SPOILAGE MOULDS IN CURED VANILLA BEANS IN TANZANIA: A CASE STUDY OF KILIMANJARO REGION

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN FOOD QUALITY AND SAFETY ASSURANCE OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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ABSTRACT

A study was carried out to identify and control the moulds responsible for spoilage of cured vanilla (*Vanilla planifolia*) beans and assessment of food safety knowledge, good manufacturing practices (GMP) and good hygiene practices (GHP of vanilla handlers in Kilimanjaro region. Five moulds namely, *Aspergillus fumigatus, Aspergillus tubingensis, Aspergillus aculeatus, Byssochlamys spectabilis (*anamorph: *Paecilomyces variotii)* and *Penicillium polonicum* were isolated from cured vanilla beans. The control of mould growth was carried out by using 30%, 50% and 70% ethanol (vol/vol.) for 5 min and 10 min. Sterile green vanilla beans inoculated with spores of *A. tubingensis, A. aculeatus* and *B. spectabilis* were incubated overnight, followed by blanching at 65ºC for 3 min and continuation of regular processes, i.e. sweating also known as fermentation and drying. Ethanol concentration of 50% and 70% completely inhibited the growth of moulds. The knowledge of handlers on food safety was satisfactory, while it was unsatisfactory on GMP and GHP. In vanilla curing process, blanching of green vanilla beans was identified as a critical control point (CCP) followed by fermenting “sweating”, drying and conditioning “curing maturation” steps as operational prerequisite programme (OPRP).

DECLARATION

I, KIBUNJE MAGEME KULWA, do hereby declare to the senate of the Sokoine University of Agriculture that this dissertation is my original work, done within the period of registration and that it has neither been submitted nor been concurrently submitted for a higher degree award in any other institution.

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The above declaration is confirmed by;

Prof. Jovin K. Mugula Date

(Supervisor)

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DEDICATION

This work is dedicated to my beloved wife, Magreth J. Mboyi, daughter Elina N. Kibunje and my parents Mr. and Mrs. Mageme.

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LIST OF ABBREVIATIONS AND ACRONYMS

µL	microlitre
ANOVA	Analysis of Variance
$_aw$	Water activity
BLAST	Basic Alignment Search Tool
bp	base pair
CAC	Codex Alimenterious Commission
ddH_2O	double distilled water
DNA	Deoxyribonucleic Acid
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FDA	Food and Drugs Authority
g	gravitation
GHP	Good Hygienic Practices
GMP	Good Manufacturing Practices
GRAS	Generally Recognized As Safe
h	hour
ISO	International Organization for Standardization
ITS	Internal Transcribed Spancer
min	Minutes
OPRP	Pre-requisite programme
PCR	Polymerase Chain Reaction
PDA	Potatoes Dextrose Agar
PE	Polyethylene
pmol	Picomole
RH	Relative Humidity

rpm	Revolution per minutes
SACIDS	Southern African Centre for Infectious Disease Surveillance
SPSS	Statistical Package for Social Sciences
SUA	Sokoine University of Agriculture
USA	United States of America
vol/vol.	volume by volume

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

1.1.1 Vanilla plant

Vanilla (*Vanilla planifolia*) belongs to the orchid family. It is a large green-stemmed creeping or climbing perennial found in the shade of humid, evergreen tropical forest and watershed areas climbing up trees (Anuradha *et al.*, 2013; Lim, 2018). However, vanilla flowers are hermaphrodite, but not able to self-pollinate, instead are hand-pollinated (Gallage and Møller, 2018; Lim, 2018). After pollination, the fruit, which is known as bean, takes 8-9 months to mature (Odoux, 2011; Gallage and Møller, 2018; Lim, 2018).

Natural vanilla flavour is a complex mixture of more than 200 flavour components and is obtained primarily from cured beans of either *Vanilla planifolia* or *V. tahitensis* (Cai *et al.*, 2019). It is the highest-priced flavour, labour-intensive, time consuming both in growing and processing and second most expensive spice after saffron, (BTC, 2013; Van Dyk *et al.*, 2014; Pardío *et al.*, 2018). It is widely used both commercially and domestically in food (60%), cosmetics (33%) and aromatherapy (7%) (BTC, 2013; Van dyk *et al.*, 2014; Cai *et al.*, 2019).

In East Africa, particularly Tanzania, vanilla is an alternative cash crop and supplements farmers' incomes in integrated cropping systems (BTC, 2013; Busungu, 2009). In Tanzania, it is entirely produced under conventional plantations intercropped with banana, jackfruit, bread tree, orange, coconut and or coffee which provide shade (Busungu, 2009; Maerere and Wilhelmus, 2014). It is mainly produced in Kagera, Kilimanjaro, Arusha, Morogoro regions and to some extent in Zanzibar.

1.1.2 Harvesting and post harvesting handling of vanilla beans

Fresh matured green vanilla beans are harvested odourless and lack the characteristic vanilla flavour. However, in order to initiate the formation of their distinctive flavour, vanilla beans are subjected to a specific process, called curing (Pérez-Silva *et al.,* 2011).

Fresh matured green vanilla beans are harvested when they reach their commercial maturity (Anuradha *et al.,* 2013). Green vanilla beans when harvested immature weigh less, do not develop the requisite full-bodied aroma and proper colour during processing and are more susceptible to fungal attack, notably *Penicillium* and *Aspergillus* spp. and when cured, they yield smaller quantities of vanillin (Odoux, 2011).

1.1.3 Curing of vanilla beans

Curing of beans starts within three days after harvest (Lim, 2018). The curing techniques are of broad range, each vanilla growing region/country has devised its own method. The most known curing methods are Mexan, Buorbon and Tahitian methods (Havkin-Frenkel and Belanger, 2018). However, the Bourbon curing method is practiced in Tanzania and involves four separate steps, that is, blanching (also referred to as killing), fermentation or " sweating", drying and conditioning steps (Odoux and Grisoni, 2011; Pérez Silva *et al.,* 2011). According to De Guzman and Zara (2012), the successive steps after killing are more or less similar in different countries producing vanilla. Fermentation is carried out to develop the proper texture and flexibility and is terminated when beans become pliable; followed by slow drying process and finally conditioning and storage (Brunschwig *et al.,* 2017).

1.1.4 Microbiological quality

Like other food spices in the value chain, vanilla is also susceptible to a number of fungal and a few viral diseases which cause considerable damage to the beans or to the whole plant, resulting into heavy crop losses (Sundaramoorthy *et al.*, 2017). Microbial contamination of vanilla beans, mainly by moulds and bacteria, can occur at harvest and through the several steps of handling and processing (-Sarter, 2011; Havkin-Frenkel and Belanger, 2018). During curing, after blanching by immersion in hot water 65–70°C for 2 min, the moulds mainly black *Aspergillus* and green *Penicillium* spp. and several bacteria such as *Bacillus* spp (*Bacillus subtilis, Bacillus firmus, Bacillus licheniformis, Bacillus pumilis, Bacillus smithii*) are found on vanilla beans (Odoux, 2011).

1.1.5 Use of ethanol to control growth moulds

Ethanol is safe to use for sterilization, fungicidal and insecticidal treatments since it is a natural product. For example, Dao *et al.* (2008) observed that under more drastic conditions such as a_w of 0.7, 30°C, 10% ethanol vol/vol, spores of *P. digitatum, P. italicum* and *P. chrysogenum* were inactivated.

1.2 Problem Statement and Justification

Vanilla production in Tanzania is increasing rapidly, especially in Kilimanjaro, Arusha, Morogoro and Kagera region. Although there is limited information on vanilla processing by smaller processors in the country (Fehr, 2011), the vanilla industry is facing a challenge of failure to meet quality and safety requirements due to microbial, especially mould contamination.

Vanilla is exposed to a wide range of microbial contamination during processing, storage, distribution, sale and/or use (Sagoo *et al.*, 2009). The curing process, which takes place for several months (Odoux, 2011), involves drying of cured vanilla by spread out on the ground or lifted trays to dry under the sun which potentially exposes the product to the risk of contamination. The cured vanilla beans have moisture content in the range of 25 - 30%, and are kept in cartons or wooden boxes for conditioning for 3 - 6 months and subsequent storage. The conditions prevailing in these boxes are conducive for fungal growth (Moosa *et al.*, 2014).

According to Fehr (2011), there is no accurate information and figures on vanilla production and quality and safety of cured vanilla beans especially from the smaller producers in the country. However, the current situation in fields indicated that cured vanilla processors were unable to compete on market because of poor microbiological quality, particularly spoilage by moulds. There is no adequate information on control of spoilage of cured vanilla beans and causative moulds in Tanzania.

The identification of spoilage moulds, conditions which facilitate spoilage and those which control it, would be important in the improvement of the quality of the product and subsequent income generation. The aim of this study was to identify spoilage moulds and assess awareness of best practices in vanilla value chain and use of ethanol to control fungal contamination during curing process in order to improve the quality of cured vanilla beans hence improvement of income and livelihood of vanilla stakeholders in Tanzania.

1.3 Objectives

1.3.1 Overall objective

The aim of this study was to identify, control spoilage moulds in cured vanilla and assess awareness of best practices among vanilla processors in Tanzania.

1.3.2 Specific objectives

The specific objectives of the study were to:

i. identify mould species responsible for spoilage of cured vanilla.
ii. assess efficacy of ethanol in the control of growth of moulds.
iii. identify critical control point of moulds contamination in vanilla post-harvest supply chain.

1.4 List of Manuscripts

i. Use of ethanol to control spoilage moulds in vanilla beans
ii. Awareness of good practices in vanilla value chain in Tanzania

The findings of this research were reported in two manuscripts presented in chapter two and three.

References

Anuradha, K., Shyamala, B. N. and Naidu, M. M. (2013). Vanilla-its science of cultivation, curing, chemistry and nutraceutical. *Critical Reviews in Food Science and Nutrition* 53(12): 1250–1276.

BTC Trade for Development (2013). An assessment of market potential for vanilla in East Africa Tanzania, Uganda and Kenya. [http://www.befair.be/en/publication/market-studies/assesment-market-potential-vanilla-products-east-africa] site visited on 29/4/2018.

Busungu, C. (2009). Genetic Diversity of Vanilla *planifolia G.* Jackson, *syn. V. fragrans* Crop grown in Tanzania using molecular techniques. Dissertation for Award of MSc Degree at Sokoine University of Agriculture, Morogoro, Tanzania, 90pp.

Cai, Y., Gu, F., Hong, Y., Chen, Y., Xu, F. and An, K. (2019). Metabolite Transformation and Enzyme Activities of Hainan Vanilla Beans During Curing to Improve Flavor Formation. *Molecules* 24: 2781-2796.

Dao, T., Bensoussan, M., Gervais, P. and Dantigny, P. (2008). Inactivation of conidia of Penicillium chrysogenum , P . digitatum and P . italicum by ethanol solutions and vapours. *International Journal of Food Microbiology* 122: 68–73.

Fehr, C. (2011). Vanilla production in East Africa Uganda, Tanzania, Kenya, and Eastern Democratic Republic of Congo. In: *Vanilla: Medicinal and Aromatic Plants — Industrial Profiles.* (Edited by Odoux, E. and Grisoni, M.), CRC Press Taylor and Francis Group, Boca Raton. pp. 327 – 332.

Gallage, N. J. and Møller, B. L. (2018). Vanilla: The most popular flavour. In:*Biotechnology of Natural Products.* (Edited by Schwab, W., Lange, B. M.

and Wüst, M.), Springer International Publishing, New York. pp. 3 – 24.

Lim, T. K. (2018). Vanilla planifolia. In: *Edible Medicinal and Non-Medicinal Plants*. Springer Science+Business Media, New York. pp. 106 – 116.

Maerere, P. and Wilhelmus, C. J. (2014). Tanzania spices sub sector strategy. international trade center. [http://hdl.handle.net/20.500.12018/7198] site visited on 20/5/2018.

Moosa, S., Yusof, S. C. M., Bahrin, R. and Nasir, M. A. M. (2014). Effect of gamma radition on major aroma coumpunds and vanillin glucosides of cured vanilla glucoside of cured vanilla beans (Vanilla planifolia). *Jurnal Sains Nuklear Malaysia* 26(1): 18–24.

Odoux, E. (2011). Vanilla Curing. In: *Vanilla: Medicinal and Aromatic Plants — Industrial Profiles.*(Edited by Odoux, E. and Grisoni, M.), CRC Press Taylor and Francis Group, Boca Raton. pp. 173 – 183.

Pérez Silva, A., Gunata, Z., Lepoutre, J. P. and Odoux, E. (2011). New insight on the genesis and fate of odor-active compounds in vanilla beans (Vanilla planifolia G. Jackson) during traditional curing. *Food Research International* 44(9): 2930 –2937.

Sagoo, S. K., Little, C. L., Greenwood, M., Mithani, V., Grant, K. A., McLauchlin, J. and Threlfall, E. J. (2009). Assessment of the microbiological safety of dried spices and herbs from production and retail premises in the United Kingdom. *Food Microbiology* 26(1): 39–43.

Van Dyk, S., Holford, P., Subedi, P., Walsh, K., Williams, M. and McGlasson, W. B. (2014). Determining the harvest maturity of vanilla beans. *Scientia Horticulturae* 168: 249 –257.

CHAPTER TWO

2.0 Use of Ethanol to Control Spoilage Moulds in Vanilla Beans

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2.1 Abstract

The aim of this study was to identify and control spoilage moulds in cured vanilla beans using ethanol. Five moulds, namely *Aspergillus fumigatus, Aspergillus tubingensis, Aspergillus aculeatus, Byssochlamys spectabilis (*Amanorph *Paecilomyces variotii)* and *Penicillium polonicum* were identified in cured vanilla. Sterile green vanilla beans were inoculated with spores of *A. tubingensis, A. aculeatus* and *B. spectabilis* (Amanorph *Paec. variotii*), kept overnight at ambient conditions, then treated with ethanol at concentration of 0% (control), 30%, 50% and 70% for 5 and 10 min. The inoculated beans were then blanched at 65^{0}C for 3 min and incubated. Treatment with 50% and 70% ethanol completely inhibited growth of moulds therefore; these concentrations can be used as controlling moulds on vanilla beans during curing process.

Key words: vanilla, curing, moulds, DNA sequence, mycotoxin, quality, safety

2.2 Introduction

Vanilla (*Vanilla planifolia*) is a spice highly valued for its aroma and flavour in the food, pharmaceutical and fragrance industries. Also, due its high value, vanilla is a source of income in producing countries like Madagascar, Indonesia, Uganda and others (Kumar and Balamohan, 2013). In East Africa, vanilla is produced in Kenya, Tanzania, Congo and Uganda however; the current leading producer is Uganda (Fehr, 2011).

Although the production of vanilla in Tanzania is increasing, vanilla processors face challenges of proliferation of moulds during curing (Maerere and Wilhelmus, 2014). Moulds contamination in the commodity value chain undermines the overall competitiveness of the contaminated products in the world market (Havkin-Frenkel and Belanger, 2018). Generally, spoiling fungi might be toxigenic or pathogenic (Al-hindi *et al*., 2011). Besides causing direct pathogenesis, they may produce mycotoxins which pose hazards to human and animal health (Al-hindi *et al.,* 2011; Hue *et al.,* 2013).

Currently, there is no standard procedure that has been established to control postharvest mould contamination across vanilla value chain unlike in other fruits such as mangoes, guavas or oranges for which fumigation method is mostly used. However, Sagoo *et al.* (2009) and Schaarschmidt *et al.* (2016) highlighted that the control of microbial contamination in spices including vanilla can be done by application of GHP and GMP in production areas.

On the other hand, use of chemicals to control fungi has global concerns about environmental pollution and health risks (Ponzo *et al.,* 2018). Thus, in order to control postharvest fungal contamination in spice industry including vanilla, it is important to identify contamination control points and develop safe, effective, economical, alternative strategies compatible with commercial handling (Homaida *et al.,* 2017).

Ethanol is designated as Generally Recognized as Safe (GRAS) (Ji *et al.,* 2019) It is a common food component with potent antifungal activity that has been used for a long time for treatment against many mould species that contaminate and spoil food products (Yuen *et al.,* 1995; Karabulut *et al.,* 2004; Berni and Scaramuzza, 2013). Also, many studies have reported ethanol treatment controlled fungal postharvest diseases of blueberries, table grape, mango, loquat, guava, grapes (Lichter *et al.,* 2002; Karabulut *et al.,* 2004; Akgun *et al.,* 2005; Chervin *et al.*,2005; Pinto *et al.*,2006; Romanazzi *et al.,* 2007; Osuna *et al.,* 2012; Groot *et al.*, 2018; Ponzo *et al.,* 2018; Ji *et al.,* 2019).

Karabulut *et al.* (2004) reported a complete inhibition of *Botrytis cinerea* spores germination in table grapes by ethanol of 30% at 50ºC for 30 sec and Ponzo *et al.* (2018) reported significant reduction of anthracnose and severity on guavas by ethanol of 40% for 2 min at 25ºC. Furthermore, Gabler *et al.* (2004) found that 20% of ethanol at 50º C was completely inhibited germination of *Rhizopus stolonifer, Aspergillus niger, Botrytis cinerea* and *Alternaria alternate*. Therefore, the aim of this study was to characterize spoilage moulds in cured vanilla beans and assess the efficacy of ethanol at different concentrations to control mould contamination during vanilla curing process.

2.3 Materials and Methods

2.3.1 Samples collection

Twenty samples of cured vanilla beans from 20 different batches (about 50g each batch) were collected from the curing centres in Kilimanjaro region, Tanzania. The samples were aseptically collected in sterile polyethylene bags, labelled and transported to African Seed Health Centre Laboratory, at Sokoine University of Agriculture, Morogoro and stored at

4°C until analysis.

2.3.2 Isolation of moulds

Three methods were used for isolation of moulds. These were: the blotter test (Narayanasamy, 2017), direct plating of vanilla cuts into potatoes dextrose agar (PDA) with incubation at 25±3°C and washing off the surfaces of intact cured vanilla bean (Nega, 2014). The blotter method gave the maximum growth of moulds compared to the other methods. Therefore, blotter method was chosen for isolation of moulds. Non-sterilized samples were evenly placed at the rate of 4 pieces per Petri plate at equal distance in each Petri plate on three layers of sterile moistened 9 cm diameter Whiteman filter paper in sterilized Petri dishes. The plates were incubated for 7-15 days at 25±3°C. After incubation the samples were examined under microscope and the fungi developing on samples were transferred to sterile PDA (HI media, India) for purification and identification (Toma and Abdulla, 2013; El-Gali, 2014). The isolates were maintained at 4°C until when used for DNA extraction.

2.3.3 Morphological identification of moulds

The sub-cultured isolates were investigated for pigment production and colony characteristics (Kim *et al.*, 2013). The conidia, hyphae, conidial head, conidiophores, spores, and colour were observed under ×400 microscope magnification (Leica GME, Switzerland) for morphological identification at the African Seed health Centre, Sokoine University of Agriculture.

2.3.4 Identification of moulds by using molecular biological technique

2.3.4.1 DNA extraction

The isolates were transferred into PDA and incubated at 25±3°C for 5 days. Total DNA was extracted from mould mycelia using the procedure described by (Don Liu *et al.,* 2000) with modification. Briefly, a small lump of mycelia was disrupted by using a sterile scalpel and added into a 150 µL Eppendorf tube containing 500 µL of lysis buffer (400 mM Tris-HCl [pH 8.0], 60 mM EDTA [pH 8.0], 150 mM NaCl,1% sodium dodecyl sulfate), and the tube was left at room temperature for 10 min.

Thereafter, 150 µL of potassium acetate (pH 4.8; which was made of 60 mL of 5 M potassium acetate, 11.5 mL of glacial acetic acid, and 28.5 mL of distilled water) was added into the tube before being vortexed at >10 000 x g for 1 min The supernatant was transferred to another 150 µL Eppendorf tube and centrifuged again, as described above. After transferring the supernatant to a new 150µL Eppendorf tube, an equal volume of isopropyl alcohol was added and the tube was mixed by inversion briefly.

The tube was centrifuged at >10 000 x g for 2 min, and the supernatant was discarded. The resultant DNA pellet was washed in 300 µL of 70% ethanol. After the pellet was centrifuged at 10 000 rpm for 1 min, the supernatant was discarded. The DNA pellet was air dried, re-suspended in 100 µL ddH_2O and stored at -20^{0}C (Lin *et al.,* 2014) until further analysis.

2.3.4.2 PCR Amplification and Sequencing

The PCR amplifications were carried out in a total volume of 25 µL, containing 10 ng template DNA, 10X PCR buffer premix (puReTaq Ready-To-Go™ PCR kit, Germany) and 10 pmol of ITS1/ITS4. The sequences of the ITS1 and ITS4 primers were 5‘-TCCGTAGGTGAACCTGCGG-3‘and5-TCCTCCGCTTATTGATATGC-3, respectively (Hussain *et al.*, 2018).

The PCR amplification was carried out according to the following temperature profile as was described by (Khare *et al.*, 2018) with modification. In brief, the polymerase chain reaction was performed in 25 μL of reaction mixture using template DNA isolated by the method described earlier. The template DNA was amplified in Applied Bio system (GeneAmp PCR system 9700, Singapore) with 30 cycles, each cycle at 94ºC for 3 min for denaturation, 0.45 min at 55ºC for annealing, 1.25 min at 72ºC for extension and a 10 min final extension at 72ºC.

The amplified PCR products were resolved by gel electrophoresis in a 1.5% agarose (Model: 08-1214, Rated 150V, 100 mA Class II, Galileo Bioscience) gel stained in 0.5 mg/mL ethidium bromide in TBE buffer at 100V for 45 min. The DNA bands resolved on agarose gel were visualized in UV transilluminator and photographed. The sizes of the amplicon was estimated after comparing with a commercial 100 bp DNA ladder on agarose gel. The PCR products were stored at 4ºC till sequencing (Olagunju *et al***.**, 2018).

The DNA sequencing was carried out at Southern African Centre for Infectious Disease Surveillance (SACIDS), SUA, Morogoro) and five amplicons were sequenced. The sequences were assembled, edited and aligned by using the Geneous software (Version no.10.2.3, Biomatters Ltd, New Zealand) then blasted against known sequences in the GenBank using BLAST to find regions of local similarity between sequences in order to identify the species (Bechem and Afanga, 2017).

Table 2.1: The ITS primer pair used in this study

Primer ⟶	Sequence 5’ 3’
ITS 1 (Forward)	TCCGTAGGTGAACCTGCGG
ITS 4 (Reverse)	TCCTCCGCTTATTGATATGC

2.3.5 Control of moulds by using ethanol

2.3.5.1 Inoculation of green vanilla beans

From the culture in PDA medium incubated at 25°C for 7±1 day, spores of three fungal species identified before (*A. tubingensis, A. aculeatus, B.spectabilis* (anamorph: *Paec. variotii*) were collected by flooding the surface of the plates with sterile saline solution (NaCl, 9 g per litre of water containing Tween 80 (0.1% vol/vol; Prolabo, Paris, France) and spore suspensions was counted on haemocytometer using a compound microscope before standardized to $1x10^5$spores/mL.

The inoculation process was done by the procedure described by Karabulut *et al.* (2004) with modification. Briefly, the green vanilla bean was washed three times by dipping in a sterile ddH_2O for 2-3 min. then dried under laminar flow (Holten LaminAir, type HH 1.2 basis, Denmark) at ambient temperature. Artificial inoculation of green vanilla was done by puncturing the beans (3 mm deep approximately on both apex and blossom ends) with a sterilized scalpel and directly inoculated by dipping into a beaker containing 500mL spore suspension of each fungal specie ($1x10^5$ spores/mL).

Inoculated green vanilla beans were wrapped with sterile aluminium foils and left overnight under a sterile laminar air flow cabinet for fungi sticking on punctured beans. Three replicate units were used per treatment for each fungus. The treatments were as follows (1) inoculated green vanilla beans were dipped into sterile distilled for 5 and 10 min. and (2) Inoculated: green vanilla beans were dipped into ethanol solution of 30, 50, and 70% (vol/vol) for 5 and 10 min per each. After treatment, all samples were blanched at temperature of 65º C for 3 min. The blanched vanilla beans were incubated at 48±2ºC for 72 h before stored at 30±2ºC for drying to approximately 25% of moisture content.

2.3.5.2 Statistical analysis

Experiments were performed using a completely randomized design. All statistical analyses were performed with SPSS software (version 21, IBM Corporation, New York, USA). The data were analysed by two-way analysis of variance (ANOVA). Mean separations were performed by Boniferon range tests. Differences at $P < 0.05$ were considered as significant.

2.4 Results and Discussion

2.4.1 Morphological identification of spoilage moulds

In the current study, various fungal mycelia were observed on the surface of vanilla cuts and the isolated fungi were examined on the basis of molecular and morphological characteristics of which five isolates were identified; that is *Aspergillus aculeatus, Aspergillus fumigatus Aspergillus tubingensis*, *Byssochlamys spectabilis* (anamorph: *Paecilomyces variotii*) and *Penicillium polonicum.*

On the morphological features, *A. tubingensis* revealed a black colour colony with whitish on the top and pale-yellow colonies on the reverse while the features of *A. aculeatus* were yellow to dark brown/grey tones colour on the top and pale yellow on the reverse (Silva *et al.*, 2011). On the other hand *B. spectabilis* (anamorph: *Paec. variotii*), the colour of the colonies was pale yellow and white at the margins nearly similar with those of *A. aculeatus*. The *A. fumigatus* colonies were dark green with white mycelia at the edges although white thick mycelia formed under the colonies and green yellow on reverse while *Penicillium polonicum* had white bluish colour and yellowish cream on reverse. All of these features were observed on PDA.

2.4.2 Molecular identification of the isolated fungi

Five fungal isolates were identified on the basis of their molecular characteristics. The amplification of 18S rRNA with ITS1 and ITS4 primers was successfully performed and 18S rRNA gene was chosen as a target for PCR amplification as is widely used in the molecular analysis (Hussain *et al.,* 2018). Thus, the ITS region of the rRNA gene is generally believed to represent a convenient target for the molecular identification of specific species of fungi (Zhang *et al.*, 2011).

Based on sequence similarity to corresponding sequences in the GenBank, all the five isolates were identified to species as shown in the Table 2.2 Sequence analysis of the ITS regions of the nuclear encoded rDNA showed significant alignments of 81.96-100 % with the isolated fungal species.

Of all isolates identified, *B. spectabilis (*Anamorph*: Paec. variotii), P. polonicum* and *Aspergillus fumigatus* have food safety concern as they can cause mycotoxin in food stuffs. *B. spectabilis* and *A. fumigatus* are among of the heat-resistant fungi important to the food industry because they can resist heat treatments used for food processing and can grow and spoil the products during storage at room temperature (Moreira *et al.,* 2018).

Table 2.2: Identification of mould isolates by ITS region of rRNA gene sequence

Identified fungal species	Length (bp)	Identity (%)	Coverage (%)	Access number (GenBank)
Aspergillus fumigatus	602	100	100	MH892837.1
Aspergillus tubingensis	603	100	100	MH045586.1
Aspergillus aculeatus	577	100	100	MN187974.1
Byssochlamys spectabilis (Paecilomyces variotii)	615	100	99	KC157706.1
Penicillium polonicum	592	81.96	92	MH382817.1

These species are ubiquitous thermo-tolerant that commonly found in food products (including pasteurized), soil, indoor air environments and woods (Houbraken *et al.,* 2008). In general, *B. spectabilis (*Anamorph*: Paec. variotii)* can survive considerable periods of heat above 85 °C and can grow under very low oxygen conditions and produce mycotoxins such as viriditoxin and deoxynivalenol (Houbraken *et al.,* 2005; Casas-Junco *et al.,* 2018; Urquhart *et al.,* 2018).

P. polonicum has been reported by (Núñez *et al.,* 2000; Polizzi *et al.,* 2012) as an important food spoilage and airborne fungus found in indoor environments, grows better at 0.99 aw, and extreme temperatures, such as 4, - 37 ºC and RH of 97–100% and produce secondary metabolites including the potent neurotoxin verrucosidin and nephrotoxic compounds. So far, contamination of *B. spectabilis (*Anamorph: *Paec. variotii)* in cured vanilla was not reported before; however these species have been studied as spoilage in food industry (Moreira *et al.,* 2018). During curing process at a stage of sweating (fermenting), incubation temperature, relative humidity and time are about 45-50 ºC, RH of 95 - 100% and 48-72 h respectively (Röling *et al.,* 2001) however, regardless of high temperature fungal growth on blanched vanilla beans has been observed at this stage. Probably identified moulds*, A. fumigatus, B. spectabilis (*Anamorph: *Paec. variotii)* and *Penicillium polonicum* are among of the fungal species that grow on blanched vanilla beans during sweating “fermentation” process.

On the other hand, identification of *Aspergillus* and *Penicillium* species in this study was not surprising as previous studies have reported on their presence in spices including vanilla. For example, El-Gali, (2014) found *Aspergillus* and *Penicillium* genera are more frequently detected than other genera of fungi in spices. Furthermore, Röling *et al.* (2001) found that black *Aspergillus* and green *Penicillium* strains are the major fungi found on vanilla beans during curing. In addition, Kumar and Balamohan (2013) indicated that vanilla beans are susceptible to infection by storage moulds like *Aspergillus, Fusarium* and *Penicillium* due to harvesting of immature beans, improper killing and drying and high relative moisture content in beans.

Also, Sarter, (2011) in his study reported isolation of *Penicillium lividum, Penicillium vanillae, Penicillium rugulosum Aspergillus niger, Aspergillus oryzae* and *Aspergillus amstelodami* in vanilla beans from Madagascar and Comoros. Therefore, the isolation of *Aspergillus tubingensis (*which was confused with *A.niger* on microscopic identification*), Aspergillus aculeatus, Aspergillus fumigatus* and *Penicilium polonicum* in this study has proved that *Aspergillus* and *Penicillium* genera are the common spoilage and probably toxicogenic contaminants in cured vanilla beans than other genera. This is because *Aspergillus* and *Penicillium* spp are most xerotolerant or xerophilic and have ability to grow within a wide range of temperature (Berni and Scaramuzza, 2013).

2.4.3 Efficacy of ethanol on fungal growth control

As shown in Table 2.3, immersion of inoculated green vanilla beans in 50% and 70% ethanol for 5 and 10 min and blanching the same beans at temperature of 65^{o}C for 3 min completely eliminated the proliferation of *A. tubingesis, A.aculeatus* and *B. spectabilis*. This suggested that ethanol inhibited all development stages of the fungi that are; spore germination, germ tube elongation as well as sporulation (Yuen *et al.,* 1995). On the other hand, treatment of vanilla beans with 30% ethanol was less effective for inhibition of the growth of fungi but resulted to significant lower spores counts (P>0.05) compared to control treatment.

Gurtovenko and Anwar (2009) observed that the interaction of ethanol with biological membranes at concentrations below 30.5% (vol/vol) induces expansion of the membranes together with a reduction of their thickness, as well as causing disorders and enhancement of the inter-digitation of lipid acyl chains. However, the bilayer structure of the membranes is maintained.

In previous study, Lichter *et al.* (2002) observed that ethanol at 30% reduced survival of *B. cinerea* spores while at 40% concentration completely inhibited the spore germination. Also Sequeira *et al.* (2017) found that temporary contact of *P. chrysogenum* spores with ethanol resulted in significant reduction of conidia germination and mycelia development in samples treated with 70% and 100% than 0 and 30% ethanol solution. High ethanol concentration is efficient to control moulds since is associated with expansion and reduction of membrane thickness along with increasing hydrophilicity of the membrane interior due to accumulation of ethanol molecules which make the lipid/water interface unstable and prone to formation of defects (Gurtovenko and Anwar, 2009).

Table 2.3: Efficacy of ethanol in controlling growth of spoilage moulds in vanilla beans

Time (min)	***A. tubingesis***				***A.aculeatus***				***B. spectabilis***			
	Control	30%	50%	70%	Control	30%	50%	70%	Control	30%	50%	70%
5	4.55±2.06[a]	1.33±1.53[a]	0	0	4.11±1.76[a]	1.33[a]±1.53	0	0	7.11±2.09[a]	1.67± 0.58[a]	0	0
10	4.55±2.29[a]	1.33±0.58[a]	0	0	3.67±2.54[a]	1.67[a]±2.08	0	0	6.22±2.38[a]	1.33 ± 1.53[a]	0	0

All data with same superscript letter across columns have same mean difference significant at (P<0.05).

The results of this study indicated that *Byssochlamys* and *Paecilomyces* could be responsible for spoilage observed during fermentation in curing process. In addition, *Aspergillus* and *Penicillium* and *Byssochlamys* spp. are mycotoxigenic. Therefore, prevention measures based on Good Agricultural Practices (GMP) and Good Manufacturing Practices (GMP) should be observed to mitigate pre-harvest and post-harvest contamination by mycotoxins in vanilla beans. Also, the study indicated that 50% ethanol could be applied as disinfectant agent in combination with other hurdles such as temperature to control spoilage and improve safety in vanilla processing.

References

Akgun, O., Romanazzi, G., Smilanick, J. L. and Lichter, A. (2005). Postharvest ethanol and potassium sorbate treatments of table grapes to control gray mold. *Postharvest Biology and Technology* 37: 129 – 134.

Al-hindi, R. R., Al-najada, A. R. and Mohamed, S. A. (2011). Isolation and identification of some fruit spoilage fungi : Screening of plant cell wall degrading enzymes. *African Journal of Microbiology Research* 5(4): 443 – 448.

Anuradha, K., Shyamala, B. N. and Naidu, M. M. (2013). Vanilla- its science of cultivation, curing, chemistry and nutraceutical. *Critical Reviews in Food Science and Nutrition* 53(12): 1250 – 1276.

Bechem, E. T. and Afanga, Y. A. (2017). Morphological and molecular identification of fungi associated with corm rot and blight symptoms on plantain (Musa paradisiaca) in macro-propagators. *International Journal of Biological and Chemical Sciences* 11: 2793–2808.

Berni, E. and Scaramuzza, N. (2013). Effect of ethanol on growth of chrysonilia sitophila ('the red bread mould') and Hyphopichia burtonii ('the chalky mould') in sliced bread. *Letters in Applied Microbiology* 57(4): 344 – 349.

Casas-Junco, P. P., Ragazzo-Sánchez, J. A., Ascencio-Valle, F. de, J. and Calderón-Santoyo, M. (2018). Determination of potentially mycotoxigenic fungi in coffee (Coffea arabica L.) from Nayarit. *Food Science and Biotechnology* 27(3): 891–898.

Chervin, C., Westercamp, P. and Monteils, G. (2005). Ethanol vapours limit Botrytis development over the postharvest life of table grapes. *Postharvest Biology and Technology* 36(3): 319–322.

De Guzman, C. C. and Zara, R. R. (2012). Vanilla. *Handbook of Herbs and Spices: Second Edition* 1: 547–589.

El-Gali, Z. I. (2014). Detection of fungi associated with some spices in original form. *Global Journal of Scientific Researches* 2(3): 83–88.

Gabler, F. M., Mansour, M. F., Smilanick, J. L. and Mackey, B. E. (2004). Survival of spores of Rhizopus stolonifer, Aspergillus Niger, Botrytis cinerea and Alternaria alternata after exposure to ethanol solutions at various temperatures. *Journal of Applied Microbiology* 96: 1354 – 1360.

Gallage, N. J. and Møller, B. L. (2018). Vanilla: The most popular flavour. In: *Biotechnology of Natural Products.* (Edited by Schwab, W., Lange, B. M. and Wüst, M.), Springer International Publishing, New York. pp. 3 – 24.

Groot, M. N., Abee, T. and Veen, H. V. B. De. (2018). Inactivation of conidia from three *Penicillium* spp. isolated from fruit juices by conventional and alternative mild preservation technologies and disinfection treatments. *Food Microbiology* 81: 108 – 114.

Gurtovenko, A. A. and Anwar, J. (2009). Interaction of ethanol with biological membranes: The formation of non-bilayer structures within the membrane interior and their significance. *Journal of Physical Chemistry* 113(7): 1983–1992.

Havkin-Frenkel, D. and Belanger, F. C. (2018). Curing of Vanilla. In: *Handbook of Vanilla Science And Technology*. Wiley-Blackwell, Chichester. pp. 191 – 208.

Homaida, M. A., Yan, S. and Yang, H. (2017). Effects of ethanol treatment on inhibiting fresh-cut sugarcane enzymatic browning and microbial growth. *Food Science and Technology* 77: 8–14.

Houbraken, J., Varga, J., Rico-Munoz, E., Johnson, S. and Samson, R. A. (2008). Sexual reproduction as the cause of heat resistance in the food spoilage fungus Byssochlamys spectabilis (anamorph Paecilomyces variotii). *Applied and Environmental Microbiology* 74(5): 1613–1619.

Hue, N. T., Nhien, N. H., Thong, P. M., Thanh, C. N. and Van An, P. (2013). Detection of toxic Aspergillus species in food by a multiplex PCR. In: *4th International Conference on Biomedical Engineering in Vietnam Proceeding.* pp. 184-189.

Hussain, O. A., Sobhy, H. M., Hathout, A. S., Sayed, A. and Fouzy, M. (2018). Isolation and molecular identification of fusarium fungi from some Egyptian Grains. *Asian Journal of Plant Science* 17(4): 182–190.

Ji, Y., Hu, W., Jiang, A., Xiu, Z., Liao, J., Yang, X. and Feng, K. (2019). Effect of ethanol treatment on the quality and volatiles production of blueberries after harvest. [https://doi.org/10.1002/jsfa.9904] site visited on 20/7/2019.

Karabulut, O. A., Gabler, F. M., Mansour, M. and Smilanick, J. L. (2004). Postharvest ethanol and hot water treatments of table grapes to control gray mold. *Postharvest Biology and Technology* 34(2): 169–177.

Khare, R., Agarwal, M. K., Bhagayavant, S. S., Verma, P. and Nagar, D. P. (2018). Detection of Aspergillus flavus using PCR method from fungus infested food grains collected from local market. *Annals of Plant Sciences Research* 7(2): 2073–2077.

Kim, J. Y., Lee, S. Y. and Choi, H. S. (2013). Molecular and morphological identification of fungal species isolated from rice Meju. *Food Science Biotechnology* 22(3): 721–728.

Kumar, R. B. K. and Balamohan, T. N. (2013). Factors affecting the quality of vanilla - A review. *Journal of Agriculture and Allied Sciences* 2(3): 37 – 41.

Lichter, A., Zutkhy, Y., Sonego, L., Dvir, O., Kaplunov, T., Sarig, P. and Ben-Arie, R. (2002). Ethanol controls postharvest decay of table grapes. *Postharvest Biology and Technology* 24(3): 301–308.

Lim, T. K. (2018). Vanilla planifolia. In: *Edible Medicinal and Non-Medicinal Plants.* Springer Science+Business Media, New York. pp. 106 – 116.

Liu, D., Coloe, S., Baird, R. and Pedersen, J. (2000). Rapid mini-preparation of fungal deoxyribonucleic acid for polymarase chain reaction. *Journal of Clinical Microbiology* 38(1): 471 – 471.

Moreira, D., Oliveira, M., and Borba, C. (2018). Human pathogenic paecilomyces from food. *Microorganisms* 6(3): 1 – 64.

Narayanasamy, P. (2017). Detection and Differentiation Of Fungal. In: *Microbial Plant Pathogens: Detection and Management in Seeds and Propagules.* John Wiley and Sons Ltd, Chichester. pp. 12–133.

Nega, A. (2014). Isolation and identification of fungal pathogens associated with cold storage type of (Coffee Arabica) Ethiopia. *Journal of Biology,Agriculture and Healthcare* 4(25): 20–27.

Núñez, F., Díaz, M. C., Rodríguez, M., Aranda, E., Martín, A. and Asensio, M. A. (2000). Effects of substrate, water activity, and temperature on growth and verrucosidin production by Penicillium polonicum isolated from dry-cured ham. *Journal of Food Protection* 63(2): 231–236.

Odoux, E. (2011). Vanilla Curing. In: *Vanilla: Medicinal and Aromatic Plants — Industrial Profiles.*(Edited by Odoux, E. and Grisoni, M.), CRC Press Taylor and Francis Group, Boca Raton. pp. 173 – 185.

Olagunju, O., Mchunu, N., Venter, S., Guibert, B., Durand, N., Métayer, I. and Ijabadeniyi, O. (2018). Fungal contamination of food commodities in Durban, South Africa. *Journal of Food Safety* 38(6): 1–10.

Pinto, R., Lichter, A., Danshin, A. and Sela, S. (2006). The effect of an ethanol dip of table grapes on populations of Escherichia coli. *Postharvest Biology and Technology* 39: 308–313.

Polizzi, V., Adams, A., De Saeger, S., Van Peteghem, C., Moretti, A. and De Kimpe, N. (2012). Influence of various growth parameters on fungal growth and volatile metabolite production by indoor molds. *Science of the Total Environment* 414: 277 – 286.

Ponzo, F. S., Benato, E. A., Marçon, B. and Cia, P. (2018). Ethanol on the postharvest control of anthracnose in ‘ Kumagai ’ guava. *Bragantia* 77(1): 160 –167.

Rodríguez, A., Rodríguez, M., Andrade, M. J. and Córdoba, J. J. (2015). Detection of filamentous fungi in foods. *Current Opinion in Food Science* 5: 36 – 42.

Röling, W. F., Kerler, J., Braster, M., Apriyantono, A., Stam, H. and van Verseveld, H. W (2001). Microorganisms with a Taste for Vanilla : Microbial ecology of traditional Indonesian Vanilla Curing. *Applied and Environmental Microbiology* 67(5): 1995–2003.

Romanazzi, G., Karabulut, O. A. and Smilanick, J. L. (2007). Combination of chitosan and ethanol to control postharvest gray mold of table grapes. *Postharvest Biology and Technology* 45(1): 134–140.

Sagoo, S. K., Little, C. L., Greenwood, M., Mithani, V., Grant, K. A., McLauchlin, J. and Threlfall, E. J. (2009). Assessment of the microbiological safety of dried spices and herbs from production and retail premises in the United Kingdom. *Food Microbiology* 26(1): 39–43.

Sarter, S. (2011). Microbial safety of cured vanilla beans. In: *Vanilla: Medicinal and Aromatic Plants — Industrial Profiles.* (Edited by Odoux, E. and Grisoni, M.), CRC Press Taylor and Francis Group, Boca Raton. pp. 229 – 235.

Schaarschmidt, S., Spradau, F., Mank, H., Banach, J. L., van der Fels-Klerx, H. J., Hiller, P. and Mader, A. (2016). Public and private standards for dried culinary herbs and spices – Part II: Production and product standards for ensuring microbiological safety. *Food Control* 70: 360–370.

Sequeira, S. O., Phillips, A. J. L., Cabrita, E. J. and Macedo, M. F. (2017). Ethanol as an antifungal treatment for paper: Short-term and long-term effects. *Studies in Conservation* 62(1): 33 – 42.

Shapaval, V., Schmitt, J., Møretrø, T., Suso, H. P. and Skaar, I. (2012). Characterization of food spoilage fungi by fourier-trnasform infrated spectroscopy. *Journal of Applied Microbiology* 114: 788–796.

Silva, L. R. (2011). Identification of fungi of the genus. *Brazilian Journal of Microbiology* 42: 761 – 773.

Sundaramoorthy, S., Sudha, D. R., Prakasam, V., Devi, R., Plant, R. and Station, Q. (2017). Fungi associated with spoiled vanilla bean. *Mycopath* 15(1): 51 – 53.

Toma, F. M. and Abdulla, N. Q. F. (2013). Isolation and Identification of Fungi from Spices and Medicinal Plants. *Research Journal of Environmental and Earth Sciences* 5(3): 131–138.

Urquhart, A. S., Mondo, S. J., Mäkelä, M. R., Hane, J. K., Wiebenga, A., He, G. and Idnurm, A. (2018). Genomic and genetic insights into a cosmopolitan fungus, paecilomyces variotii (Eurotiales). *Frontiers in Microbiology* 9: 1–21.

Yuen, C. M. C., Paton, J. E., Hanawati, R., Shen, L. Q., Paton, J. E., Hanawati, R. and Shen, L. Q. (1995). Effects of ethanol, acetaldehyde and ethyl formate vapour on the growth of Penicillium italicum and P. digitatum on oranges vapour on the growth of Penicillium italicum and P. digitatum on oranges. *Journal of Horticultural Science* 70(1): 81 – 84.

Zhang, Z., Wang, C., Yao, Z., Zhao, J., Lu, F., Yu, G. and Lu, Z. (2011). Isolation and identification of a fungal strain QY229 producing milk-clotting enzyme. *European Food Research and Technology* 232(5): 861 – 866.

CHAPTER THREE

3.0 Awareness of Best Practices in Vanilla Value Chain in Tanzania

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3.1 Abstract

A survey was carried out to assess awareness of vanilla handlers in Kilimanjaro, Tanzania, on microbiological safety, food safety attitudes, good manufacturing practices (GMP) and good hygienic practices (GHP). A total of 38 respondents from different stages of vanilla value chain were interviewed. The microbiological safety and food safety attitudes of vanilla handlers were satisfactory (81.6%) while GMP and GHP were unsatisfactory (13.2%). In vanilla curing using HACCP hazard approach blanching stage was identified as a critical control point followed by fermenting (sweating), drying and conditioning (curing maturation) as operational perquisite programmes (OPRP). Therefore, there was need for use of best practices to improve the microbiological quality and safety in vanilla value chain.

Keywords: Vanilla, awareness, best practices, CCP, OPRP, Hazard analysis

3.2 Introduction

Vanilla (*Vanilla planifolia*) is an edible fruit that contains flavour and aroma compounds and known by different names; vanilla in English, vanilla in Hindi, fanilya in Arabic and lavani in Swahili (TUKI, 2000; Ahmed *et al.*, 2019). It is a native orchid grown for food, perfumes or pharmaceutical purposes and second most famous scents and expensive spice after saffron, which is sweet, comforting, warm, and complex (BTC, 2013; Van Dyk *et al.*, 2014; Baqueiro-Pe˜na, 2016; Pardío *et al.*, 2018; Ahmed *et al.*, 2019).

Although all steps in vanilla processing are important, some of the stages are more important than others because of critical quality or safety concerns for the final product (Pardio *et al.*, 2009). Also, knowledge, attitude and practices of curing operators could be of more importance in particular curing stages since failure of operators to adhere to hygienic best practices can cause microbial contamination or foodborne disease such as food poisoning (Moreb *et al.*,2017).

Operators' education level, work experience, culture and training have impacts different degrees of impact on knowledge and attitudes regarding safety handling of foods (Lee *et al.* 2017). On the other hand, food safety has become a constant concern all over the world, leading food industry, healthcare institutions and governments of several countries find ways to monitor production chains in order to control, reduce or minimize food safety hazards to minimum acceptable level (De Oliveira *et al.*, 2016). Therefore, the aim of the study was to assess the knowledge and awareness on vanilla microbial safety, food safety attitude, GHP and GMP of food handlers and identifying microbial critical control points in r vanilla beans curing stages.

3.3 Methods and Materials

3.3.1 Description of the study area

This study was carried out in Kilimanjaro region (Fig. 3.1). The region is located on the North Eastern part of mainland Tanzania, (20 25’ and 40 15’ S; 360 25’ 30’’ and 380 10’ 45’’ E) and is bordered to the North and East by Kenya, to the South by the Tanga Region, to the Southwest by Manyara Region, and to the West by the Arusha Region.

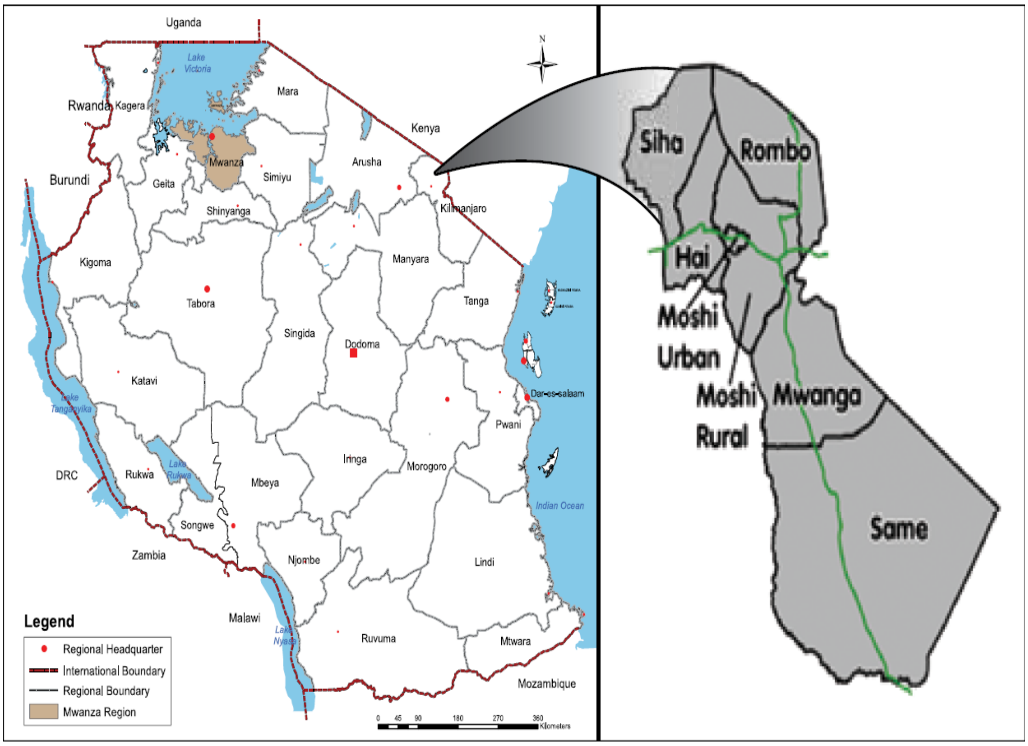

Figure 3.1: Pictorial description of the study area-Kilimanjaro region, Tanzana. (Source: Kilimanjaro region investment guide)

3.3.2 Sampling plan

A case study involving one vanilla processing centre in Kilimanjaro was conducted between April to June 2019. Two vanilla processing centres (in Moshi rural and Hai Districts) were to be involved in this study. However, prior to the main sampling, only Moshi rural vanilla processing centre was operating while the other centre in Hai district was not in operation.

The processing centre in Moshi Rural District operated full-time and received vanilla from the entire area of Kilimanjaro, Arusha and some areas from Morogoro and Kagera regions in Tanzania. The processing centre had 12 curing operators, 4 agronomists, 49 farmer champions (leaders) and 1705 registered farmers.

The design of the study consisted of two parts. The first part was designed to evaluate awareness knowledge about vanilla microbial safety, food safety attitude, good personal hygiene and good manufacturing practices of the vanilla handlers. The second dealt with development of vanilla process flow chart. The participation of Vanilla handlers, agronomists and famer champions was conducted on a voluntary basis. Therefore, we only managed to get consent from 38 vanilla handlers to take part in the study.

3.3.3 Data collection

The questionnaire was developed in English and pretested before using. It consisted of 55 questions on which demographic information (4 questions), vanilla microbial safety knowledge (18 questions), food safety attitude (15 questions), Good hygiene Practices (11 questions) and Good manufacturing Practices (7 questions). Face-to-face interviews were conducted to collect all information mentioned above through a semi-structured questionnaire by using questions as described by (Ali and Immanuel, 2017; Lee *et al.,* 2017; Shori, 2017).

The section of the questionnaire dealing with vanilla microbial safety knowledge comprised of 18 questions with three possible answers; “yes”, “no” and “do not know”. A scale ranging between 0 and 18 (representing the total number of questions on microbial safety knowledge) was used to evaluate the overall vanilla microbial safety knowledge of the vanilla handlers. The vanilla handlers that obtained total score ≤ 11 points were considered to have “insufficient” knowledge and those that had scores ≥ 12 points (≥ 64% accuracy) were considered to have “good knowledge” of vanilla microbial safety. The attitudes section of the questionnaire comprised of 15 questions with three possible answers; “yes”, “no” and “do not know”. Vanilla handlers that answered 9 or fewer questions correctly were considered to have “insufficient” understanding whereas handlers that answered 10 or more questions correctly were considered to have “good understanding. The good hygiene practices (GHP) section had 11 questions with three possible responses; “yes”, “no” and “sometimes”. The respondents were assessed and evaluated based on self-reporting of personal hygiene, and observation of other safe food handling practices. Each correct practice reported or observed scored one (1) out of the total 11 points. The individual respondent scored ≥ 72% (n=8) was considered as having “good food hygiene practice.

Lastly, in Good Manufacturing Practices (GMP) section, respondents were assessed and evaluated as that of GHP section. The section had 7 questions with two possible responses; “yes” and “no”. Each correct practice reported scored one (1) point. For evaluation, a score ≥ 70% (n=5) by an individual respondent was considered as having “good” GMP. All responses regarding the GMP and GHP practices were validated by the researcher’s observations of the vanilla handling, working area of the respondents, and responses were corrected by the researcher in situations where observations did not match with the responses (e.g., where indicated they wash hands, wear hairnet, mask or do not eat, drink or smoke while handling vanilla).

3.3.4 Vanilla process flow chart

In order to identify the critical control points for contamination, a process flow diagram was prepared using hazard analysis critical control points (HACCP) approach (Sabbithi *et* *al.*, 2017). The process chart flow was developed through brainstorming discussion meeting with vanilla handlers and verified by the researcher through actual participation in the process on site. The discussion was based on how vanilla beans are harvested, transported from the farms to the processing centre, reception procedures, post-harvest handling and curing procedures. After designing a process flow chart (Fig. 3.2), hazard analysis was done by involving field procurement officers, vanilla receptionists and curing operators. Hazards (physical, chemical and biological) alongside the vanilla value chain were identified and the likelihood of the hazards to occur was considered based on historic occurrence records, product characteristics and nature of operating/handling environments Before setting critical limits, the identified possible practices which contribute to contamination were assessed and refined by removing the least important causes/practices through voting, the most voted practices/causes were taken by the researcher Microbial assessment was done by taking samples before and after from vanilla reception, sorting/grading, washing/cleaning, blanching, sweating and drying stages to verify the level of microbial contamination at each stage.

3.3.5 Data analysis

The collected data were analysed using simple descriptive statistics like frequency, mean and percentage using SPSS (version 21.0, IBM corporation, New York, USA) package software, and data were coded before analysed. Descriptive statistics was used to describe quantitative factors. Frequencies and percentages were used for describing qualitative characteristics.

3.4 Results and Discussion

3.4.1 Demographic information of the participants

The interview was conducted for all thirty eight respondents working with vanilla from different niches in vanilla value chain system (farmer champions, agronomist/field officers and curing operators) of the vanilla processing centre. Out of the 38 individual respondents (Table 3.1), 63.2 % (n=24) were male and 36.8 % (n=14) were female of which more than half of them 73.7% (n = 28,) were between 18 to 39 years of age. Also 42.1% (n=16) of the respondents had 1-2 years of experience in the vanilla industry. On the other hand, 100% (n=38) of the respondents had formal education that is, 31.6%, 28.9%, 15.8% and 23.7% for primary, secondary, diploma and higher education, respectively.

Table 3.1: Demographic Information

Variables	Item	Number	Percentage
Work experience	< 1 year	9	23.7
	1-2 years	16	42.1
	3-5 years	9	23.7
	> 5 years	4	10.5
Sex	Female	14	36.8
	Male	24	63.2
Level of education	Primary	12	31.6
	Secondary	11	28.9
	Diploma	6	15.8
	Higher education	9	23.7
Age	18-29 years	15	39.5
	30-39 years	13	34.2
	40-49 years	4	10.5
	≥ 50 years	6	15.8

3.4.2 Knowledge about vanilla microbial safety

In this study, the vanilla handlers demonstrated moderate knowledge about vanilla microbial safety (Table 3.2). Of the eighteen on food safety knowledge, 44.7% of respondents did not know if microorganisms can be found on the skin, hair, in the nose or mouth of healthy vanilla handlers. The respondents had poor knowledge about temperature abuse during blanching in relation to fungal infection; since more than half (73.7%) of respondents didn’t know the recommended blanching temperature for green vanilla beans. On the other hand only 60.5% of respondents agreed that improper blanching of vanilla increases a risk of fungal infection. Additionally, 50% of respondents did not know about toxins which may be caused by fungal contamination in cured vanilla beans while about 42.1% of the respondents did not know that cured vanilla beans at a stage of drying are at higher risk of contamination.

Table 3.2: Knowledge on vanilla microbial safety

	Response (n) %		
Variables	**Don't know**	**No**	**Yes**
Washing hands before handling vanilla reduces the risk of food contamination	5(13.2)	1(2.6)	32(84.2)
Using gloves while handling vanilla reduces the risk of food contamination	8(21.1)	3(7.9)	27(71.1)
Improper cleaning and sanitization of utensils increase the risk of food contamination	5(13.2)	0	33(86.8)
Eating and drinking during handling semi/processed vanilla increase the risk of contamination	12(31.6)	1(2.6)	25(65.8)
Vanilla pods handled in clean environment reduces the risk of microbial contamination	9(23.7)	1(2.6)	28(73.7)
Re-blanching cured pods can contribute to microbial contamination	19(50)	9(23.7)	10(23.3)
Temperature have a significant effect on the curing of vanilla pods	14(26.8)	2(5.4)	22(57.9)
Whether separation of bucket and bags used for handling washed and unwashed green vanilla pods prevent cross-contamination	8(21.1)	4(10.5)	26(68.4)
Cured vanilla can be chopped/placed on the same table that used in receiving green vanilla	12(31.6)	24(63.2)	2(5.3)
Improper blanching of vanilla increases a risk of fungal infection	14(36.8)	1(2.6)	23(60.5)
Improper cured vanilla storage causes contamination	13(34.2)	1(2.6)	24(63.2)
Microbes are on the skin, hair, in the nose and mouth of healthy vanilla handlers	17(44.7)	4(10.5)	17(44.7)
Cross contamination is when microorganisms from a contaminated vanilla are transferred by the vanilla handler’s hands or processing equipment/utensils to other vanilla pods"	9(32.7)	3(7.9)	26(68.4)
Green vanilla blanching temperature should be kept at a 50°C	28(73.7)	7(18.4)	3(7.9)
Correct sweating (fermentation) temperature for keeping of blanched vanilla is above 45°C	27(71.1)	6(15.8)	5(13.2)
Cured vanilla pods at stage of sun drying are at higher risk of contamination	16(42.1)	4(10.5)	18(47.4)
Have ever heard about toxins that may be present in cured vanilla which can be caused by moulds	19(50.0)	14(36.8)	5(13.2)
Is okay to ignore slight injuries and go straight back to work	10(26.3)	26(68.4)	2(5.3)

Table 3.3: Food safety altitude of vanilla handlers

	Response (n) %		
Variables	**Don't know**	**No**	**Yes**
Well cured vanilla pods are less infected by microorganism	7(18.4)	1(2.6)	30(78.9)
Proper hand hygiene can prevent cross contamination on vanilla processing	6(15.8)	1(2.6)	31(81.6)
Cured vanilla pods and green vanilla pods should be stored separately to prevent the risk of contamination	3(7.9)	1(2.6)	34(89.5)
Fungal infected and non-infected cured vanilla pods should be stored separately to reduce the risk of contamination	7(18.4)	0	31(81.6)
Is necessary to check the temperature of blanching water periodically to ensure proper blanching and reduce the risk of contamination	16(42.1)	1(2.6)	21(55.3)
Health status of production workers should be evaluated before employment	10(26.3)	0	28(73.7)
Wearing mouth masks is an important practice to reduce the risk of cured vanilla contamination	7(18.4)	2(5.3)	29(76.3)
Wearing gloves is an important practice to reduce the risk of cured vanilla contamination	6(15.8)	2(5.3)	30(78.9)
Wearing hairnets and clean cloths/coats is an important practice to reduce the risk of contamination	5(13.2)	2(5.3)	31(81.6)
Long and painted fingernails could contaminate cured vanilla pods with foodborne pathogens	7(18.4)	1(2.6)	30(78.9)
Vanilla handlers can be a source of microbial contamination and food pathogen outbreaks	9(23.7)	4(10.5)	25(65.8)
Knives, cutting boards and other food contact working surface should be properly sanitized to prevent cross contamination	5(13.2)	1(2.6)	32(84.2)
Vanilla handlers with abrasions or open cuts on their hands should not handle cured vanilla and/or green vanilla	9(23.7)	3(7.9)	26(68.4)
Personal protective equipment reduces contamination risk	6((15.8)	1(2.6)	31(81.6)
Employee's (vanilla handlers) personal items should be kept outside the production area	8(21.1)	4(10.5)	26(68.4)

3.4.3 Food safety altitude of vanilla handlers

A reduction in the incidence of microbial contamination or foodborne illness is strongly influenced by the attitudes of food-handlers towards the implementation of food safety plans. Therefore, there is a strong linkage between positive behaviour, attitudes and education of food-handlers in maintaining safe food handling practices (Akabanda *et al.,* 2017).

In this study (Table 3.3), about 78.9 % of respondents agreed that well processed vanilla pods are less infected by microorganism and 81.6% indicated that proper hand hygiene can prevent cross contamination on vanilla processing. Similarly, majority of respondents (89.5%) agreed that cured vanilla pods and green vanilla pods should be stored separately in order to prevent the risk of contamination. In addition, 81.6% the respondents agreed that fungal infected and non-infected cured vanilla pods should be stored separately to reduce the risk of contamination.

Also, about 76.3%, 78.9 and 81.6 of respondents agreed that wearing mouth-mask, using gloves, and hear nets, clean cloth/apron respectively are the important practices to reduce risk of contamination when handling or processing vanilla. This was similar to the results of Elobeid *et al*. (2019), who found that food handlers had a positive attitude toward safe food in terms of separation of raw and processed foods (87.5 %); use of head caps, masks and gloves (94.4 %); and covering of cut hands or fingers (80 %). Wearing gloves can reduce the risk of food contamination coming from food handlers; however, does not affect or replace the importance of washing hands (El-Nemr *et al.,* 2019).

The majority (78.9%) of vanilla handlers were aware that long and painted fingernails could contaminate cured vanilla pods with foodborne pathogens. They were also aware of the fact that vanilla handlers can be a source of microbial contamination and food pathogen outbreaks (65.8 %). Also, about 68.4% of respondents agree that, employees with abrasions or open cuts on their hands should not handle cured vanilla and/or green vanilla and that health status of production workers should be evaluated before employment (73.7 %). The respondents presented good understanding about cleaning and sanitation equipment as 84.2% agreed that knives, cutting boards and other vanilla contact working surface should be properly sanitized to prevent cross contamination. Thus, the general attitudes of the vanilla handlers toward food safety was satisfactory, except on issues relating to blanching temperature abuse, about 55.5% of the respondents agreed that was necessary to check the temperature of blanching water periodically to ensure proper blanching and reduce the risk of contamination. Therefore, training of the operators is necessary to equip them with best knowledge about good practices (Aung *et al.*, 2019).

3.4.4 Food hygiene practices of vanilla handlers

In assessing the food hygiene practices of the vanilla handlers (Table 3.4) only 52.6% of the respondents reported that they use gloves or wear a coat/apron when working or handling vanilla. Wearing gloves, mask and hair nets are important practices to prevent cross contamination nevertheless, gloves can also become a source of contamination through contact of contaminated materials and other food contact surfaces (Osaili *et al.*,2018). In this study only 55.3% of the respondent reported to use hairnet and mouth mask when handling, sorting or packing vanilla beans, this was because some of vanilla handlers work from the fields and collection centres than the curing/processing centre thus no proper monitoring. Majority of the vanilla handlers (86.8%) said they wash their hands properly after visiting toilet. Additionally, 76.3 % of the respondents reported to wash their hands properly before touching cured vanilla however almost half of respondents

(55.3%) reported to wash their hands properly after touching cured vanilla, this disparity between washing hands before and after touching vanilla caused by other factors such as time pressure, lack of knowledge concerning the risks of not washing hands properly or because they did not feel to wash their hands by working with the same type of food (Gemeda, 2018). Microorganisms can be introduced during food processing by cross contamination from any raw agricultural product or from infected humans handling the food and the practice of not washing hands in between handling of raw and cured greatly increase the chances of such cross contamination (Aung *et al*., 2019).

Lack of knowledge on cleaning and sanitizing the operational items (i.e. Polyethylene bags, buckets, knives, tables) between preparation of green vanilla and cured vanilla pods was obvious in the current study, only 34.2% of respondents reported to clean or sanitize their items between preparation of green vanilla and cured vanilla. The same findings were reported by Ali and Immanuel, (2017) who observed that same utensils used for preparing raw materials were used to handle cooked/processed food. Also, about 76.3% of respondents said did not eat, drink (including water) or smoke in their work place while 71.1% of responded said did not wear jewellery or polish nail when handling vanilla or in production area.

Table 3.4: Food hygiene practices of vanilla handlers

	Response (n) %		
Variables	**Sometimes**	**No**	**Yes**
Whether use gloves during the handling of cured vanilla pods	4(10.5)	14(36.8)	20(52.6)
Whether wear a coat/apron while working	7(18.4)	11(28.9)	20(52.6)
Whether wear a hairnet while working	6(15.8)	11(28.9)	21(55.3)
Whether wear a mask when you sort, handle, grade or pack cured pods	8(21.1)	9(23.7)	21(55.3)
Whether wash your hands properly after visiting toilet	3(7.9)	2(5.3)	33(86.8)
Whether wash your hands properly before touching cured vanilla	6(15.8)	3(7.9)	29(76.3)
Whether wash your hands properly after touching cured vanilla	9(23.7)	8(21.1)	21(55.3)
Whether eat, drink(including water) or smoke in your work place	5(13.2)	29(76.3)	4(10.5)
Whether wear nail polish or jewellery when handling or in production area	8(21.1)	27(71.1)	3(7.9)
Whether use equipment of different colours or Whether sanitize the operational items (i.e PE bags, buckets, knives, tables) between preparation of green vanilla and cured vanilla pods	7(18.4)	18(47.4)	13(34.2)
Whether properly clean the vanilla storage area before storing new products	12(31.6)	1(2.6)	25(65.8)

3.4.5 Good manufacturing practices for vanilla handlers

The majority of respondents (Table 3.5) showed unsatisfactory awareness knowledge about GMP. According to CODEX (2017) requirements, GMP awareness is a mandatory recommendation to control a contamination problem in food industry. About 86.8% of respondents are not aware of required microbiological quality parameters for cured vanilla pods while 78.6% of respondents did not know the microbiological quality of water used in washing vanilla and other items.

In addition, 73.6% of respondents had never attended any training related to food processing. It has reported in previous studies that, low knowledge levels about food safety practices including GMP is a barrier to implementing safe food hygiene practices

(Mullan *et al.,* 2015). On the other hand, respondents (63.2%) were aware of having a schedule for cleaning and disinfecting equipment and facility while 68.4% said to implement the schedule for cleaning and disinfecting equipment and facility according to the timeline. Also, about 76.3% and 73.7% of respondents reported to clean a work place before and after executing any vanilla handling activities. Equipment and work place if not cleaned and hygienically handled are main means of cross contamination in food industries, therefore awareness of food handlers about hygienic handling of equipment and cleaning work place is a vital practices to prevent food contamination and foodborne disease (Meleko *et al.*, 2015).

Table 3.5: Good manufacturing practices for vanilla handlers

	Response (n) %	
Variables	**No**	**Yes**
Aware of required microbiological quality parameters for cured vanilla pods	33(86.8)	5(13.2)
Whether ever attended any training related to food processing?	28(73.7)	10(26.3)
Whether knows the microbiological quality of water used in washing vanilla and other items?	30(78.9)	8(21.1)
Whether has a schedule for cleaning and disinfecting equipment and facility?	14(36.8)	24(63.2)
Whether implement the schedule for cleaning and disinfecting equipment and facility according to the timeline?	12(31.5)	26(68.4)
Whether clean a work place before executing any vanilla handling activities?	9(23.7)	29(76.3)
Whether clean a work place after finishing doing any vanilla handling activities?	10(26.3)	28(73.7)

3.4.6 Vanilla processing flow chart

In this study, about nine vanilla bean processing steps were identified which include: harvesting and handling, transportation, reception, grading, washing, disinfecting blanching/killing, fermenting/sweating and drying. These steps were identified by using Hazard Analysis Critical Point Control (HACCP) approach.

HACCP is a structured approach for identification, assessment of risk (likelihood of occurrence and severity) and control of hazards associated with a food production process or practice (Yalcin and Çapar, 2017) and process flow diagram (Fig. 3.2), defined by ISO 22 000, (2018) as schematic and systematic presentation of the sequence and interactions of steps in the process.

3.4.6.1 Harvesting and handling

The quality of the raw materials such as green vanilla beans is very important to keep the quality of the final product (Manolopoulou, 2017). Before harvesting, the green vanilla beans must be mature and must be at least 9 months after day of pollination suitable for processing and should be of high quality (Dignum *et al.*, 2002). Visual inspection is done at this stage to ensure green vanilla beans are mature, no immature, no split (or less), free from mechanical injuries, decay, insects or other damages, that may affect the further handling and storage procedures. It was observed that, injured, immature and overripe (split) were easily attacked by fungi more than well matured and wholesome pods (Havkin-Frenkel and Belanger, 2018). The main contaminant identified at this stage were foreign bodies (e.g. threads, soils, leaves, plastics and wood fragments,), handling defects (damages, bruising, smashing), immature and overripe pods.

3.4.6.2 Transportation

At this stage after harvesting green vanilla beans are transported to processing centre using public transport or designated vehicle in cardboard boxes or PE bags within 24h after harvesting. CODEX, (2001) recommends where appropriate to use single commodity food transportation units in order to avoid contamination.

Identified contaminants at this stage include; packaging and handling defects (damages, bruising, smashing), foreign materials (e.g. fur, feather, dungs, hair, plastics, metal) from a transporting vehicle or packaging materials. Therefore, care should be taken to prevent, deterioration and spoilage through appropriate measures which may include controlling temperature, humidity, and/or other controls (CODEX, 2011).

3.4.6.3 Reception

The vanilla bean packaging bags/boxes are unpacked and subjected to a visual inspection. At this stage collection, documentation, maintenance and application of information related to all processes in the supply chain such as batch number, vehicle number, procurement personnel and/or name, transaction ID and weight is done (Opara and Mazaud, 2001).

These aspects are considered important because they guarantee the adequate quality control including traceability on raw materials and processed foods (Silva and Abud, 2017). Most of hazards identified at this stage are the same as those at transportation stage.

3.4.6.4 Temporary storage

At this stage, vanilla beans held in an appropriate storage condition for a given time (less than 18h) until moved to the next step**.** In the study of Krishnakumar *et al.* (2007) indicated that **s**toring green vanilla beans for more than 3 days after harvesting before blanching/killing increases the risks of fungal contamination from splitting and decreasing of vanillin content. Also, microbes contamination due to dirty food contact surfaces and uncontrolled storage temperature environment at this stage is possible.

3.4.6.5 Grading and sorting

During this stage, visual inspection control is carried out to determine vanilla bean fruit suitability (Manolopoulou, 2017).The selection (grading and sorting) is one of the most important stages, because it is responsible for the final classification of the fruit that will be processed (Silva *et al.,* 2015). At this stage, the fruits are exposed onto a clean table where are graded into four categories depending on their length and physical appearance and sorted based on their maturation, firmness, bruised, defects caused by fungi, rodents and insects. All defective fruits and contaminants are separated and removed from the whole non-defective fruits to prevent quality deterioration of the final products.

3.4.6.6 Cleaning

The washing and disinfecting are the mandatory processes because the raw material tends to arrive at the industry with a burden of microorganisms, dirtiness and, in particular, soil acquired during the harvest and the transportation. The washing and disinfecting processes aim to reduce the number of initial microorganisms to a minimum acceptable level (Silva *et al.,* 2015).

At first, the green vanilla beans are submitted to immersion in water without disinfectant, to remove the excess of dirtiness before, they are submitted to the next processing stage. But in this study found that, in contrast to Codex (2001) requirements, neither disinfectant nor microbiological quality of washing water that used in cleaning were known. In most fruit industry, washing is done using tap water followed by a dip in chlorinated water to reduce effectively the microbial loads on the fruit surface (Manolopoulou, 2017). Chlorinated water cannot be used in vanilla industry, therefore, ethanol of 50% or 70% vol/vol was proposed in this study to be used as a disinfectant for green vanilla beans after pre washing.

3.4.6.7 Blanching/killing (CCP)

The fundamental purpose of the killing stage is to bring about the cessation of the vanilla bean vegetative life and, furthermore, to disrupt cellular and tissue organization in the green bean, such that previously segregated enzymes and their corresponding substrates can come in contact and interact (Havkin-Frenkel and Belanger, 2018). The green vanilla beans are submerged in hot water for a specified time and temperature depending on their length (cm). For instance grade I (>17cm) is blanched at 70ºC for 5 min, grade II (15-16.9 cm) at 68ºC for 4 min, grade III (12-14.9 cm) at 65ºC for 3 min and grade IV(10-11.9 cm) at 63ºC for 2 min. Based on the microbial assessments (data not shown) conducted in each stage of curing process, microbial contamination, notably aerobic bacteria found too high at sorting and after washing but was significantly reduced blanching however microbial counts increased exponentially on the next curing stages depending of handling environments This stage is critical point as live active enzymes stopped and microorganisms are killed. Therefore, under or over blanching will affect the quality and safety of the final products.

3.4.6.8 Fermenting/sweating (OPRP)

The main objective of the sweating stage is to restrain enough wetness, which is necessary to allow enzymes to catalyse different oxidative and hydrolytic processes (Ahmed *et al.*, 2019). At this stage, the blanched vanilla beans while wrapped into blankets or PE bags are incubated into fermentation box/chamber maintained at temperature of about 45-50ºC and relative humidity close to saturation 95 to 100% (Roling *et al.*, 2001) for 48-72 h. Beans contain nearly 60% - 70% moisture content at the end of this stage, however moisture is allowed to escape rapidly to attain a certain safety level which reduces the risk of microbial spoilage and to block further enzymatic activity during the subsequent operations (Anuradha *et al.*, 2013), any fluctuation of temperature and humidity (low temperature and humidity) may lead to fungal growth.

Therefore, the control of sweating temperature and relative humidity is of important to prevent spoilage microorganism and favours the occurrence of non-enzymatic reactions that enhance development of aromatic chemical compounds.

3.4.6.9 Drying (OPRP)

Drying is the most difficult stage in the curing process to control (Gil *et al.*, 2015). Sometimes, uneven drying may be possible due to the differences in bean moisture content, variable environmental situations, and varying bean size (Ahmed *et al.*, 2019). After sweating/fermenting, the vanilla beans come out with moisture of >70% (Sreedha *et al.*, 2007; Anuradha *et al.*, 2013). Therefore, at this stage, the fermented vanilla beans are slowly dried down to a moisture of 25-35% by spreading them on the racks on open sun drying space or into a heat controlled drying container at temperature of 30-32°C and RH of <40%. This stage was identified as operational prerequisite (OPRP) during hazard analysis and risk assessment as because the probability of cross contamination from ambient environment was high if GMP/GHP not properly adhered.

At sun drying, vanilla beans are usually roughly spread out on blankets or PE mats and set on racks in the sun for part of the day. After a 1-1.5 h of sun exposure depending on weather condition, the vanilla is enveloped in blankets and piled up in the wooden box until the next day. This procedure is repeated daily for several weeks. Beans that are considered dry enough are removed and the shade drying phase begins; this is continued until the entire batch is sufficiently dry (Odoux, 2011). The level of bean drying (during either the sun drying or shade drying phase) is assessed empirically by touch, so the operator has to be quite experienced in vanilla curing to be able to pass a proper judgment on the extent of pod stability. In this study high total plate count was found on vanilla beans and hand of operators (data not shown). Operators who were sorting and judging vanilla on the sun drying, had higher microbial counts on their hands than operators who were not working on same area, probably this was caused by microbial contamination from the drying environments. Roling *et al.* (2001) found an increase in fungal numbers between cycle of sunning and sweating and were associated with weather and environmental conditions of where the vanilla was dried.

3.4.6.10 Conditioning and storage

For conditioning, the beans are stored in closed boxes. This step lasts from 1 month to several months, vanilla beans are tight up into bundles and stored in cool and dry condition, the main risk at this stage is fungal growth if close monitoring is not adhered to. Various chemical and biochemical reactions such as esterification, etherification, and oxidative degradation take place during this step, which produce various aroma constituents and further enhance overall flavour quality of cured beans (Anuradha *et al.*, 2013).

Findings of the present study provide very important information on the level of microbial safety knowledge, food safety practices and major knowledge gaps among vanilla handlers in Kilimanjaro region. Vanilla handlers in this study have insufficient knowledge on essential concepts of GMP and GHP, and that in turns may increase the risk of microbial contamination and/or foodborne illnesses. Continuous education and effective training should be provided to improve vanilla handlers' knowledge in aspects which seem to be lacking. Areas of most concern are temperature control during vanilla blanching and quality of water used in washing. Vanilla handler employees need also education on toxin produced by fungi and its impacts on vanilla quality and safety, as this would encourage vanilla handlers to practice safe when processing and handling vanilla by following both process flow chart and HACCP requirement.

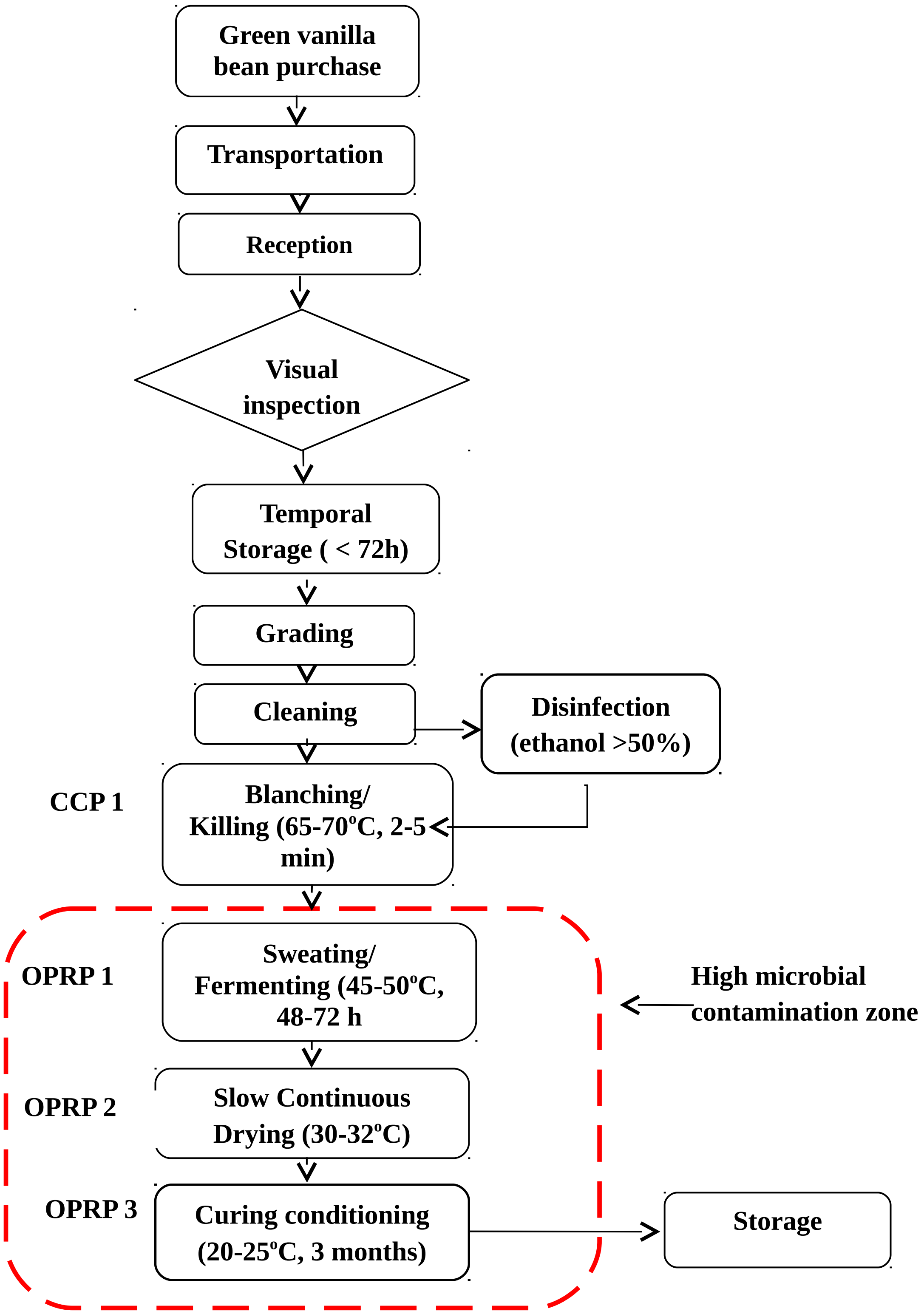

Figure 3.2: Vanilla process flow chart

References

Ahmad, H., Khera, R. A., Hanif, M. A., Ayub, M. A. and Jilani, M. I. (2019). Vanilla. In: *Medicinal Plants of South Asia.* Elsevier Ltd, Amsterdam. pp. 657-669.

Akabanda, F., Hlortsi, E. H. and Owusu-Kwarteng, J. (2017). Food safety knowledge, attitudes and practices of institutional food-handlers in Ghana. *BioMed Central Public Health* 17(1): 1 – 9.

Anuradha, K., Shyamala, B. N. and Naidu, M. M. (2013). Vanilla- its science of cultivation , curing, chemistry and nutraceutical. *Critical Reviews in Food Science and Nutrition* 53(12): 1250–1276.

Aung, S. T., Nwe, A. A., Shan, W. W., Naing, S. M. and Kyaw, K. (2019). Food Handling Practices among Food Handlers of Eating Establishments in the Government Hospitals, Mandalay City, Myanmar. *Archives of Current Research International* 16(2): 1-14.

BTC Trade for Development (2013). An assessment of market potential for vanilla in East Africa Tanzania, Uganda and Kenya. [http://www.befair.be/en/publication/market-studies/assesment-market-potential-vanilla-products-east-africa] site visited on 19/4/2018.

CODEX (2001). *Recommended International Code of Hygienic Practice for the Transport of Food in Bulk and Semi-Packed Food*. Codex Alimentarius Commission No. 47. Food and Agriculture Organization, Rome. 6pp.

CODEX (2011). *Recommended International Code of Practice General Principles of Food Hygiene.* Codex Alimentarius Commission No. 1969. Food and Agriculture Organization, Rome. 19pp.

CODEX (2017). *General Standard for Contaminants and Toxins in Food and Feed*. Codex Stan No. 193. Food and Agriculture Organization, Rome. 54pp.

De Farias Silva, C. E., Moura, E. M. de oliveira, De Souza, J. E. A., Abud, A. K. and de, S. (2015). Quality control of tropical fruit pulp in Brazil. *Chemical Engineering Transactions* 44: 193 – 198.

De Oliveira, C. A. F., Da Cruz, A. G., Tavolaro, P. and Corassin, C. H. (2016). Food safety good manufacturing practices, sanitation standard operating procedures, hazard analysis and critical control point. In: *Antimicrobial Food Packaging*. (Edited by Barros-Velázquez, J.) Academic Press, Amsterdam. pp. 129 – 139.

Dignum, M. J. W., Kerler, J. and Verpoorte, R. (2002). Vanilla curing under laboratory conditions. *Food Chemistry* 79(2): 165–171.

El-Nemr, I., Mushtaha, M., Irungu, P., Asim, H., Tang, P., Hasan, M. and Goktepe, I. (2019). Assessment of Food Safety Knowledge, Self-Reported Practices, and Microbiological Hand Hygiene Levels of Produce Handlers in Qatar. *Journal of Food Protection* 82(4): 561-569.

Elobeid, T., Savvaidis, I. and Ganji, V. (2019). Impact of food safety training on the knowledge, practice, and attitudes of food handlers working in fast-food restaurants. *British Food Journal* 121(4): 937-949.

Gemeda, T. E. (2018). Assessment of knowledge, attitude and practices of food handlers in nekemte referral hospital, Wollega, Ethiopia. *Journal of Nutritional Health and Food Engineering* 8(1): 87 – 92.

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

Havkin-Frenkel, D. and Belanger, F. C. (2018). Curing of Vanilla. In: *Handbook of Vanilla Science and Technology*. Wiley-Blackwell, Chichester. pp. 191 – 208.

Idris, A. A. and Immanuel, G. (2017). Assessment of hygienic practices and microbiological quality of food in an institutional food service establishment. *Journal of Food Processing and Technology* 8: 1 – 8.

ISO 22000 (2018). *Food Safety Management Systems: Requirements for Any Organization in the Food Chain*. International Organization for Standardization, Geneva. 32pp.

Krishnakumar, V., Bindumol, G. P., Potty, S. N. and Govindaraju, C. (2007). Processing of vanilla (Vanilla planifolia Andrews) beans - Influence of storing fresh beans , killing temperature and duration of killing on quality parameters. *Journal of Spices and Aromatic Crops* 16(1): 31–37.

Lee, H. K., Abdul Halim, H., Thong, K. L. and Chai, L. C. (2017). Assessment of food safety knowledge, attitude, self-reported practices, and microbiological hand hygiene of food handlers. *International Journal of Environmental Research and Public Health* 14(1): 1 – 55.

Manolopoulou, E. and Varzakas, T. (2017). Comparison of hazard analysis critical control point and international organization for standardization, 22000 in the ready-to-eat fruit and vegetable industry in conjunction with application of failure mode and effect analysisand ishikawa diagrams. In: *Minimally Processed Refrigerated Fruits and Vegetables*. Springer, Boston. pp. 685 – 721.

Meleko, A., Henok, A., Tefera, W. and Lamaro, T. (2015). Assessment of the sanitary conditions of catering establishments and food safety knowledge and practices of food handlers in Addis Ababa University Students' Cafeteria. *Science Journal of Public Health* 3(5): 733-43.

Moreb, N. A., Priyadarshini, A. and Jaiswal, A. K. (2017). Knowledge of food safety and food handling practices amongst food handlers in the Republic of Ireland. *Food Control* 80: 341–349.

Mullan, B. A., Wong, C., Todd, J., Davis, E. and Kothe, E. J. (2015). Food hygiene knowledge in adolescents and young adults. *British Food Journal* 117(1): 50 – 61.

Odoux, E. (2011). Vanilla Curing. In: *Vanilla: Medicinal and Aromatic Plants — Industrial Profiles.* (Edited by Odoux, E. and Grisoni, M.), CRC Press Taylor and Francis Group, Boca Raton. pp. 173 – 185.

Opara, L. U. and Mazaud, F. (2001). Food traceability from field to plate. *Outlook on Agriculture* 30(4): 239 – 247.

Osaili, T. M., Al-Nabulsi, A. A. and Krasneh, H. D. A. (2018). Food safety knowledge among foodservice staff at the universities in Jordan. *Food Control* 89:167 – 176.

Pardío, V. T., Flores, A., López, K. M., Martínez, D. I., Márquez, O. and Waliszewski, K. N. (2018). Effect of endogenous and exogenous enzymatic treatment of green vanilla beans on extraction of vanillin and main aromatic compounds. *Journal of Food Science and Technology* 55(6): 2059 – 2067.

Röling, W. F., Kerler, J., Braster, M., Apriyantono, A., Stam, H. and van Verseveld, H. W (2001). Microorganisms with a Taste for Vanilla : Microbial ecology of traditional Indonesian Vanilla Curing. *Applied and Environmental Microbiology* 67(5): 1995–2003.

Sabbithi, A., Reddi, S. G. D. N. L., Naveen Kumar, R., Bhaskar, V., Subba Rao, G. M. and Rao, V. S. (2017). Identifying critical risk practices among street food handlers. *British Food Journal* 119(2): 390 – 400.

Sharangi, A. B. (2018). Indian spices: The legacy, production and processing of India's treasured export. In *Indian Spices: The Legacy, Production and Processing of India's Treasured Export*. Springer, Cham. pp. 249–275.

Shori, A. B. (2017). Awareness and knowledge about food spoilage and principles of food preservation among Saudi Women in Jeddah. *Journal of Food: Microbiology, Safety and Hygiene* 2(2): 2–5.

Silva, C. E. de F. and Abud, A. K. de S. (2017). Tropical Fruit pulps: Processing, product standardization and main control parameters for quality assurance. *Brazilian Archives of Biology and Technology* 60: 1–19.

TUKI (2000). English-Swahili Dictionary. Institute of Kiswahili Research,University of Dar Es Salaam.

Van Dyk, S., Holford, P., Subedi, P., Walsh, K., Williams, M. and McGlasson, W. B. (2014). Determining the harvest maturity of vanilla beans. *Scientia Horticulturae* 168: 249 – 257.

CHAPTER FOUR

4.0 CONCLUSIONS AND RECOMMENDATIONS

The vanilla global market demands high and consistent quality and safety of cured beans. This study concludes that prevention of fungal growth as well as training the vanilla processers on GMP and GHP about vanilla processing, will ensure production of safe and high quality vanilla products. Also, identifying point of contamination in vanilla value chain and using of ethanol as mean of disinfection during curing process will ensure control and significant contaminant reduction of fungi in cured vanilla to an acceptable minimum safety level.

The study reviewed effective blanching, fermenting (sweating), drying and adherence of vanilla handlers to GMP and GHP could be a solution to control of microbiological hazards of cured vanilla beans. Also, it is important to establish Hazard Analysis Critical Control Point (HACCP) to help the vanilla producers and processors build safety knowledge about vanilla processing.

Microbial safety knowledge, food safety practices and best practices awareness are very important to vanilla processors. Therefore, awareness training about GMP and GHP is highly recommended to vanilla processors. It should be done regularly and made mandatory to all processors to build knowledge capacity that will help them to prevent and control microbial contaminations in vanilla products.

APPENDIX

Appendix 1: Survey questionnaire

Dear sir/ madam
My name is Kibunje Mageme from Sokoine University of Agriculture, Morogoro. I am a student and researching on knowledge and practices related to GMP and GHP in vanilla indsutry regarding microbiological safety standards, mould infection in particular. I am interviewing vanilla processor/farmers on knowledge about food safety, altitude and practices that can influence the quality of vanilla products.
I will ask you some questions but also seek your permission to walk me around your facility/work area/farm to observe your daily activities. Your name will not appear in the final published research. The information obtained here will be helpful in identifying main cause and will have influence in on the market. You may ask questions at any time throughout our interview. If you have further questions about the research,you can contact me through my number + 255 784 867 626

	Date

A. DEMOGRAPHIC INFORMATION (Mark only one box)

Name (Any identification you may use is acceptable)				
Work experience	☐ <1 year	☐ 1-2 year	☐ 3-5 years	☐ > 5 years
Age (years)	☐ 18-29	☐ 30-39	☐ 40-49	☐ Above 50
Sex	☐ Female	☐ Male	☐ Others	
Level of education	☐ Not attended	☐ Primary	☐ Secondary	☐ Diploma ☐ Higher eduction

B.	KNOWLEDGE ABOUT VANILLA MICROBIAL SAFETY	Yes	No	I don't know
1	Washing hands before handling vanilla reduces the risk of food contamination			
2	Using gloves while handling vanilla reduces the risk of food contamination			
3	Improper cleaning and sanitization of utensils increase the risk of food contamination			
4	Eating and drinking during handling semi/processed vanilla increase the risk of contamination			
5	Vanilla pods handled in clean environment reduces the risk of microbial contamination			

6	Reblanching cured pods can contribute to microbial contamination			
7	Temperature have a significant effect on the curing of vanilla pods			
8	To prevent cross-contamination, separate buckets and bags should be used for handling washed and un washed green vanilla pods			
9	Cured vanilla can be chopped/placed on the same table used to receive green vanilla			
10	Improper blanching of vanilla increases a risk of fungal infection			
11	Improper cured vanilla storage causes contamination			
12	Microbes are on the skin, hair,in the nose and mouth of healthy vanilla handlers			
13	Cross contamination is when microorganisms from a contaminated vanilla are transferred by the vanilla handler’s hands or processing equipment/untensil to another vanilla pods			
14	Green vanilla blanching temperature should be kept at a 50°C			
15	The correct sweating (fermention) temperature for keeping of blanched vanilla is above 45°C			
16	Cured vanilla pods at stage of sun drying are at higher risk of contamination			
17	Have you ever heard toxins that may present in cured vanilla which can be caused by mouldy			
18	It is okay to ignore slight injuries and go straight back to work			
C	**FOOD SAFETY ATTITUDES OF VANILLA HANDLERS**	**Yes**	**No**	**I don't know**
1	Well cured vanilla pods are less infected by microorganism			
2	Proper hand hygiene can prevent cross contamination on vanilla processing			
3	Cured vanilla pods and green vanilla pods should be stored separately to prevent the risk of contamination			
4	Fungal infected and non infected cured vanilla pods should be stored separately to reduce the risk of contamination			
5	It is necessary to check the temperature of blanching water periodically to ensure proper blanching and reduce the risk of contamination			
6	The health status of production workers should be evaluated before employment			
7	Wearing masks is an important practice to reduce the risk of cured vanilla contamination			
8	Wearing gloves is an important practice to reduce the risk of cured vanilla contamination			

9	Wearing hairnets and clean cloths/coats is an important practice to reduce the risk of contamination			
10	Long and painted fingernails could contaminate cured vanilla pods with foodborne pathogens			
11	Vanilla handlers can be a source of microbial contamination and food pathogen outbreaks			
12	Knives, cutting boards and other food contact working surface should be properly sanitized to prevent cross contamination			
13	Vanilla handlers with abrasions or open cuts on their hands should not handle cured vanilla and/or green vanilla			
14	Personal protective equipment reduces contamination risk			
15	The personal items of employees (production) should be kept outside the production area			
D	**GOOD HYGIENE PRACTICES OF VANILLA HANDLERS**	**Yes**	**No**	**Sometimes**
1	Do you use gloves during the handling of cured vanilla pods			
2	Do you wear a coat/apron while working?			
3	Do you wear a hairnet while working?			
4	Do you wear a mask when you sort,handle,grade or pack cured pods?			
5	Do you wash your hands properly after visiting toilet?			
6	Do you wash your hands properly before touching cured vanilla?			
7	Do you wash your hands properly after touching cured vanilla?			
8	Do you eat, drink (including water) or smoke in your work place?			
9	Do you wear nail polish or jewerly when handling or in production area?			
10	Do you use equipment of different colours or do you sanitize the operational items (i.e LDPE bags,buckets,knives,tables) between preparation of green vanilla and cured vanilla pods?			
11	Do you properly clean the vanilla storage area before storing new products?			
E	**GOOD MANUFACTURING PRACTICES OF VANILLA PROCESSORS**		**Yes**	**No**
1	Are you aware of required microbiological quality parameters for cured vanilla pods?			
2	Have you ever attended any training related to food processing?			
3	Do you know the microbilogical quality of water used in washing vanilla and other items?			

4	Do you have a schedule for cleaning and disinfecting equipment and facility?		
5	Do you impliment the schedule for cleaning and disinfecting equipment and facility according to the timeline?		
6	Do you clean a work place before excuting any vanilla handling activities?		
7	Do you clean a work place after finishing doing any vanilla handling activities?		