the grapes were collected, this is confirmed by, the difference between the expected count and actual count in table 4.2a. In other words, assuming the Total Viable Count of microbial load on fresh grapes is independent of the source from which the grapes were collected, there would not be any difference between the expected count and actual count.

In addition, Table 4.2a also shows that, though there appears to be a relationship between the Total Viable Count and the source of collection, in other words the (TVC) of microbial load on a fresh

grape is dependent on the source of the grapes, this relationship is by chance and not significant as implicated in Table 4.2a, where the small Chi-square value of 14.000 and its significance value 0.16 which is greater than the significance level of 0.05

Table 4.2a Cross-tabulation between source of grapes collection and determination of Total Viable Counts

	Total Viable count	Total acceptance	exceed limit		
Source of Sample	MWV	Count	0	4	4
		Expected Count	2.0	2.0	4.0
	LMRV	Count Expected Count	0	4	4
		Count	2.0	2.0	4.0
	DRV	Count Expected Count	2	2	4
		Count	2.0	2.0	4.0
	JM		4	0	4
			2.0	2.0	4.0
	AM	Count Expected Count	3	1	4
		Count	2.0	2.0	4.0
	KS	Count Expected Count	3	1	4
		Count	2.0	2.0	4.0
Total		Count	12	12	24
		Expected Count	12.0	12.0	24.0

Source: Microbiological Test Results (2017)

Table 4.2b Chi-Square Tests of TVC

	Value	df	Asymp. Sig. (2-sided)

Pearson Chi-Square	14.000[a]	5	0.016
Likelihood Ratio	18.729	5	0.002
Linear-by-Linear Association	9.255	1	0.002
N of Valid Cases	24		

Significant at 0.05

In the case of *Staphylococcus aureus* load on the grapes collected the study shows once again that, there appears to be a relationship between *Staphylococcus aureus* load on the fresh grapes and the source from which the grapes were collected, this is confirmed again by the difference between the expected count and actual count in Table 4.3a. This suggests that, *Staphylococcus aureus* load on fresh grapes is related to the source from which the grapes were collected. In addition, Table 4.3b also shows that, though there appears to be a relationship between *Staphylococcus aureus* and the source from which the grapes were collected. This relationship is by chance and not significant as implicated in Table 4.3b, where the small Chi-square value of independence 10.500 and its significance value of 0.062 which is greater than the significant level of 0.05.

Table 4.3a Cross tabulation between source of grapes collection and determination of *Staphylococcus aureus*

	Staphylococcus aureus		**Total**
	acceptable	**exceed limit**	

Source of Sample	**MWV**	Count	1	3	4
		Expected Count	2.7	1.3	4.0
	LMRV	Count	1	3	4
		Expected Count	2.7	1.3	4.0
	DRV	Count	4	0	4
		Expected Count	2.7	1.3	4.0
	JM	Count	4	0	4
		Expected Count	2.7	1.3	4.0
	AM	Count	3	1	4
		Expected Count	2.7	1.3	4.0
	KS	Count	3	1	4
		Expected Count	2.7	1.3	4.0
Total		Count	16	8	24
		Expected Count	16.0	8.0	24.0

Source: Microbiological Test Results (2017)

Table 4.3b Chi-Square Tests of *Staphylococcus aureus*

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	10.500[a]	5	0.062
Likelihood Ratio	12.558	5	0.028
Linear-by-Linear Association	3.943	1	0.047
N of Valid Cases	24		

Significant at $p < 0.05$

Regarding the internal *Escherichia coli* load the fresh grapes collected table 4.4 clearly shows that, there were no variations in the input values recorded. Which means that all inputs where constant and shows an acceptance of *Escherichia coli* load on the grapes collected internally.

Due to these results Chi-square could not be computed since all inputs were constant or the same.

Table 4.4Cross tabulation between source of grapes collection and determination of *Escherichia coli*

			Escherichia coli acceptable	Total
Source of Sample	**MWV**	Count	4	4
		Expected Count	4.0	4.0
	LMRV	Count	4	4
		Expected Count	4.0	4.0
	DRV	Count	4	4
		Expected Count	4.0	4.0
	JM	Count	4	4
		Expected Count	4.0	4.0
	AM	Count	4	4
		Expected Count	4.0	4.0
	KS	Count	4	4
		Expected Count	4.0	4.0
Total		Count	24	24
		Expected Count	24.0	24.0

Source: Microbiological Test Results (2017)

Regarding the internal *Salmonella typhimurim* load the fresh grapes collected table 4.5 clearly shows that, there were no variations in the input values recorded. This means that all inputs were constant which indicates internal *Salmonella typhimurim*load was not detected on the grapes collected. Due to these results for the cross tabulation Chi-square could not be computed since all inputs were constant or the same.

Table 4.5: Cross tabulation between source of grapes collection and determination of *Salmonella typhimurim*

			Salmonella typhimurim ND	**Total**
Source of Sample	**MWV**	Count	4	4
		Expected Count	4.0	4.0
	LMRV	Count	4	4
		Expected Count	4.0	4.0
	DRV	Count	4	4
		Expected Count	4.0	4.0
	JM	Count	4	4
		Expected Count	4.0	4.0
	AM	Count	4	4
		Expected Count	4.0	4.0
	KS	Count	4	4
		Expected Count	4.0	4.0
Total		Count	24	24
		Expected Count	24.0	24.0

Source: Microbiological Test Results (2017)

The study also shows in table 4.6 that, there is statistically significant difference between the mean values of internal Total Viable Count load on fresh grapes and the source of collection. These resultsare implicated by the significance value of 0.005 in table 4.6 which is less than the significance level of 0.05 being tested. These findings suggest that, the internal Total Viable Count of microbial load on fresh grapes depends on the source from which the grapes are collected. In addition, table 4.6 once again revealed significance difference in the mean values of *Staphylococcus aureus* load on fresh grapes with respect to the source from which the fresh grapes are collected. This is confirmed by the significance value of 0.038 in the table, which less than the level of significance being tested.

Table 4.6 Internal Total Viable Count ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Total Viable count	Between Groups	3.500	5	0.700	5.040	0.005
	Within Groups	2.500	18	0.139		
	Total	6.000	23			
Staphylococcus aureus	Between Groups	2.708	5	0.542	3.000	0.038
	Within Groups	3.250	18	0.181		
	Total	5.958	23			

Source: Microbiological Test Results (2017) Significant at p< 0.05

From figure 4.7a the study revealed that, the internal Total Viable Count (TVC) on the grapes collected had an equal score of (50.0%) for both acceptable and exceed limits. While *Staphylococcus aureus* for the internal microbial count on the grapes, also show in the same figure 4.6a that, the acceptable microbial count formed (54.2%), while the exceed limit count scored (45.8%).

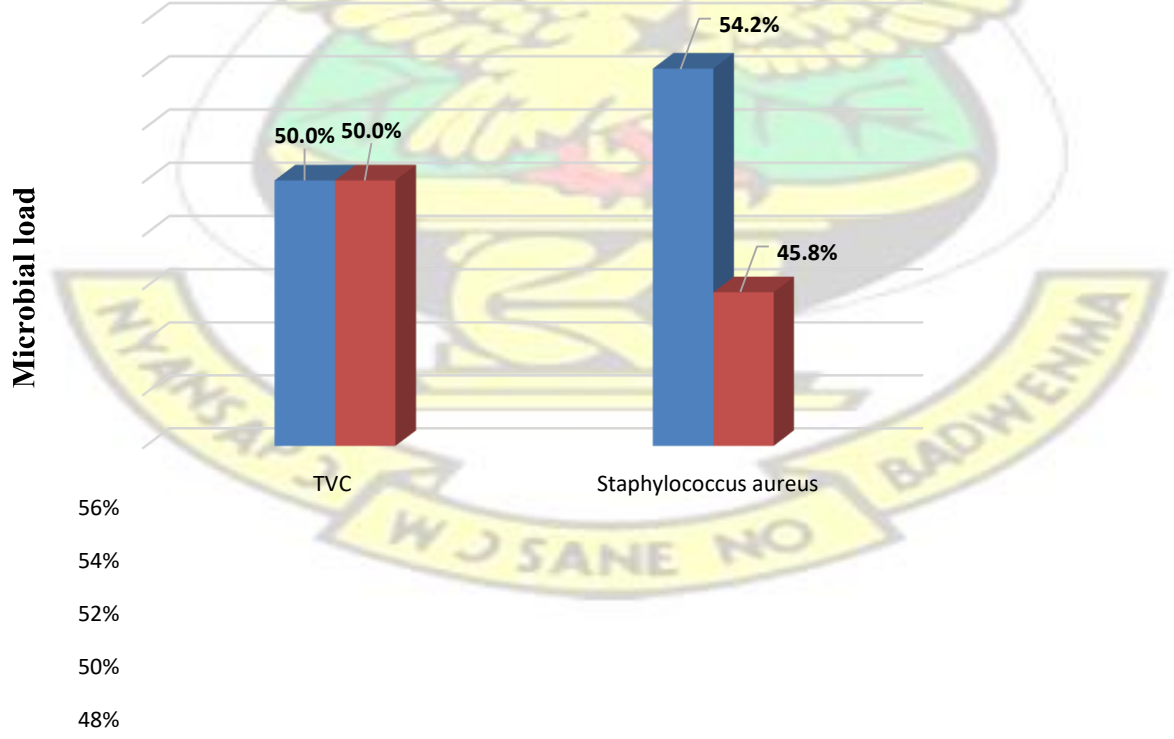

Figure 4.7a Internal Microbial Distribution of the Grapes
Source: Sampled Data (2018)

4.2 External Microbiological Test

Table 4.8a indicates that, with respect to the external microbiological test performed on the samples, the table indicates that for weeks one and two mesophilic bacteria results recorded for the street vendors exceeded the limit value of $1x10^3$CFU/g while that of the grocery shops were within the limit. *Salmonella typhimurium* and *Escherichia coli* were not detected in all the samples from week one to four. In week one and two *Staphylococcus aureus* count for Motor Way Vendor and Legon Madina Road Vendor exceeded the limit $1x10^2$. The table also shows that for week three and four TVC results as well as Staphylococcus aureus for Motor Way Vendor and Legon Madina Road Vendor exceeded the limit $1x10^3$CFU/g.

Table 4.8aExternal Microbiological Test Results On Fresh Grapes

	Parameter	Sample source					
	(CFU/g)	MWV	LMRV	DRV	JM	AM	KS
1	TVC	$3.6x10^5$	$3.6x10^4$	$4.38x10^4$	$6.5x10^1$	$1.52x10^4$	$3.65x10^3$
	S.aureus	$5.2x10^3$	$4.8x10^2$	$1.0x10^2$	$<10x10^1$	$4.3x10^2$	$<10x10^1$
	Salmonella	ND	ND	ND	ND	ND	ND
	E.coli	ND	ND	ND	ND	ND	ND
2	TVC	$2.85x10^6$	$4.2x10^3$	$3.34x10^3$	$1.16x10^2$	$9.5x10^2$	$3.4x10^2$
	S.aureus	$5.0x10^3$	$<10x10^1$	$5.0x10^1$	$<10x10^1$	$<10x10^1$	$<10x10^1$

	Salmonella	ND	ND	ND	ND	ND	ND
	E.coli	ND	ND	ND	ND	ND	ND
3	TVC	$1.85x10^6$	$5.65x10^3$	$1.45x10^2$	$<10x10^1$	$3.15x10^2$	$1.12x10^2$
	S.aureus	$9.85x10^2$	$1.85x10^4$	$<10x10^1$	$<10x10^1$	$<10x10^1$	$1.35x10^2$
	Salmonella	ND	ND	ND	ND	ND	ND
	E.coli	ND	ND	ND	ND	ND	ND
4	TVC	$2.65x10^6$	$5.35x10^3$	1.30x10	$5.5x10^1$	$2.3x10^2$	$1.25x10^2$
	S.aureus	$1.0x10^3$	$1.65x10^3$	$<10x10^1$	$<10x10^1$	$<10x10^1$	$<10x10^1$
	Salmonella	ND	ND	ND	ND	ND	ND
	E.coli	ND	ND	ND	ND	ND	ND

Source: Sampled Grapes (2017): Standard Specification : Total viable count limit ---- $1x10^3$, *Staphylococcus aureus* limit---- $1x10^2$, *Escherichia coli limit*----- $1x10^2$, *Staphylococcus aureus*----- Absent:ND: Not Detected

4.2.1 Test for Relationship between Source of Sample and Microbial Load

In the case of Total Viable Count of microorganisms on the grapes collected, the study revealed once again that there appears to be a relationship between the external Total Viable Count of microbial load on the fresh grapes and the source from which the grapes were collected, this is confirmed by, the difference between the expected count and actual count in table 4.9a. In other words, assuming the external Total Viable Count of microbial load on fresh grapes is independent of the source from which the grapes where collected, there would not be any difference between the expected count and actual count.

In addition, table 4.9a also implies that, though there appears to be a relationship between the Total Viable count and the source of collecting the grapes, in other words though the Total Viable Count of microbial load on fresh grapes is dependent on the source of the grapes this relationship is by chance and not significant. As implicated in table 4.9b where the small Chi-square value of

independence 8.000 and its large significance value 0 .156 which is greater than the significance level of 0.05 being tested.

Table 4.9aCross tabulation between source of grapes collection and determination of Total Viable count

			Total Viable count		Total
			acceptable	exceed limit	
Source of Sample	MWV	Count	0	4	4
		Expected Count	1.5	2.5	4.0
	LMRV	Count	0	4	4
		Expected Count	1.5	2.5	4.0
	DRV	Count	2	2	4
		Expected Count	1.5	2.5	4.0
	JM	Count	3	1	4
		Expected Count	1.5	2.5	4.0

	AM	Count	2	2	4
		Expected Count	1.5	2.5	4.0
	KS	Count	2	2	4
		Expected Count	1.5	2.5	4.0
Total		Count	9	15	24
		Expected Count	9.0	15.0	24.0

Source: Microbiological Test Results (2017)

Table 4.9b Chi-Square Tests of TVC

	Value	**df**	**Asymp. Sig.(2-sided)**
Pearson Chi-Square	8.000[a]	5	0.156
Likelihood Ratio	10.621	5	0.059
Linear-by-Linear Association	4.220	1	0.040
N of Valid Cases	24		

Significant at p< 0.05

The external *Staphylococcus aureus* load on the fresh grapes collected is related to the source from which the grapes were collected. This is confirmed again by the difference between the expected count and actual count in table 4.10a. Which suggests that, *Staphylococcus aureus* load on fresh grapes is dependent on the source from which the grapes where collected. In addition table 4.10a also shows that, though there appears to be a relationship between *Staphylococcus aureus* and the source from which the grapes were collected, once again this relationship happened by chance and not significant. This is further implicated in table 4.10a below, where the small Chi-square value of independence 10.909 and its significance value of 0.53 which is greater than the significant level of 0.05 being tested.

Table 4.10a Cross tabulation between source of grapes collection and determination of *Staphylococcus aureus*

			Staphylococcus aureus		**Total**
			acceptable	**exceed limit**	
Source of Sample	**MWV**	Count	0	4	4
		Expected Count	2.2	1.8	4.0
	LMRV	Count	1	3	4
		Expected Count	2.2	1.8	4.0
	DRV	Count	4	0	4
		Expected Count	2.2	1.8	4.0
	JM	Count	3	1	4
		Expected Count	2.2	1.8	4.0
	AM	Count	3	1	4
		Expected Count	2.2	1.8	4.0

KS	Count	2	2	4
	Expected Count	2.2	1.8	4.0
Total	Count	13	11	24
	Expected Count	13.0	11.0	24.0

Source: Microbiological Test Results (2017)

Table 4. 10b Chi-Square Tests of *Staphylococcus aureus*

	Value	df	Asymp. Sig. (2sided)
Pearson Chi-Square	10.909[a]	5	0.053
Likelihood Ratio	14.063	5	0.015
Linear-by-Linear Association	3.102	1	0.078
N of Valid Cases	24		

Significant at p< 0.05

Regarding the external *Escherichia coli* load the fresh grapes collected table 4.11 clearly shows that, there were no variations in the input values recorded. This means that all inputs were constant and shows an acceptance of the external Escherichia coli load on the grapes collected. Due to this result Chi-square could not be computed since all inputs were constant or the same.

Table 4.11 Cross tabulation between source of grapes collection and determination of *Escherichia coli*

	***Escherichia coli* acceptable**	**Total**

Source of Sample	**MWV**	Count	4	4
		Expected Count	4.0	4.0
	LMRV	Count	4	4
		Expected Count	4.0	4.0
	DRV	Count	4	4
		Expected Count	4.0	4.0
	JM	Count	4	4
		Expected Count	4.0	4.0
	AM	Count	4	4
		Expected Count	4.0	4.0
	KS	Count	4	4
		Expected Count	4.0	4.0
Total		Count	24	24
		Expected Count	24.0	24.0

Source: Microbiological Test Results (2017)

Regarding the external *Salmonella typhimurim* load the fresh grapes collected Table 4.12 clearly shows that, there were no variations in the input values recorded. Which again means that, the inputs where constant indicating that, external *Salmonella typhimurim* load was not detected on the grapes collected. Due to this result for the cross tabulation, Chi-square could not be computed since all inputs were constant.

Table 4.12 Cross tabulation between source of grapes collection and determination of*Salmonella typhimurim*

			Salmonella typhimurim **none detected**	**Total**
Source of Sample	**MWV**	Count	4	4
		Expected Count	4.0	4.0
	LMRV	Count	4	4
		Expected Count	4.0	4.0
	DRV	Count	4	4
		Expected Count	4.0	4.0
	JM	Count	4	4
		Expected Count	4.0	4.0

	AM	Count	4	4
		Expected Count	4.0	4.0
	KS	Count	4	4
		Expected Count	4.0	4.0
Total		Count	24	24
		Expected Count	24.0	24.0

Source: Microbiological Test Results (2017)

Furthermore, the study also shows in table 4.13 that there is no statistically significant difference between the mean values of the external Total Viable Count load on fresh grapes and the source of collection. This results are implicated by the large significance value of 0.164 associated to the small F statistics of 1.800 in the table, which greater than the significance level of 0.05 being tested. This finding suggests that, the external Total Viable Count of microbial load on fresh grapes does not depend on the source from which the grapes are collected. In addition Table 4.13 revealed that, there is a significant difference in the mean values of the external *Staphylococcus aureus* load on fresh grapes, with respect to the source from which the fresh grapes are collected. This is confirmed by the significance value of 0 .038 in the table, which less than the level of significance being tested.

Table 4.13: External Total Viable Count ANOVA

		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Total Viable count	Between Groups	1.875	5	0.375	1.800	0.164
	Within Groups	3.750	18	0.208		
	Total	5.625	23			
Staphylococcus aureus	Between Groups	2.708	5	0.542	3.000	0.038
	Within Groups	3.250	18	0.181		
	Total	5.958	23			

Source: Microbiological Test Results (2017) Significant at p< 0.05

Figure 4.14 below also reveals that, the external Total Viable Count(TVC) on the grapes collected had a score of (62.5%) for counts that exceeded the limit, while (37.5%) were within the acceptable limits. Also for the *Staphylococcus aureus* on the external microbial count on the grapes, it is as well showed in figure 4.6b below that, the acceptable microbial count formed (54.2%), while the counts that exceeded the limit count scored (45.8%).

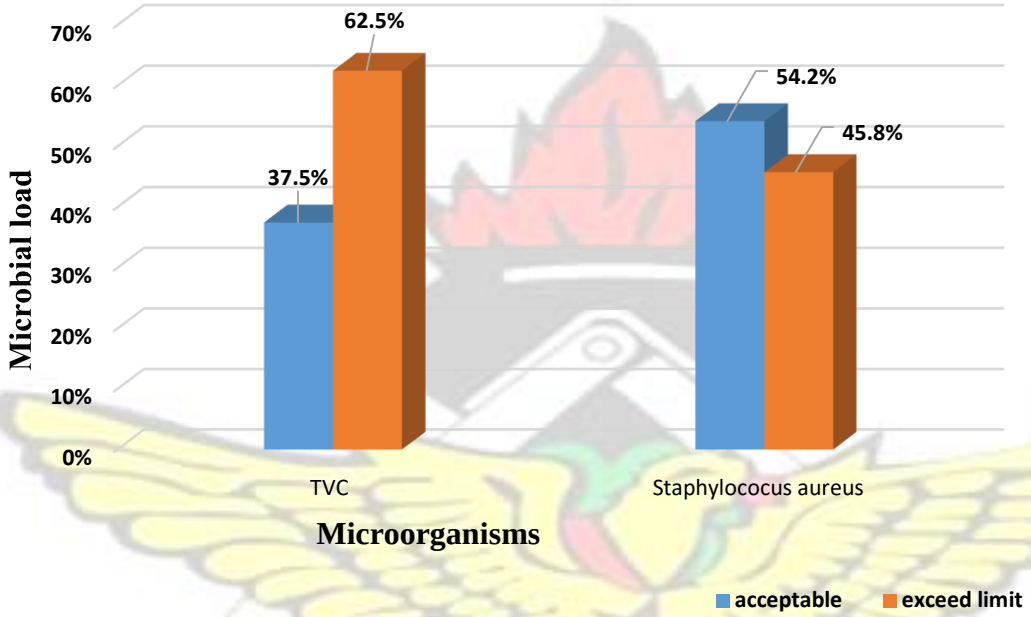

Figure 4.14External Microbial Distribution of Fresh Grapes

Source: Sampled Data (2018)

4.3 DISCUSSION OF FINDINGS

4.3.1 Internal Microbiological Test

From the study, there appear to be a significant difference between the internal Total Viable Count of microbial load on fresh grapes and the retail source from which they were collected. Which means that, the internal Total Viable Count of microbial load on the fresh grapes depends strongly on the source from which the grapes are collected, this results suggests that grapes from the street

vendors, are more likely not have gone through best practices at the various stages of preparation before sales, since the internal Total Viable Count microbial load from the street vendors recorded a higher load as compared to those from the grocery shops. Also the ANOVA results which revealed a significant relationship in the internal TVC and the source of collection implies that, most vendors do not handle their grapes properly at various stages of preparation before selling.

Also the insignificant relationship between the internal *Staphylococcus aureus* load on fresh grapes and the source, from which it was collected, implies that the internal load of *Staphylococcus aureus* on fresh grapes, is not dependent on the source from which the grapes were collected. Furthermore, the study showed that, the internal load of *Salmonella typhimurim* load on fresh grapes was within the limit of the load while, *Escherichia coli* load internally on the fresh grapes was not detected as per this research.

4.3.2 External Microbiological Test

Findings from the study shows that there is no significant relationship between the external Total Viable Count load on fresh grapes and the source from which they were collected, as well as the external *Staphylococcus aureus* load on fresh grapes is insignificantly related to the source from which the grapes were collected. This suggests that, most of the microbial load were more of internal than external, which mean that, microorganisms are most likely to enter the grapes at the production level, than when with the retailers. Also the external *Salmonella typhimurim*load on fresh grapes and the external *Escherichia coli l*oad on the fresh grapes were not detected on the grapes collected.

This results suggests that, the significant relationship between the internal Total Viable Count load and the source from which the grapes were collected, as opposed to the insignificant relationship between the external Total Viable Count load and the source from which the fresh grapes were

collected suggests that, the Total Viable Count load internally on fresh grapes could be attributed to the developmental process or stage of the grapes rather than, the retail vendors from which the fresh grapes were collected.

Also high load of *Staphylococcus aureus* from the street vendors implies that probably, poor personal hygienic practice was employ such as not wearing of gloves, hair nets and covering of nose and mouths before packaging them on to the market as compared to, grapes from the grocery shops. This result confirms literature cited earlier in this study by Cantwell and Suslow (2002) who revealed that there is a significantly higher bacterial counts during processing of fruit crops. Furthermore, the results confirm the study by Lequeu *et al*. (2003) who purported that, spoilage microorganisms can enter plant tissues during fruit development, either through the calyx (flower end) or along the stem.

Additionally, the significant relationship established by this study regarding the external *Staphylococcus aureus* load and the vendors from which the grapes were collected once again confirms that, the external microbial load on fruit crops are more dependent on the vendors, with respect how they practice proper hygiene in the washing and packing of the grapes before sales. Also the absence of*Staphylococcus aureus* and *Escherichia coli* as side the Total Viable Count, do not disprove the study of Tambekar and Mundhada (2006) who stated that, foodborne bacterial pathogens commonly detected in fresh vegetables are coliform bacteria, *E. coli*, *Staphylococcus aureus and Salmonella sp.*

CHAPTER FIVE

SUMMARY OF FINDINGS, CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The study concludes based on the findings that, the TVC results which indicates that, the grapes contain large bacterial load, might be contributed from the different sources such as from, the prewarehousing, harvesting and poor handling practice at the post-harvest activities. Also the significant relationship between the presence of *Staphylococcus aureus* load externally on the grapes and the retail source of collection indicates poor hygienic practice, which may be because the handlers do not use gloves, hair net, etc. In addition, the study concludes that, the absence of *E. coli* in the grapes indicates the absence of feacal contamination at the various stages of fruit processing.

5.2 Recommendation

The study suggests the following recommendations based on its findings:

- Authorities who supervise the growth of food crops safety and public health should endeavor to check, the various stages of fruit processing to ensure they are in conformity with best practices.
- Also vendors should be educated on personal hygiene and how to relate it to the handling of fruits crops before bringing them to their various places of sales.
- Consumers should be encouraged to purchase fresh grapes often from the grocery shops than the street vendors.
- Future studies should investigate the relationship between the microbial load on readyto-eat foods and the developmental stages of the fruits.
- Future studies, should investigate the predominate internal microbial load in the Total Viable Count of fruits and its effect on the health of humans.

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APPENDIX

Appendix A:Media Preparation

Plate Count Agar Medium (Oxoid CM0325)

Component	g/l
Tryptone	5.0
Yeast Extract	2.0
Glucose	1.0
Agar	9.0 (top it up to 17.5g)

Suspend 17.5g in distilled water and bring to the boil to dissolve the medium completely. Dispense into McCartney bottles and sterilize by autoclaving at 121°C for 15 minutes. Adjust pH to 7.0± 0.2 before and after sterilization.

Buffered peptone water (CM0509)

Component	g/l
Peptone	10.0
Sodium chloride	5.0
Di – sodium phosphate	3.5
Potassium hydrogen phosphate	1.5

Suspend 20g in 1 litre of distilled water. Mix well and distribute into sampling bottles. Sterilize by autoclaving at 121°C for 15 minutes. Adjust pH 7.2± 0.2 before and sterilization.

Tryptone- bile-glucuronic medium (TBX)

Component	g/l
Enzymatic digest of casein	20.0
Bile salts No.3	1.5
5-Bromo-4-chloro-3indolylβ-D-glucuronic acid (BCIG)	144 µmol [a]
Dimethyl sulfoxide (DMSO) [b]	3ml
Agar	9g to 18g [c]
Water	1000 ml

a e.g. 0.075 g of cyclohexylammonium salt.

b Dimethyl sulfoxide is harmful by inhalation and contact. The use of a fume cupboard when handling is advised. Because of this toxicity, a diluent recommended by the manufacturer may be used.

Dissolve 36.6g in 1 litre of distilled water and bring gently to the boil to dissolve. Sterilize by autoclaving at 121ºC for 15 minutes. Cool to 50ºC and pour 15ml of medium into sterile petri dishes or hold at 45ºC when using the pour plate technique. .

Tryptone Soya Agar (Oxoid CM131)

Component	g/l
Tryptone	15.0
Soya peptone	5.0
Sodium Chloride	5.0
Agar	15.0 (top it to 17.5g)

Suspend 40.0g in 1 litre distilled water and bring to the boil to dissolve the medium completely. Dispense into flasks and sterilize by autoclaving at 121°C for 15 minutes. Adjust the pH to 7.3±0.2 before and after sterilization.

Baird Parker Rabbit Plasma Fibrinogen Agar Medium (Oxoid CM0275)

Components	g/l
Sodium pyruvate	10.0
Lithium chloride	5.0
Pancreatic digest of casein	10.0
Glycine	12.0
Yeast extract	1.0
Meat exract	5.0
Agar	12.0 to 22.0

Commercially available solution of bovine fibrinogen/ rabbit plasma

Suspend 63g in 1 litre water and bring to the boil to dissolve the medium completely. Dispense into flasks and sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add 50ml of Egg Yolk Tellurite Emulsion. Mix well and pour into plates. Adjust pH to 6.8±0.2 before and after sterilization.

Urea Agar Base (Oxoid CM0053)

Components	g/l
Peptone	1.0
Glucose	1.0

Di – Sodium Phosphate	1.2
Potassium Dihydrogen Phosphate	0.8
Phenol Red	0.012
Agar	15.0

Suspend 2.4g in 95ml of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 115ºC for 20 minutes. Cool to 50ºC and aseptically one ampoule puffs sterile urea solution. Mix well, distribute 10ml amounts into sterile container and allow setting in the slope position. Adjust pH to 6.8± 0.2 before and sterilization.

Triple Sugar Iron Agar (CM277)

Component	g/l
Lab-Lemco Powder	3.0
Yeast Extract	20.0
Sodium Chloride	5.0
Lactose	10.0
Sucrose	10.0
Glucose	1.0
Ferric citrate	0.3
Sodium thiosulphate	0.3
Agar	12.0 (top it to 17.5g)

Suspend 65g in 1 litre of distilled water. Bring to boil to dissolve completely. Mix well and distribute 10ml into McCartney bottles. Sterilize by autoclaving at 121ºC for 15 minutes. Allow the medium to set in slope form with a butt about 1 inch deep. Adjust pH to 7.4± 0.2 before and after sterilization.

Tryptone water (CM0087)

Components	g/l
Tryptone	1.0
Sodium chloride	5.0

Dissolve 15g in 1 litre of distilled water. Dispense 5ml into test tube and sterilize by autoclaving at 121^{o}C for 15 minutes. Adjust pH to 7.4± 0.2 before and after sterilization.

XLD medium (CM469)

Component	g/l
Yeast Extract	3.0
L-Lysine Hcl	5.0
Xylose	3.75
Lactose	7.5
Sucrose	7.5
Sodium desoxycholate	1.0
Sodium chloride	5.0
Sodium thiosulphate	6.8
Phenol red	12.5 (top it to 5g)

Suspend 53 in 1 litre of distilled water. Heat with frequent agitation until the medium boils. Do not overheat. Transfer to a water bath at 50^{o}C pour into plates. Adjust pH to 7.4±0.2 before and sterilization.

Rappaport Vassiliadis Broth (CM866)

Components	g/l
Sodium chloride	7.2
Potassium dehydrogen sulphate	1.26
Di-potassium hydrogen phosphate	0.8
Magnesium chloride	13.58
Malachite green	0.36

Add 2.75g to 1 litre of distilled water and heat gently to dissolve. Dispense 10ml volumes into MaCartney bottles and sterilize by autoclaving at 115^{o}C for 15 minutes and adjust pH to 2±0.2 before and after sterilization.

Tetrationate Broth (CM 0671)

Components	g/l
Casein peptone	2.5

Meat peptone	2.5
Bile salts	1.0
Calcium carbonate	10.0
Sodium thiosulphate	30.0

Dissolve 46g in 1 litre of distilled water and bring to the boil. Cool to below 45°C and add 20ml of iodine-iodide solution immediately before use. Mix continuously whilst dispensing 10ml volumes into sterile tubes. Use the complete medium (with added iodine) on the day of preparation.

Iodide- Iodine Soluttion

Iodine	6.0g
Potassium iodide	5.0g
Distilled water	20ml

The base was prepared beforehand and kept for several weeks at 4°C. Iodine-iodide solution was then added at the time of use to the quantity of medium needed.

Nutrient Agar (CM0003)

Components	g/l
Meat exract	3.0
Peptone	5.0
Agar	12 to 18

Suspend 20.0g in distilled water and bring to the boil to dissolve the medium completely. Sterilize by autoclaving at 121^{o}C for 15 minutes. Adjust pH to 7.0± 0.2 before and after sterilization. Cool to about 45^{o}C and pour 10ml to 15 ml into sterilized petri dishes.

Bismuth Sulphite Agar (CM 0201)

Components	g/l
Peptone	5.0
“Lab –lemco” powder	5.0
Glucose	5.0
Disodium phosphate	4.0
Ferrous sulphate	0.3
Bismuth sulphate indicator	8.0

Brilliant green 0.016

Suspend 20g in 500ml of distilled water in a 1 litre flask. Heat gently with frequent agitation until the medium just begins to boil and simmer for 30 seconds to dissolve the agar. Cool to 50-55°C, mix well to disperse suspension and pour thick plates (25 ml medium per plate).

Adjust the pH 7.6 ± 0.2.

Appendix 2

Ghana Standards Authority Standard Specification for fresh fruits and vegetables

Organisms'	Microbial Count Colonies/ ml
Total Viable count	10^3
E. coli	10^2
Staphylococcus	10^2
Salmonella spp	absent

Crosstabulation between source of grapes collection and detection of *S.tyhpimurium*

			Salmonella typhimurim	Total
			none detected	
Source of Sample	MWV	Count	4	4
		Expected Count	4.0	4.0
	LMRV	Count	4	4
		Expected Count	4.0	4.0
	DRV	Count	4	4
		Expected Count	4.0	4.0
	JM	Count	4	4
		Expected Count	4.0	4.0
	AM	Count	4	4
		Expected Count	4.0	4.0
	KS	Count	4	4
		Expected Count	4.0	4.0
Total		Count	24	24
		Expected Count	24.0	24.0

Table 4.9Source of Sample * Escherichia coli Crosstab

			Escherichia coli	Total
			acceptable	
Source of Sample	MWV	Count	4	4
		Expected Count	4.0	4.0
	LMRV	Count	4	4
		Expected Count	4.0	4.0
	DRV	Count	4	4

		Expected Count	**4.0**	**4.0**
	JM	**Count**	**4**	**4**
		Expected Count	**4.0**	**4.0**
	AM	**Count**	**4**	**4**
		Expected Count	**4.0**	**4.0**
	KS	**Count**	**4**	**4**
		Expected Count	**4.0**	**4.0**
Total		**Count**	**24**	**24**
		Expected Count	**24.0**	**24.0**

APPENDIX 3: SPSS Output Tables

External Total Viable Count ANOVA						
		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Total Viable count	Between Groups	1.875	5	.375	1.800	.164
	Within Groups	3.750	18	.208		
	Total	5.625	23			
Staphylococcus aureus	Between Groups	2.708	5	.542	3.000	.038
	Within Groups	3.250	18	.181		
	Total	5.958	23			

Source of Sample * Total Viable count

Crosstabulation					
			Total Viable count		Total
			acceptance	exceed limit	
Source of Sample	MWV	Count	0	4	4
		Expected Count	2.0	2.0	4.0
	LMRV	Count	0	4	4
		Expected Count	2.0	2.0	4.0
	DRV	Count	2	2	4
		Expected Count	2.0	2.0	4.0
	JM	Count	4	0	4
		Expected Count	2.0	2.0	4.0
	AM	Count	3	1	4
		Expected Count	2.0	2.0	4.0
	KS	Count	3	1	4
		Expected Count	2.0	2.0	4.0
Total		Count	12	12	24
		Expected Count	12.0	12.0	24.0

Chi-Square Tests			
	Value	df	Asymp. Sig. (2sided)
Pearson Chi-Square	14.000[a]	5	.016
Likelihood Ratio	18.729	5	.002
Linear-by-Linear Association	9.255	1	.002
N of Valid Cases	24		

Source of Sample * Staphylococcus aureus					
			Staphylococcus aureus		Total
			acceptable	exceed limit	
Source of Sample	MWV	Count	1	3	4
		Expected Count	2.7	1.3	4.0
	LMRV	Count	1	3	4
		Expected Count	2.7	1.3	4.0
	DRV	Count	4	0	4
		Expected Count	2.7	1.3	4.0
	JM	Count	4	0	4
		Expected Count	2.7	1.3	4.0
	AM	Count	3	1	4
		Expected Count	2.7	1.3	4.0
	KS	Count	3	1	4
		Expected Count	2.7	1.3	4.0
Total		Count	16	8	24
		Expected Count	16.0	8.0	24.0

Chi-Square Tests			
	Value	df	Asymp. Sig. (2sided)
Pearson Chi-Square	10.500[a]	5	.062
Likelihood Ratio	12.558	5	.028
Linear-by-Linear Association	3.943	1	.047
N of Valid Cases	24		

Crosstabulation between source of grape collectionand detection of *S. typhimurium*				
			Salmonella typhimurim	**Total**
			none detected	
Source of Sample	**MWV**	Count	4	4
		Expected Count	4.0	4.0
	LMRV	Count	4	4
		Expected Count	4.0	4.0
	DRV	Count	4	4
		Expected Count	4.0	4.0
	JM	Count	4	4
		Expected Count	4.0	4.0
	AM	Count	4	4
		Expected Count	4.0	4.0
	KS	Count	4	4
		Expected Count	4.0	4.0
Total		Count	24	24
		Expected Count	24.0	24.0

Internal Total Viable Count LoadANOVA						
		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Total Viable count	Between Groups	3.500	5	.700	5.040	.005
	Within Groups	2.500	18	.139		
	Total	6.000	23			
Staphylococcus aureus	Between Groups	2.708	5	.542	3.000	.038
	Within Groups	3.250	18	.181		
	Total	5.958	23			

Table 4.9b	**Chi-Square Tests**		
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	8.000[a]	5	0.156
Likelihood Ratio	10.621	5	0.059
Linear-by-Linear Association	4.220	1	0.040
N of Valid Cases	24		
Significant at p< 0.05			

Crosstabulation between source of grape collection and determination of *E.coli*				
			Escherichia coli **acceptable**	**Total**
Source of Sample	**MWV**	Count	4	4
		Expected Count	4.0	4.0
	LMRV	Count	4	4
		Expected Count	4.0	4.0
	DRV	Count	4	4
		Expected Count	4.0	4.0
	JM	Count	4	4
		Expected Count	4.0	4.0
	AM	Count	4	4
		Expected Count	4.0	4.0
	KS	Count	4	4
		Expected Count	4.0	4.0
Total		Count	24	24
		Expected Count	24.0	24.0