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TEST OF PERFORMANCE FOR A STORAGE SYSTEM OF RAW COCOA BEANS

by

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CERTIFICATION/DECLARATION

I, ANTHONY OPPONG KYEKYEKU, declare that this submission is my own work towards the MSc and that to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

ISO standard 8402 defines validation as "confirmation by examination and provision of objective evidence that particular requirements for a specific intended use are fulfilled". In this non-experimental, observational and cross sectional study, the performance of a known conventional storage system was tested and evaluated for fitness for its intended purpose. The system has a scope extended for the storage of dry cocoa beans. System sensitivity, reproducibility and uncertainties are not known in details. This research discussed the system performance in the context of existing literature on factors that influence the quality of agrocommodity during storage.

Controlled conditions were defined precisely for the system as much as possible to enable generalization to be formulated given specific established procedures. Minitab® 17 and R statistical software (R-3.3.1) were used for the statistical Analyses. The approach to the storage system testing was to observe and compare through laboratory test methods the quality of the storage samples before and after storage. The samples were kept in Kilner jars and the temperature of the storage environment controlled and monitored over a period of Four hundred and eight (408) days. Standard test methods use in international trade of cocoa such as the cut test analysis (ISO 1114:1977), moisture determination with Aqua boy KAM III model and bean count determination were used for quality assessment. The data analysis assumed the entire population as a sample in order to attribute generality to the data collected.

The study concluded a statistically significant mean value at 95% CI for the performance data analysed before and after storage for all variables observed. Correlational graphs showed a strong positive correlation for all variables investigated with the exception of All Other Defect

(AOD). The weak relationship between the before and after data for AOD had an explained variability of 51.8% with the unexplained variability attributable to the uncontrolled condition of hidden infestation before storage. The research concluded with a high-performance criterion for the storage system after testing due to its ability to maintain the initial quality after storage within industry accepted standard.

DEDICATION

This work is dedicated to the Almighty God who knows what was and what is to come. To my lovely wife, Sussana Antwi-Boasiako, my unborn children; Alexis, Hillary and Hilda, Friends and my entire Family for their love, support, understanding and contribution for who I am today and would be tomorrow.

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CHAPTER ONE

INTRODUCTION

1.1 Background to Quality Control

Quality control and assurance principles according to Philip Crosby (as cited Suarez, 1992) is doing things right the first time and every time whereas quality assurance is a systematic approach that monitors and evaluates all aspect of a process to ensure that specified standards of quality are being meet. In measuring quality, norms, conventions, statutory and international standards are used. These standards provide industry players with information about the product and also assures product quality uniformity. ISO/IEC 2451:2014 specifies the quality specification of cocoa beans for the international market.

The focus of every quality assurance system is to protect the product or service from undue variations so to please the customer. For sample storage, quality assurance systems are important to protect the stored sample from undue variation during storage. Quality assurance requires performance qualification protocols for systems that have relevance on quality integrity such as sample storage. The only way to be sure a system is performing as intended is to validate the system. Validation as defined by ISO 9001 is assuring that a method, system or process fits for its intended purpose by providing objective evidence. A validated system goes through performance qualification in order to addresses the performance criteria of the system to be fit for its intended purpose.

1.2 Problem Statement

Working in a sample storage laboratory, it was common practice to keep samples in sample carrier bags for storage purposes. Most samples ninety (90%) percent of the time had changes in the initial quality after four (4) months of storage when the before and after data collected were compared. Literature has shown that, scientists have attributed quality integrity of an agro commodity in storage to initial minimal or zero infestation and good conditions (environmental) of storage. The difficulty in identifying hidden infestation in the early stages of development and poor storage environment possess a threat to sample integrity during storage. The problem investigated was to determine whether a storage system for dry cocoa beans was fit to perform its intended purpose of maintaining the quality integrity of retention samples.

1.3 Objectives

The main objectives of the work basically were to observe through laboratory test methods a storage system with an extended scope for dry cocoa beans in a testing laboratory to determine its performance by:

i). measuring the relationship between the before and after quality of dry cocoa beans.

the Null hypothesis would be true if;

1. The mean frequency count of before and after data for TM, TS, AOD, MC and BW does not differ.
2. The frequency count of before and after data for TM, TS, AOD, MC and BW correlate strongly.

Other objective of the study was to

ii). Develop regression models as system performance benchmarks to enable raw cocoa beans storage performance testing through verification, sensitivity analysis and precision check. This can possibly lead to reference material development for storage system validation.

1.4 Research Questions

The questions used to assist in determining whether the quality of dry cocoa bean changed or maintained grade after storage were whether;

1. the mean frequency count of performance data for TM, TS, AOD, MC and BW before and after storage differed.
2. the correlation of the frequency count of performance data for TM, TS, AOD, MC and BW before and after storage was weak or strong.

1.5 Hypothesis

The hypothesis of the work was that, at controlled conditions of temperature and an uncontrolled situation of hidden infestation, the quality of dry cocoa beans in a sterilized food storage container should remain in acceptable range per industry standard.

The null hypothesis is true when;

1. Ho: The mean frequency count of before and after data for TM, TS, AOD, MC and BW does not differ.
2. Ho: The frequency count of before and after data for TM, TS, AOD, MC and BW correlate strongly

The alternate hypothesis is true when the null is rejected.

1.6 Methodology

The laboratory test methods use to observe the storage system was in conformity to ISO 1114: 1977. Dry cocoa beans that were used for the study were samples from fumigated consignments ready for shipment. The controlled conditions provided were standard conditions of temperature monitored with Testo 623 thermohygrometer recorded and analysed with Minitab® 17 control charts. An uncontrolled situation of hidden infestation was assumed for each storage system. The quality and safety requirement of the samples were audited against international cocoa standards using the cut test method and cocoa moisture determination technique. The initial quality of the sample before storage were determined and stored for four (4) months (121 days). The after storage quality was determined and compared to the initial using Minitab® 17 paired T and Correlation analysis tool.

CHAPTER TWO

LITERATURE REVIEW

2.1 Background to Cocoa Beans

Linnaeus system of classification describes cocoa as Theobroma, the Genus and cacao, the species name. Wood and Lass (1985) first put cocoa in the family Sterculiacea (Costa et al., n.d.). Alverson et al., (1999) placed cocoa into the family Malvaceae (Osobase, 2012). The Genus to which cocoa belongs to have twenty-two (22) members with cocoa standing out because of its economic importance (Kongor et al., 2016). The Mayans of South America called the cash crop Theobroma, ‘‘foods for the gods’’ (Rusconi & Conti, 2010). This literal interpretation was as a result of the role cocoa played in rituals of marriage performed for their gods. It is believed that, the association of cocoa during the Romantic era had its root from this ancient ritual (Martin & Sampeck, 2015).

Some economic importance of cocoa (cited by Kongor et al., 2016) has been documented by scientist like Krähmer et al., (2015); Ho, Zhao, & Fleet, (2015) on the role cocoa beans play in the manufacturing sector of the confectionery, pharmaceutical and cosmetic industry. Apart from cocoa being the raw material for these industries, the cash crop serves as the major foreign exchange earner for leading producing countries such as Ghana (Essegbey & Ofori-Gyamfi, 2012). Europe and USA imports most of these cocoa with new emerging markets in Eastern Europe and Asia (Distr, 2012). As at 2013, Ghana COCOBOD reported that, it employs about 70% of the national agricultural labor force (Melorose, Perroy, & Careas, 2015a). IFDC (2014) reported that, cocoa exports in West and Central Africa supports over $8 billion for the Region’s national economies significantly contributing to the Gross Domestic Product (GDP) of these producing countries (Jahan, Karuri, Lazo, Vega, & Makepeace, n.d.). According to FAO (2001), more than 20 million people benefit from the Cocoa trade.

2.2 Quality Attributes of Dry Cocoa Beans

Levai et al., (2015) in their work on postharvest practices and farmers' perception of cocoa bean quality in Cameroon explains how high quality traits of cocoa beans is obtained when postharvest treatment of the cocoa bean is taken seriously. He used cross sectional study approach to evaluate the knowledge gap of these farmers in Cameroon. Two broad categories of nutritional and fermentative quality among others was described by Kongor et al., (2016) on their review on factors influencing quality variation in cocoa bean (Theobroma cacao) bean flavour profile. Parcel of cocoa with a very high fermentative and nutritional quality higher than average enjoys premium price. Roelofsen (1958) also described the three major primary processes that make up the postharvest handling of cocoa in the Journal of advances in Food Research where he discussed pre-conditioning of the pulp, fermenting of the cocoa beans and drying. He explained that drying occurred after fermentation to reduce the moisture content from 60% to 7.5%. Research indicates that bean moisture drying temperature should not exceed 65^{o}C and can also be done using the sun or an artificial source (ICCO, 2000). Emmanuel et al., (2012) reported pulp pre-conditioning to be an important precursor for a good fermentation regime for cocoa beans and that pod storage before fermentation was essential for fermentation to be beneficial. According to the Transport Information Service (TIS) (Anonymous, 2016) "a well fermented cocoa, is one that has good flavor and no signs of germination and slaty defect". Adequate fermentation time according to Guehi et al., (2007) is crucial for high quality cocoa beans. Under fermentation results in cocoa beans that are very bitter, whereas over fermented cocoa beans are very dark making such cocoa beans identifiable by their slaty outlook and astringent taste according to the Transport Information Service (TIS) (Anonymous, 2016). Camu et al., (2008) in their work on fermentation of cocoa bean: influence of microbial activity and polyphenol concentrations on the flavour of chocolate added that, well fermented cocoa beans are fully brown, partly fermented are partly purple, and under fermented are purple, cocoa beans that are not fermented are slaty. Other quality attributes are described as physical and encompass bean count, bean weight, bean size, total moulds, infested or germinated beans, presence of foreign materials, flat beans etc. (Niemenak, Ariel, & Effa, 2014). These quality attributes are enforced by importing countries because their presence in the consignment will promote deterioration of the parcel being exported ("Quality Certification for Dry Cocoa beans," n.d.).

2.3 Quality Specification of Dry Cocoa beans

Cocoa Quality Specification in the Cocoa trade enables a level playing ground for all stakeholders. Specification confers quality uniformity understanding by all in the Cocoa trade business. In the Cocoa trade, suppliers of cocoa are bent on staying in competition with other exporting countries. Whiles a country like Ghana is already enjoying premium price from applying stringent standards, enabling them stay on top of the market, according to Quarmine et al., (2012), others use the standards merely as guidelines. Below is Table 2.1 showing the Quality specification guidelines by Ghana COCOBOD

Table 2.1: Quality Specification for Ghana Cocoa (COCOBOD)

Maximum Defect Levels			
GRADE	TM	TS	AOD
Grade I	3%	3%	3%
Grade II	4%	8%	6%

2.4 Measuring Quality for Cocoa Beans

Lowe, (1994) in his journal article on Performance Measurement explains the need to ascertain established requirements by verifying against agreed specification to ensure conformity.

Established specifications are stated, implied or statutory requirement that denotes acceptable quality. In Cocoa trade, international standards together with country specific standards are used to safe guard the quality of the commodity. For example, the International Organization for Standardization (ISO) Cocoa beans – Sampling (ISO 2292:1973) is the most widely use sampling methodology for Cocoa beans sampling (Costa et al., n.d.). Some countries have country specific standards in place to enable them better meet the international standard. These country specific standards such as MS 293:2005 is used in Malaysia for the grading of Cocoa beans (Standard, 2014). Standards specifies the requirement for methods, process and systems to ensure the supply of quality products. Example of standard requirement in the Cocoa trade is that from Geneva, 2001 where the International Trade Center specified that ‘‘the complete consignment shall be examined in lots of not more than 25 tons on dispatch’’ with a minimum of 300 beans taken per ton (International Trade Centre, 2001). When measured quality is different from quality specification, Cocoa trade quality disputes arise. There are instances when the supply of the produce to the international market warrants the rejection of the parcel at destination, or the determination of a discount for an inferior parcel (International Trade Centre, 2001). In such disputes, due diligence in following standard test methods and procedures for quality determination are given much attention during resolution. These trade disputes are inevitable and resolved by arbitration councils outside the court of law (Edition, n.d.). Retention samples become very important for retesting and quality assessment of such consignment, and as also indicated by the European Commission (2006) in the EudraLex Journal on GMP Guidelines Annex 19: Reference and Retention Samples, the need for a reliable storage for these samples. Good storage preempts quality alteration of samples hence retaining the ability to generate valid results.

2.5 Food Storage Systems

Hauri & Niece, (2011) published their work on food containers that could assure higher shelf life of the food under storage in the Journal of Chemical Education. They investigated the after quality of the food stored to check whether the storage system applied could maintain the initial quality within acceptable limits. They use two styles of the food container for a period of one week (7 days) and compared the results from their study. The less expensive style consistently had reduced quality of the food stored after storage. This was because it leached more silver into the food that was tested compared to the alternative that had less silver impregnated into the storage container. In effect, the quality of food after storage with higher silver impregnated container changed drastically after storage as juxtapose with the less silver impregnated container. The cheaper containers were silver laden and affected the quality of the food that was stored and hence the quality alteration after seven (7) days of storage. Olabode, (2012) in his work on the "Impact of different storage containers on free fatty acid formation in stored cocoa beans" used jute bags, woven sack (bagco), tin coated container and plastic bucket as storage systems for the storage of dry cocoa beans for a period of five months. He wanted to find out whether these storage systems can affect the quality of the stored cocoa beans Free Fatty Acid (FFA) levels after a five (5) month period. He allowed air to freely move between the storage environment naturally and in the end observed the loss of moisture content in some of the storage containers. He concluded cocoa beans stored in woven sack to have experienced the least moisture lost compared to the other storage systems and attributed generality to moisture lost due to ventilation. Essien et al., (2010) in their work on "Hermetic storage: a novel approach to the protection of Cocoa beans" discussed the use of hermetic technology to safeguard the quality of dry cocoa beans without the use of fumigants or need for refrigeration technology. Their work discussed hermetic storage for bulk cocoa (10-1000 tons) and small portable containers (60kg) of cocoa beans in Cocoon bags and SuperGrainbags respectively.

The hermetic technology discussed operated on the concept of modified atmosphere creation and the work concluded a successful storage method that could assure the quality of the sample after storage for both bulk and small portable containers. All these scientist; Hauri & Niece (2011), Olabode (2012) and Essien et al., (2010a), sought to investigate the storage system that could maintain the quality of the stored product from undue variation. They use different approach and food material but in the end compared the initial quality to after storage quality drawing conclusion on the performance of the system that produced expected results. Dano et al., (2013) in their work on the influence of fermentation and drying materials on the contamination of cocoa beans by Ochratoxin A, published in the Journal of Toxins affirms a good storage system for cocoa beans as one that maintains its quality integrity after storage. Several investigators have also worked on storage systems for diverse agro commodities and have either suggested alternative to storage or endorse conventional storage systems. Cocoa beans are vulnerable to infestation and early detection is essential, yet pest of stored products tend to be elusive and their presence is often not obvious (Stubbs, Chambers, Schofield, & Wilkins, 1985). Azalekor (1999) in his work on Assessment of damage cause by *Corcyra cephalonica* (Stainton) (Lepidoptera: *Pyralidae*) and *Araecerus fasciculatus* (Degeer) (Coleoptera: *Anthribidae*) to stored Cocoa Beans in Ghana confirm the association of dry cocoa beans with hidden infestation that is difficult to detect. Unlike fruits and vegetables, defects in cocoa beans are basically caused by poor fermentation and infestation problems (Rajendran, 2005). Mould and insect infestation affect cocoa in storage and reduces the quality of the parcel. According to Traoré (2009), a storage system for cocoa sample should have the ability to maintain the quality of the sample during a period of six (6) months. This research has similarity of purpose but different approach to compare the quality of stored raw cocoa beans in a system that assumes a real life situations simulated in the laboratory so that results from the simulation will parallel its real life counterpart. Most of the initial research review shows that, researchers have been interested in the role the storage containment contributed to quality change after storage. This work analyses the inherent physical qualities of the food material to be stored and how its initial quality before storage might affect the quality after storage in an air-tight storage vessel devoid of residual infestation with a scope extended for raw cocoa bean storage.

2.6 Storage Systems Validation

The performance of any system can be guaranteed when the system is duly qualified after it has been installed and operational. The design, installation and implementation of a reliable storage system is done through the validation process. ISO 9001:2000 defines validation as confirmation, through the provision of objective evidence, that performance requirement is achieved. The international vocabulary of Metrology 3rd edition, JCGM 200:2012 section 2.45 defines validation as verification where the specified requirement is adequate for an intended use (Joint Committee for Guides in Metrology, 2004). According to Codex Alimentarius, (2008), a system will produce valid results consistently when validated before use with established controls for systems environment monitoring. The United Nations Office On Drugs and Crime (UNODC) (2009) explains the criteria for controlling environmental conditions as one that is to be established with documented procedures during installation and implementation of the system. In storage system design and installation, protocols are developed to address the systems operation. Bedard (2008) in the technical supplement to WHO Technical Reports Series, No. 961, 2011 on Qualification of temperature-controlled storage areas illustrates that, protocols for temperature controlled storage areas includes but not limited to temperature monitoring, authorization and installation qualification. The requirement for the routine assurance that the system is working efficiently is all included in the established documented procedures (WHO, 2006).Once the development of such procedures are complete, there is a system to be implemented and monitored for normal operation. The performance of the system operations using established procedures can be used to generate data for at least a year to enable system validation. As part of the performance qualification, the operational data generated for the system should be analyzed to confirm suitability of the system to produce same quality when operated within the scope of the documented or established procedures (WHO, 2006). There is also the need for preventive maintenance to assure continuous system performance after validation. Huber, (2009) also adds the need for a scheduled validity checks on the storage system to assure performance compliance to system requirement. In determining storage system precision checks, Briggs (1996) explains how comparing results of the analyses over a period against the calculated range of the results during system validation will give the relative range and absolute differences required to determine precision of the system.

Ilyukhin et al., (2001) conducted a survey of control system validation practices in the food industry which they published in the Journal of Food control. They confess to the best of their knowledge the lack of research in the area of validation in the food industry as at that time. They therefore made it their purpose to collect information from stakeholders in the industry to find out the knowledge gap of validation among players in the industry. To their astonishment, they realized majority of the food manufacturers who partook in the survey suggested they relied on third party organisations for control system validation of their equipment’s and environment in which these equipment’s operated. These third party operatives in themselves lacked understanding of the validation process. Some food manufacturers according to their research however used equipment supplier after sales service validation resources. They advised a formal and structured equipment maintenance training for clients by these suppliers as well as monitoring and maintenance for the environment in which the equipment operated. Eames et al., (2012) in the Journal of Computers and Electronics in Agriculture evaluated the validation of a novel software used to provide a control environment for refrigerated foods. The design of this software enables the control of transient and steady state operations of the refrigerated storage system. The designers of the system according to their research claim that the software had the ability to monitor energy consumption of the storage system and hence could provide vital information for decision on energy cost reduction. Chong et al., (2013) worked on "Food sustainability by designing and modelling a membrane controlled atmosphere storage system" and published it in the Journal of Food Engineering. They evaluated an innovative storage system's ability to preserve food based on green membrane technology. They compared the new technology to the conventional method for fruits and vegetable storage and transport and concluded superiority of the new technology over the conventional with mathematical models that were also evaluated for performance under continuous operation. This research is to add onto the already existing information on storage system validation and also exemplify the enormous benefit of having a system that performs as expected in a true zero-defect approach (Crosby,1987 cited by Suarez, 1992) making every detail of an organizational operational strategies an important item.

2.7 Quality Assurance (QA) Study Design

Belli (2008) described non-experimental techniques in research in the Journal Lapan as one that is non-manipulative and enables the formulation of new hypothesis and require less resources hence its cost effectiveness. Stoller (2004) described quality assurance (QA) work as one that should be hypothesis driven and are usually before and after studies. He further explained that, to be successful with before and after studies for QA, the compared groups in the work should be similar in important features so as not to affect development of the outcome. In the book "The illusion of learning from observational studies", Gerber et al., (2004), described observational studies as research that can examine the effect of variation in a set of independent variables. They discussed in their conclusions, the approach in making strong assumptions about statistical relationship between observed and unobserved causes of dependent variables having in mind a particular statistical uncertainty given a modeling assumption and a theoretical uncertainty about which modeling assumptions would be correct. Briggs, (1996) developed regression model to assist in future predictions of quality given a set of assumptions. Song & Chung, (2010) on observational studies described how the design approach could best be in line in studies that involve investigative questions. They discussed the design suitability to provide results similar to that of randomized control trials from the plastic surgery literature. Buskirk, (1988) in the Journal of Ecology even though used non-observational research to generate dynamic models concluded the absence of strong interactions to mean the need to build complex models from simple models. Mann, (2003) in the journal of Emergency Medicine described cross sectional studies as a relatively quick and easy research design that do not permit distinction between cause and effect but could be used to determine generality. This research also used observational design approach because its cost effectiveness and ability to provide a snapshot generalization of the performance of the storage system under study. The research design also defined the system under study under specific assumptions for dependent and independent variables in order to theoretically predict after quality results using generated regression models from before and after data under those assumptions. The design was also instrumental to assess the performance of the storage system in conditions similar to its counterpart to determine what could have happen to the sample in parallel to its imitation.

2.8 Quality Assessment of Dry Cocoa Beans

The laboratory methods used for analyzing the quality of cocoa beans is the moisture determination with Aqua Boy KAM III model, cut test method (ISO 1114:1977) and bean count (ISO 2451:1973); “the average count of the beans derived from the number of cocoa beans in a given weight”. The MS 293:2005, a national standard for Malaysian Cocoa also specifies the number of beans in 100g weight of total beans. During bean count, the bulk sample is reduced by quartering of about 300 beans. Portions of the 300 beans must be weighed with coco scale to 100g and the number of whole beans, after the removal of flat beans, foreign matter and debris in 100 g must be counted (Standard, 2014).

Whiles the bean count determines the average count of the beans in 100 g, the cut test method determines the degree of fermentation, and the organoleptic characteristics of the sample representing the parcel. The degree of fermentation and the organoleptic characteristics can best be determined using the cut test to identify the corresponding defects of over fermentation or under fermentation (“EX-134-10 Fine and Bulk - edited,” n.d.). The fermentation problems are illustrated through the cut test method as slaty, purple, and germinated beans. The color of the nib also shows the degree of fermentation. A fully fermented cocoa beans is dark brown according to Camu et al., (2008). Tettey, (2014) used the cut test method to identify the extent of mould and insect infestation for organoleptic characteristics, by identifying frass, cocoon, larvae, and fungi spores. Olunloyo et al., (2011) used results from the cut test method to compare to results he generated from their work on neural-based electronic nose for cocoa beans quality assessment. The work concluded 95% accuracy. They also used the cut test method to confirm the condition of the beans to be used for electronic nose experiment citing ISO 1114:1977. They concluded that, as few as 3% visibly mouldy beans gives unpleasant musty flavors to cocoa products whiles others under certain conditions produce mycotoxins. They further went on to described slaty beans as the unfermented beans which is grey or slaty in color. Insect damaged beans were the defects that were caused by the feeding behavior of stored product pest. Germinated defects were described as those defects that happen because of late fermentation or drying process causing the cotyledon to germinate to pierce the bean before being killed. Flat beans however only added to the waste or shell content because they lack useful cotyledon. Niemenak et al., (2014) in their work on physical and chemical properties of cocoa beans used the cut test for the quality assessment. M. Fowler, a contributor in the book ‘‘industrial chocolate manufacture and use: fourth edition’’ described the key parameters in quality assessment of cocoa (Beckett, 2009). He described the cut test method as a method to assess the key parameters of cocoa quality. He explained that, during the cut test method, defects otherwise called unfavorable characteristics are identified and quantified to grade the produce. The method identifies visibly mouldy, slaty, infested, germinated and flat beans. These test methods which have been used for dry cocoa beans quality data generation was used in this research. The test methods are internationally recognized methods and effective in determining the fermentative and infestation indices for dry cocoa beans.

2.9 Quality Analysis Equipment

The equipment use for moisture determination is the Cocoa Moisture Meter (Aqua-boy). Aqua boy KAM III with cup electrode has an accuracy of plus/minus 0.1%, reproducibility of 0.2% and is easy to use and highly portable designed for the Cocoa industry (“Technical features of Aqua-boy”, n.d.). The measuring principle of the Aqua boy is based on the electrical conductivity of the material which always bears a fixed relation to the moisture (“Aqua boy, a measure of quality”, n.d.).

In the book NATO-CCMS pilot study "Conservation of historic brick structures": proceedings of the 5th expert meeting, Berlin, 17-19 October 1991 by Vianello (1992), described among other test, the use of Aqua boy to verify humidity content of a material based on electric conductivity. The author has used it to test retention samples and concluded standard values for special type of samples.

There is limited information in the open literature on the use of Kilner Jars for the storage of retention samples for reference analysis in the food industry testing laboratory. The Jar is used for preserving food in retail shops and kitchens of homes and restaurants (“Uses of Kilner Jar”, n.d.). The nearest use of the jar in the laboratory was its use for culturing insects. Oparaeke & Bunmi (2006) use Kilner jars to culture insects in their work on the Bioactivity of two powdered spices as homemade insecticides against *Callosobruchus subinnotatus* (Pic.) on stored Bambara groundnut. McCaffery A.R., (1990) in the book “Chromatography and Isolation of Insect Hormones and Pheromones” discussed the use of Kilner jars for culturing insects. According to Wikipedia, a ‘‘rubber sealed screwed top jar used for preserving foods, was invented by the Kilner family’’ (Wikipedia, 2016). Kilner jars range in capacity and made from non-tempered glass material with metal clip lid and rubber seal for air tight storage. Bechoff et al., (2011) use Kilner jars as storage systems in their work on “On-farm evaluation of the impact of drying and storage on the carotenoid content of orange-fleshed sweet potato”. They determine losses of carotenoid in the orange-fleshed sweet potato after drying and storage.

The Coco scale was also pivotal in the determination of the bean weight of whole bean in 100g. This is a customized measuring instrument supplied by H. Fereday & Son for the Cocoa industry that has a meter, weighing bridge and a weighing pan protected by a carrier case. The coco scale operates through gravitational force comparing the torque of the test object to an inbuilt or calibrated/ known mass of the weighing balance. Enyan et al., (2013) in their work on physical and organoleptic coffee bean under different drying methods and depths of drying in a tropical environment used a top-loading sensitive weighing scale of the Coco type (Coco scale) for sample weighing. Their work was published in the International Research Journal of Plant Science. Calibrated Coco scale traceable to national standard at the Ghana Standards

Authority was used for the weighing. A lock back knife called Okapi Knife was also used to cut cocoa beans lengthwise for internal defect identification. Defect identified were mould, slaty, germinated, insect damage, and flat beans. A rubber thimble (thumb protector) was used to protect the thumb to reduce cutting time during the analysis.

2.10 Statistical Analysis of Performance Data

Minitab® 17 test of significance tool (paired T) was used for data analysis of the before and after study to evaluate the results of the initial and after storage quality for dry cocoa beans samples stored in Kilner jars. The work assumed continuous dependent variables with independent variables of related groups with no significant outliers. Minitab® 17 paired T analysis was used to determine whether the two dependent groups differed. Pham & Jimenez, (2012) in their work on "accurate paired sample test for count data" published in the journal of bioinformatics explained that, paired sample testing for before and after treatment with a paired sample design assumes a constant relative change within each pair across biological samples. They used models from such comparisons as an approximation to true model in cases of heterogeneity of response in complex biological systems. They also modeled the technical variations with the help of probability distributions depending on the reproducibility of the acquisition workflow.

This QA study also assumed a constant relative change in the storage vessels and technical variations were also modeled using Regression tools in Minitab® 17. Statistical descriptive as explained by Trochim, (2006) was also use to interpret the measures of central tendencies and variability in the dataset. Pearson Product Moment Correlation in conjunction with Regression tools in Minitab® 17 was used to assess the strength of the linear association between two variables and the strength of the relationship in the dependent variables. R statistical package with powerful graphical output was also use to graphically demonstrate the nature of the relationship in the dependent variables as explained by Obe & Hsu, (2010).

2.11 Benchmarking Performance Data

Scientists have conducted research on food storage systems comparing the initial quality before storage to the final quality after storage using diverse systems. In the work of Pristouri et al., (2010), even though one system proofed to be better than the other, there was still a gradual loss of quality in the best storage system. Extra-olive oil in tin containers and dark glass had a better quality compared to plastic bottles. In the work of Knee & Aggarwal, (2000), conventional storage systems that uses the principle of modified atmosphere packaging proofed to be a better alternative to vacuum storage systems for fruits and vegetables. Using conventional sealed plastic food containers as control, the control performed better than the vacuum container. In the work of Joo et al., (2011), on the comparative shelf life study of blackberry retail storage conditions involved fruits that were stored in containers either petroleum based or bio based; cultivars demonstrated better quality in the oriented poly (styrene), OPS container with less weight loss, and decrease insoluble solids content (SSC) and Titratable Acidity(TA). Even though there was weight loss for both containers, the fruits maintained the US standard No 1 grade. Wilczynski (2008) used before and after studies in his work on the quality of reporting of diagnostic accuracy studies. His results showed that, the interaction between the two independent factors was not significant but rather had remained relatively constant. His work was published in the Journal of Radiology Issue 3 Volume 248. This research attempted to benchmark the performance of the storage system to enable future evaluation of the system for precision, sensitivity, robustness and reproducibility from system verification performance data compared to relative and absolute range derived from system validation (internal benchmark).

2.12 Food Storage Systems Dynamics

Storage of agrocommodity requires strict adherence to good storage practices if the quality of the product is to be assured. The control of stored product pest through disinfestation activities is geared at reducing the population of insects. Current trends of controlling insect is the use of modified atmospheres storage (Kader, 1994). With this technology, carbon dioxide gas is introduced to cause asphyxiation of the insects in hermetically sealed bags used to store cocoa bean (Villers, Bruin, & Navarro, 2006). Other methods to maintain quality during storage is keeping moisture content within acceptable level. During storage of cocoa beans, moisture content evaporates to a point where an equilibrium relative humidity (ERH) is formed between the air outside the storage compartment and MC in the coco bean (Mabbett, 2013). MC is directly proportional to the ERH and in the event of high ERH, there is an exponential increase in the insect and mould population (Henderson, 1984). Insect and mould population dynamic in storage is such that, an increase in MC with a corresponding increase in ERH will lead to the introduction of other organisms such as bacteria and yeast. The growth of dry moulds is evident in a storage compartment that has ERH of 75% (Rashid, Kurt, & Carl, 2013). Cocoa beans are hygroscopic in nature and can be stored safely without quality alteration if the beans are kept below or at 8.0% w/w (Codex Alimentarius Commission, 2006). The hygroscopic properties of cocoa make it vital to always assure good MC within acceptable range. Acceptable moisture content for cocoa beans should be less than 7.5 (“Drying Cocoa beans”, n.d.). Most exporting countries set their MC standard below 7.5% to protect the commodity from deteriorating (Food, Policies, & Fao, 2013). The MC in cocoa beans is represented by both the bound and unbound water which is best determined together as the water activity of the bean (Mabbett, 2013). The water activity thus, measure the activities of all the surviving life forms in the cocoa bean and their contribution to the MC. Dry cocoa beans reach critical moisture levels at 8.0 (Villers, 2010). At constant room temperature and relative humidity, MC remains constant (Cengel & Yunus, 1998). When storing cocoa beans in closed systems, air conditioners can be used to stabilize the temperature to control relative humidity of the system as explained by Inorganic, (2003). At constant temperature, relative humidity is controlled and condensation is prevented. Research has introduced hermetic cocoon bags (Grain Pro) as an alternative to store cocoa beans that will prevent infestation by insects and moulds. The technology is used by many exporting countries such as Philippines, Mexico and USA to protect and preserve dry cocoa beans. The principles underlying the storage system works for smaller containment use to store cocoa (Essien, Navarro, & Villers, 2010b). Vacuum systems are being developed by scientist to enable the removal of air from plastic bags for cocoa beans storage (Villers et al., 2006). Importers of organic cocoa beans are already using this vacuum system as a pest control strategy for organic cocoa (International Trade Centre, 2001). With all these dynamics of infestation, moisture content, environmental conditions, and quasi-hermetic storage conditions at play in a storage system, the performance of the storage system to maintained the initial quality of the cocoa beans sample under storage is put to test- assuming an uncontrolled situation of hidden infestation and controlled temperature regime- to find out whether the system is fit to perform its intended purpose of maintaining the initial quality of the sample after storage.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research Design

The design of the research took a non-experimental, observational and cross sectional form with laboratory test methods for data collection. This design has been used for quality assurance research in fields of medicine, psychology, social science etc.

Guided questions were formulated for the research. The questions were to assist in determining whether the quality of dry cocoa beans samples will change after storage. At controlled conditions of temperature and an uncontrolled situation of hidden infestation, assuming all storage vessels used in the research had equal level of performance due to equal sterilization treatment;

The alternative hypothesis was that, a change in quality of dry cocoa beans in storage is as a result of the presence of hidden infestation and/or method of storage. Assumption made were that; all storage vessels used in the research had equal level of performance due to equal sterilization treatment; the Alternate Hypothesis would be true if;

1. The mean frequency count of before and after data for TM, TS, AOD, MC and BW differs.
2. The frequency count of before and after data for TM, TS, AOD, MC and BW correlate weakly.

3.3 Sampling

3.3.1 Sample Size

Two hundred and one (201) samples from 2015/2016 Main Crop (MC) and 2016 Light Crop (LC) represented the sample size. The sample size chosen was to enable a snapshot generalization on the performance of the storage system.

3.3.2 Sample collection

Samplers performed the sampling in accordance with the requirement of ISO/DIS 2292. Samples were collected from warehouses satisfying the following requirements:

1. Devoid of structural defects
2. Had proper ventilation schedule
3. Had good sanitation programme
4. Devoid of roof leakages

Samples were accepted from stacks with the following criteria;

1. Stack on elevated pallets above ground of about seven (7) cm.
2. Fumigated stack ready for shipment
3. Stacks with maximum capacity of 320 tones.

Samples were assigned alphanumeric coded labels and transported in sample carrier bags to the laboratory for analysis.

3.4 Sample Preparation

3.4.1 Bottling

Kilner jars were used for sample storage. The jars were sterilized in warm soap water, rinsed and oven dried at 60 ^{o}C for 3 hours thoroughly before use. Cocoa beans from sample carrier bags were introduced into labelled Jars and tightly closed with a clip lid. The labels were alphanumeric coded (eg. HM001). The bottled samples were left on laboratory shelves with constant room temperature of 20-25 ^{o}C at equilibrium relative humidity of 55%-75% for 121 days (4 months).

3.5 Laboratory Analysis

Laboratory Analysis was done before and after storage and results recorded into a field note book. Test methods performed on the samples included moisture determination with Aqua-boy, bean weight determination and cut test analysis.

3.6 Moisture determination

The equipment used was Cocoa Moisture Meter (Aqua-boy)

3.6.1 Procedures in Determining the Moisture Content

Cocoa sample of about a hand full was put into the cup electrode and connected to the meter by a qualified analyst. The white button was depressed and results read directly on the meter. The white button was release to finish the operation. The process was repeated thrice and average reading taken. Results were recorded in a field note book.

3.7 Bean Weight Determination

Determination of the bean count of whole bean in 100g is required to determine the bean weight of whole bean.

3.7.1 Procedure in determining Weight of the whole bean

The coco scale balance was used in a place with good temperature and humidity (stable environment). Coco scale was exercised before actual measurement were taken. As a precautionary measure, shock loading the balance during initial exercising of the equipment is discouraged. Whole beans were gently placed in the center of the weighing pan and reading taken at the precise moment of achieving balance stability. Readings were recorded appropriately.

3.7.2 Procedure in Determining Count of the whole bean

Bean count was carried out by the determination of the number of cocoa beans to make a weight of 100 g. The bean count was expressed as the number of beans per 100 g.

The bulk sample was reduced by quartering of about 300 beans. Portion of the 300 beans were weighed with coco scale to100 g. The number of whole beans, after the removal of flat beans in 100 g was counted. An average of three counts for each sample was recorded.

Using the formula below, we derived the bean weight:

Formulae: $\{Bean\ Count\ =\ (Total\ whole\ bean\ x\ 100)/\ Weight\ of\ whole\ bean\}$

3.8 Cut test analysis

The equipment used was okapi knife.

3.8.1 Procedure in Determining Defects through the Cut Test

Cut test analysis followed ISO 1114: 1977 for cocoa grading. The cut test was carried out to determine the incidence of mouldy beans (TM), slaty beans (TS), and All Other Defects (AOD). The sample of whole beans used for the bean weight determination was used for the cut test**.** Three hundred (300) beans were cut lengthwise through the middle, so as to expose the maximum cut surface of the cotyledons. Both halves of each bean were examined visually in full daylight. Separate counts were made on the number of beans which were defective with internal moulds, slaty, insect-damaged/infested and germinated. Where a cut bean was defective in more than one defect, moulds were given much priority followed by slaty and then AOD.

3.9 Data Analysis

Before and after data collected were analyzed using Minitab® 17, R and Excel. Minitab® 17 was used for Paired t-test, Pearson product-moment correlation, Regression, Xbar-S, and Xbar-R analysis. R was used for Linear regression graphs because of its superior graphic illustrations. Excel was used for data storage and statistical descriptive analysis.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Variability and Centrality of Performance Data

With the exception of MC and TS, there was a decrease in variability in the after data for TM, AOD and BW. AOD recorded the highest standard deviation (STD) value of 1.149 given a mean value of 1.772 in the before data. The variation in the data can be attributed to expected random errors in the storage system that was created by the modified atmosphere in the closed system. Guenha et al., (2014) in their work on hermetic storage also encountered variations when a modified atmosphere was introduced in a plastic bag sealed for three (3) and six (6) months. The variations in their work however still maintained infestation, germination potential and initial weight within acceptable values of 13.9-17.5%.

MC had a maximum reading for before and after sample at values greater than (>) 8.0% indicating critical levels. In the work of Guehi et al., (2007) on enumeration, identification and evaluation of fungi isolates, the researchers described critical levels of moisture content to be the level at which the quality of raw cocoa bean cannot be guaranteed because of high risk factors with mycotoxinogenic fungi contamination. They further commented also the "complex interaction of factors, such a temperature, moisture, endogenous fungal species, storage history and storage time" that makes quality prediction of raw cocoa beans difficulty solely on MC. Three (3) samples had MC (>) 8.0% and these samples were expected to fail the moisture content monitoring with the Xbar-R analysis tool for before and after storage. Whether the three (3) samples could cause a change in quality solely on the MC levels was to be determined after paired T analysis of the performance data gathered for the entire population. Below is Table 4.1 showing descriptive statistics for performance data before and after storage.

Table 4.1: Descriptive Statistics for Performance data before and after storage

VAR.	Data	Min	1st Quartile	Median	Mean	3rd Quartile	Max.	Variance	STD
MC	B	6.100	6.600	6.900	6.878	7.100	8.300	0.163	0.404
	A	6.000	6.600	6.900	6.888	7.100	8.300	0.165	0.406
BW	B	1.020	1.140	1.180	1.174	1.210	1.340	0.003	0.060
	A	1.020	1.140	1.180	1.177	1.210	1.330	0.003	0.058
TM	B	0.000	0.000	0.300	0.671	1.000	3.000	0.510	0.714
	A	0.000	0.000	0.700	0.736	1.000	3.300	0.501	0.708
TS	B	0.000	1.000	1.000	1.366	2.000	3.700	0.701	0.837
	A	0.000	1.000	1.000	1.366	2.000	3.700	0.701	0.837
AOD	B	0.000	0.700	1.700	1.772	2.300	4.700	1.321	1.149
	A	0.000	0.300	1.000	1.134	1.700	4.300	1.008	1.004

Key: B = Before, A = After

4.2 Performance data for MC

Paired-samples t-test was run on 201 samples for performance data before and after storage to determine whether there was a statistically significant Mean difference in the data. The mean moisture content was higher after storage (6.8877 % ± 0.4069) than before storage (6.8761 % ± 0.4049); with a statistically significant mean increase of 0.01161 (95% CI, -0.02057 to -0.00264) moisture content, t (200) = -2.55, p < .011. The increase however was within levels acceptable to maintain the quality of the stored sample. Jonfia-Essien, (2012) at the Proc 9th. International Conference on Controlled Atmosphere and Fumigation in Stored Products, Antalya, Turkey stated that “The quality of cocoa beans can be sustained by ensuring general good sanitation and maintaining low Moisture Content (MC) to as low as 6.0 – 7.5%. MC mean increase after storage still maintained the initial MC within a range of 6.0 – 7.5%. Below is Table 4.2 showing Paired T for (MC) Before- After.

Table 4.2: Paired T for (MC) Before – After

	N	MEAN	StDev	SE Mean
Before	201	6.8761	0.4049	0.0286
After	201	6.8877	0.4069	0.0287
Difference	201	-0.01161	0.06445	0.00455

95% CI for mean difference: (-0.02057, -0.00264)

T-Test of mean difference = 0 (vs not = 0): T-Value = -2.55 P-Value = 0.011

A linear regression established that the after storage MC data statistically significantly predicted before storage MC data, $F (1, 199) = 5281.72$, $p < .000$, and the after storage MC data accounted for 96.4% of the explained variability in before storage MC data. The regression equation was: (MC)Before = - 0.0376 + 1.03 (MC)After. Below is Table 4.3 and Table 4.4 showing Regression Analysis and Analysis of Variance respectively for MC Before versus After.

Table 4.3: Regression Analysis: (MC) Before versus After

Predictor	**Coef**	**SE Coef**	**T**	**P**
Constant	-0.03759	0.01668	-2.25	0.025
After	1.02890	0.01416	72.68	0.000

S = 0.0115997 R-Sq. = 96.4% R-Sq. (adj) = 96.4%

Table 4.4: Analysis of Variance: (MC) Before versus After

Source	DF	SS	MS	F	P
Regression	1	0.71067	0.71067	5281.72	0.000
Residual Error	199	0.02678	0.00013		
Total	**200**	**0.73745**			

A Pearson's product-moment correlation was run to assess the relationship between before and after moisture content. There was a strong positive correlation between before and after data, r (199) = 0.982, $p < .000$. Below is Figure 4.1 of a Linear Regression showing the relationship between Before MC and After MC.

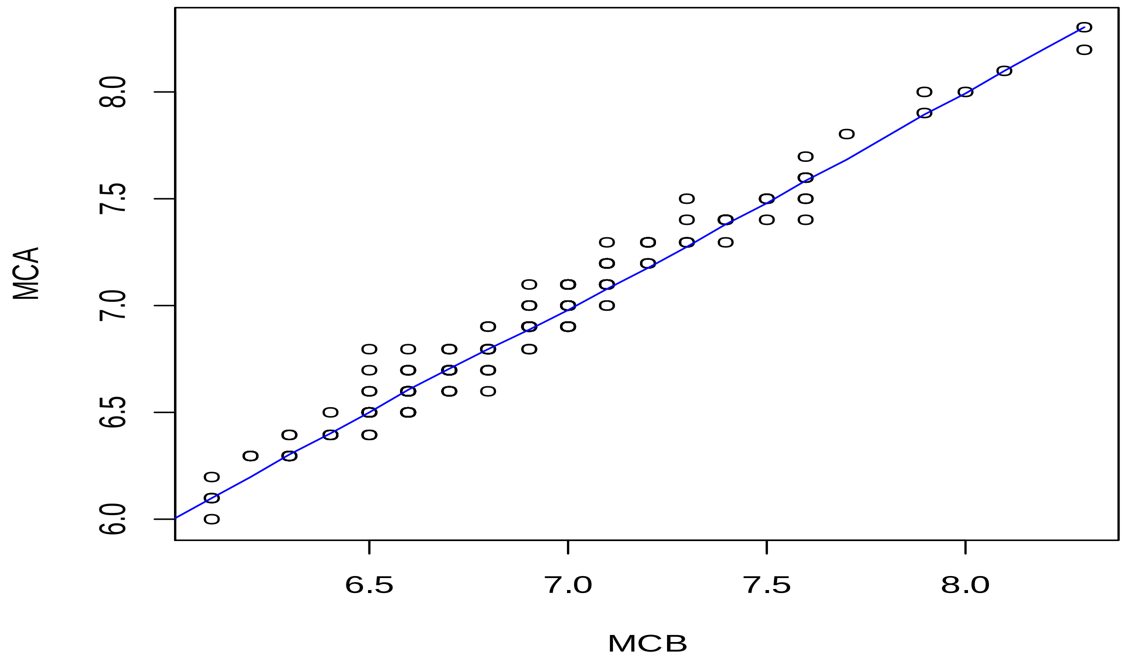

Figure 4.1: Linear regression of MCB and MCA

An Xbar-R chart for every 8th subgroup sampled failed at points that were within the critical moisture content (MC ≥ 8.1%). The Xbar-R Chart was used to determine the stability of the processes which was dependent on the moisture content of the samples. Below is Figure 4.2 showing Xbar-R Chart for moisture content of samples before storage. In the first test, no point had more than 3.00 standard deviations from the center line. In the second test, there were 9 points in a row on same side of the center line.

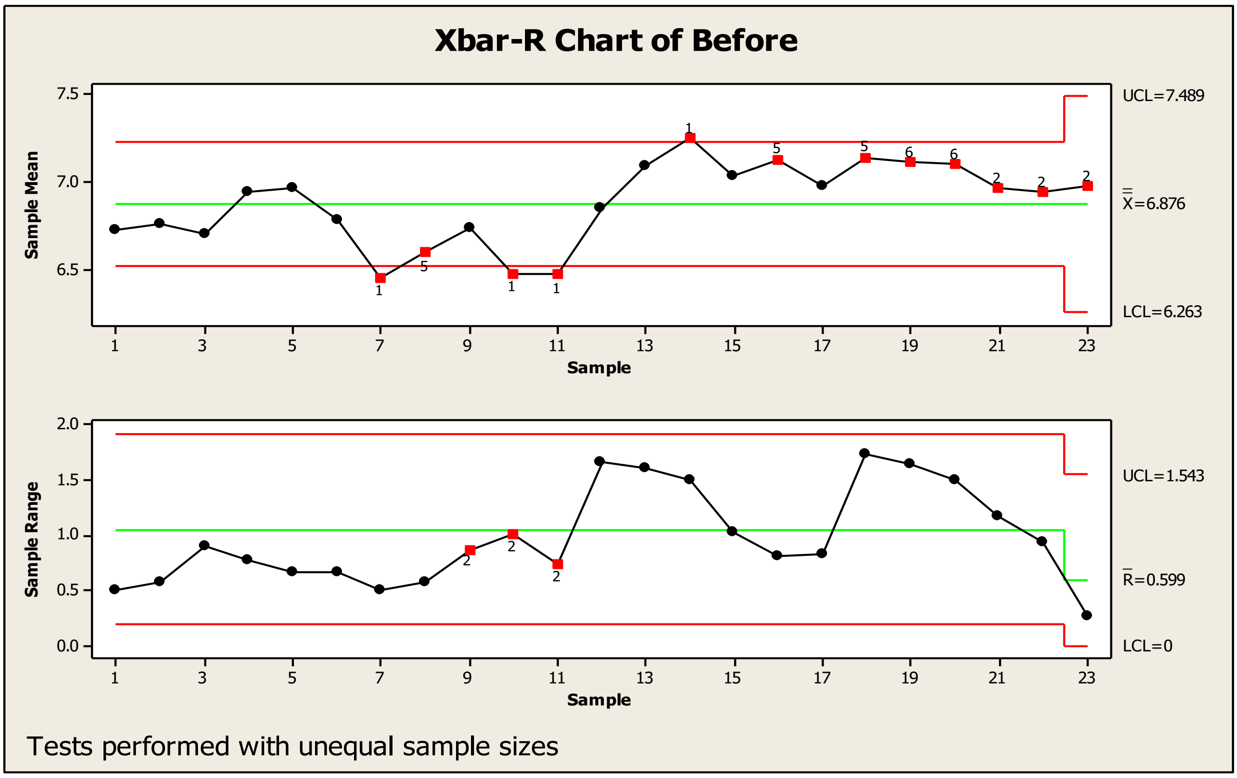

Figure 4.2: X Bar-R Chart for MC of samples before storage

4.2.1 Benchmarking Performance Data for MC

The quality of stored cocoa is controlled when moisture content remains in a range that is acceptable to prevent mould and insect infestation. For dry cocoa beans this range is 6.0 – 7.5%. Above 8.0% w/w, the safety and quality cannot be assured ("Cocoa beans", 2016) Mabbett, (2013) explains the process of evaporation and subsequent condensation of moisture content in cocoa beans where he describes cocoa beans with an initial moisture content critical to safe storage to have a high vulnerability to deteriorate when equilibrium relative humidity is formed between the air outside the storage compartment and MC in the cocoa bean. The results of this study showed that the initial moisture content remain within acceptable level because the process of evaporation and condensation were controlled using air conditioners to stabilize the storage environment relative humidity. Henderson, (1984) findings of direct proportionality between high MC values and high ERH to mould and insect infestation proves that at high MC, growth of dry moulds is promoted in storage compartment that has ERH of 75%. Rashid et al., (2013) in their work on the effect of storage parameters on an agrocommodity published in the Journal of Natural Science Research also showed the effect of not controlling moisture content, relative humidity and temperature on stored agro commodity as the driving force for fungal growth and insect pest. Codex Alimentarius Commission (2006) documents 8.0% w/w and below as the safest MC level for dry cocoa beans storage. It was expected for the three samples with MC greater than 8.0% w/w to fail after storage but interestingly, on the count of the whole population studied, the three samples could not affect the frequency count of the mean reading after storage. It also did not affect the MC quality of the samples from the entire population after storage. Each sample was tested separately but in the end a formulated generalization for the entire population was used to describe the Lot. Since the method of data analysis used did not segregate individual samples from the population studied, it is reckoned, if that had been the case, perhaps the three (3) samples could have had after storage quality to be outside acceptable limits. Below is Figure 4.3 showing Xbar-R Chart for MC monitoring after storage.

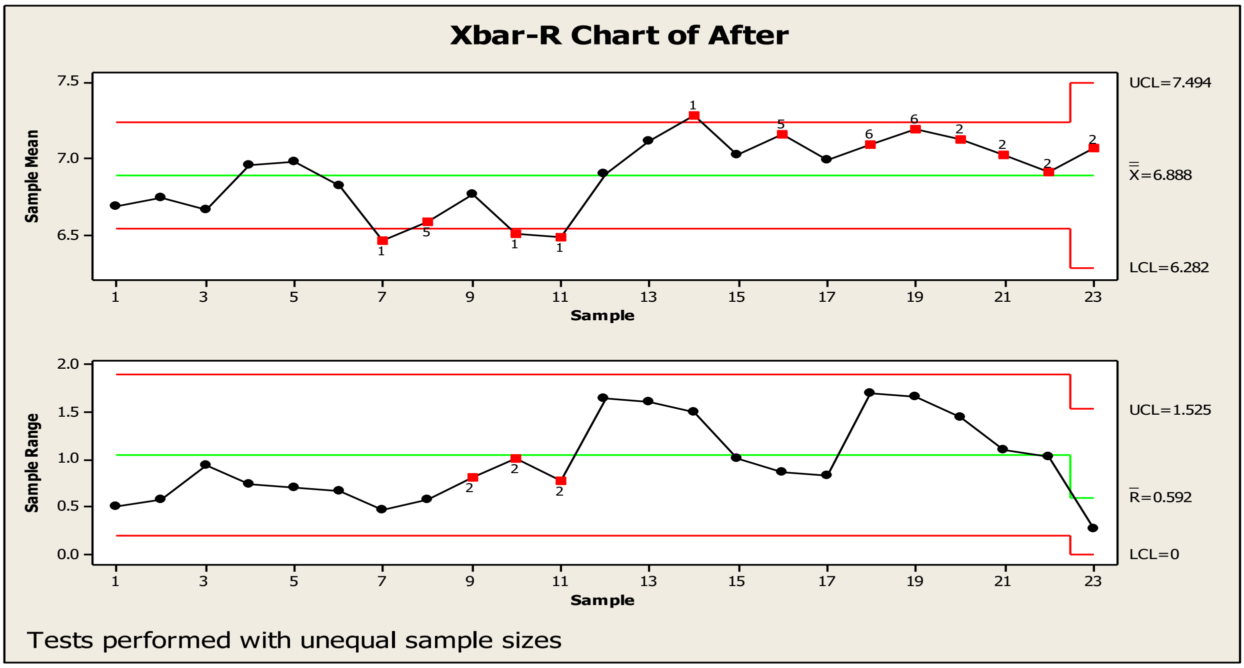

Figure 4.3: Xbar-R Chart for MC monitoring after storage

Mabbett, (2013) in the Journal of International Pest Control published quality control for stored coffee and cocoa where the determination of water activity for cocoa beans was stated as being represented by both bound and unbound water in the bean. The more the present of unbound water in the cocoa bean, the greater the chances of the bean supporting the growth of insect and moulds in a closed system. GrainPro Inc, a company dedicated to storing the future in their document on "Protection of cocoa beans and the emergence of Hermetic storage" affirms the role rising humidity contributes to deterioration of cocoa beans in storage.(Beans Storage, Villers, Avenue, & Navarro, n.d.) Cocoa is safe to be stored without quality alteration when MC is below 8.0% w/w. Controlled storage environment is also important so that the sample does not absorb moisture that will add onto its unbound water due to its hygroscopic nature. In the Scottish Museum Council Fact Sheet-adapted for use in Australia 2003, air conditioner system was suggested to stabilize temperature and relative humidity levels as possible though not recommended first approach. The first approach recommended however was reducing the adverse effect of the external conditions on the internal environment were storage would be done. This recommendation included other things such as keeping all windows closed at all times and keeping the entrance door closed as much as possible. The Fact Sheet also spelt out the use of silica gel to control internal environment which has to be monitored continuously for adverse microclimate formation. A typical air tight case for storage is the Kilner jar used in this research for the storage of dry cocoa beans. In order to monitor the stability of the room air conditioners to maintain temperatures conducive for storage, 20 data points from data collected over four hundred and eight (408) days was used to plot an X Bar-S chart to monitor the stability of the process. The X Bar-S showed a relatively constant temperature regime for the storage period of four hundred and eight (408) days. Keeping temperature constant, the relative humidity was controlled and MC was kept within acceptable range for those samples within acceptable range whereas samples with MC outside the acceptable range maintained MC outside acceptable range after storage. This perhaps is why a statistically significant Mean increase of 0.01161, with before storage Mean reading of 6.8761 % could increase or decrease by 0.4049; and still maintained the reading within acceptable range of ≤ 75%. This finding agreed to Cengel & Yunus (1998) assertion that at constant temperature, relative humidity is controlled and MC remains constant. Below is Figure 4.4 showing Xbar-S Chart of temperature monitoring during storage.

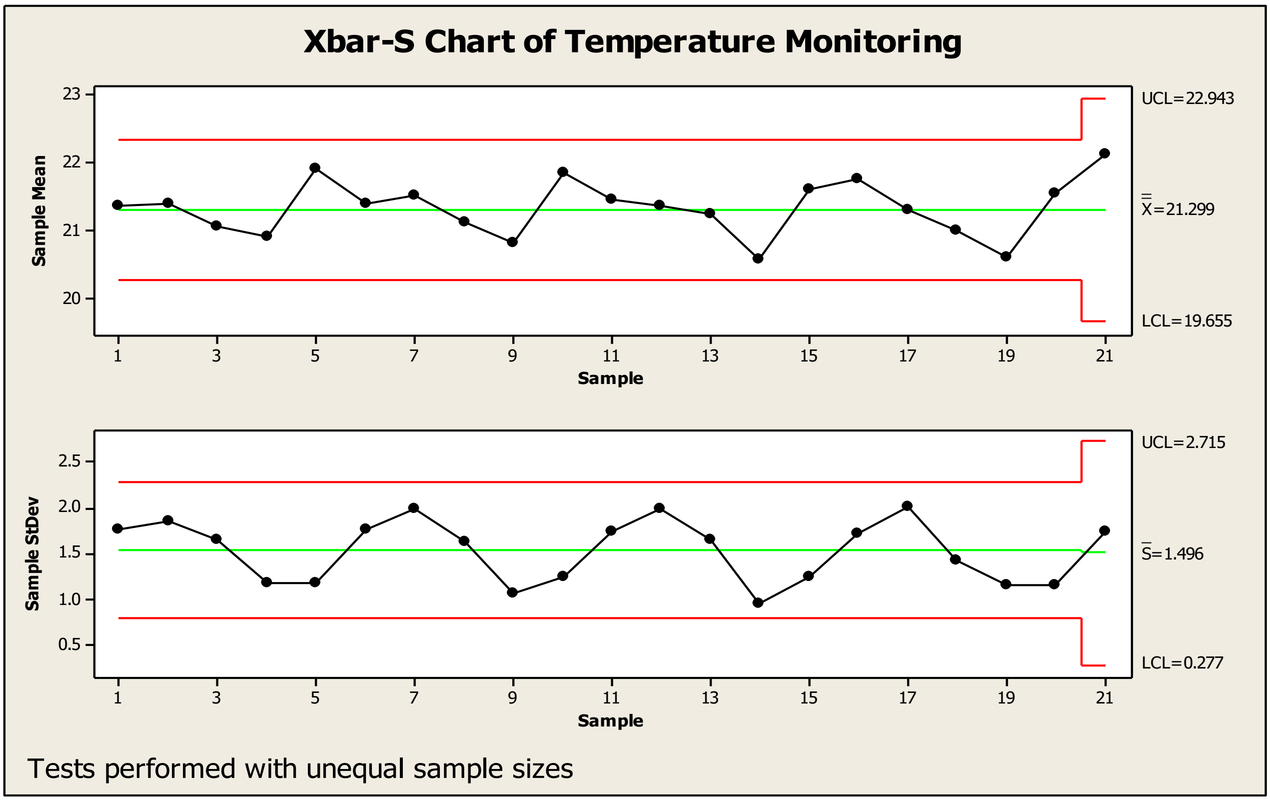

Figure 4.4: Xbar-S Chart of Temperature Monitoring during Storage

4.3 Performance data for Total Mould (TM)

Paired-samples t-test was run on 201 samples before and after storage to determine whether there was a statistically significant mean difference in the before and after data. The mean TM was higher after storage (0.7363 % ± 0.7083) than before storage (0.6711 % ± 0.7143); and statistically significant with Total Mould, t (200) = -6.49, p < .000. The increase however was within levels acceptable to maintain the TM quality of the stored sample. In the book on Cocoa Beans: Chocolate & Cocoa Industry Quality Requirement, the maximum percentage of beans defect for TM allowable was set at 3% for grade 1 and 4% for grade II (Costa et al., n.d.). The initial TM before storage though increase after storage by 0.0652 only put the final TM in the range 0.7363 % ± 0.7083 still within the acceptable limit of grade I. Below is Table 4.5 showing Paired T for (TM) Before- After.

Table 4.5: Paired T for (TM) Before – After

	N	MEAN	StDev	SE Mean
Before	201	0.6711	0.7143	0.0504
After	201	0.7363	0.7083	0.0500
Difference	201	-0.0652	0.1424	0.0100

95% CI for mean difference: (-0.0850, -0.0454)

T-Test of mean difference = 0 (vs not = 0): T-Value = -6.49   P-Value = 0.000

A linear regression using Minitab® 17 established that the after storage TM statistically significantly predicted before storage TM, $F (1, 199) = 4824.48$, $p < .000$, and the after storage TM accounted for 96.0% of the explained variability in the before storage. The regression equation was: (TM)Before = - 0.0566 + 0.988 (TM)After. Below is Table 4.6 and Table 4.7 showing Regression Analysis and Analysis of Variance respectively for (TM) Before versus (TM) After.

Table 4.6: Regression Analysis: (TM) Before versus After

Predictor	**Coef**	**SE Coef**	**T**	**P**
Constant	-0.05663	0.01452	-3.90	0.000
After	0.98840	0.01423	69.46	0.000

Table 4.7: Analysis of Variance: (TM) Before versus After

Source	DF	SS	MS	F	P
Regression	1	98.010	98.010	4824.48	0.000
Residual Error	199	4.043	0.020		
Total	200	102.053			

A Pearson's product-moment correlation was run to assess the relationship between before and after Total Mould. There was a strong positive correlation between before and after data, r (199) = 0.980, p < .000

4.3.1 Benchmarking Performance data for TM

A good post-harvest drying for cocoa beans eliminates the occurrence of mould defect in the dry cocoa beans (International Cocoa Organization (ICCO), 2009). Even though there was a significant difference (-0.0652) in the means of the before and after data, the after mean reading (0.736) was still within a range that maintained the initial quality of the bean (0- 3%) for grade I and (3% - 4%) for grade II. Appendix B-4 has details of standard mean values for grade I and II. Most fungi will require very little amount of oxygen to grow and develop, the oxygen present in the system though not measured was limited since air tightness minimizes the flow of warm air or cool air from the external environment as explained by Cammalleri & Gumpertz, (2003) in their work on condensation in a building envelope published in the proceedings of "SSPC 2003: The Industrial Protective Coatings Conference and Exhibit, Ernest N. Morial Convention Center, New Orleans, LA, 26 – 29 October 2003,". Fagbohun et al., (2011) using direct plating and slide culture methods in their work on fungi associated with spoilage of dried cocoa beans during storage identified fungi that were all obligate aerobes. In a closed system where oxygen is a limited resource, the respiratory requirement of fungi associated with the spoilage of dry cocoa beans in storage cannot be meet and hence as the limiting factor declines without being replenish, the atmosphere inside the storage system gradually delimits the fungi population that could be supported if they were already present in the before storage. Villers (2010) in his work on hermetic storage described how gradual loss of oxygen through cellular activities of life forms during hermetic storage will with time introduce carbon dioxide that could be harmful to obligate aerobes associated with the spoilage of dry cocoa beans. Essien et al., (2010) also on hermetic storage affirms how modified atmosphere creation in a storage system improves the shelf life of cocoa beans and prevents deterioration of a sample during storage. His assertions encompass not only the role fungi play in raw cocoa bean deterioration but the complexities and interplay of other factors such as MC, insect infestation and initial quality of the consignment before storage. There was a strong positive correlation for before and after TM indicating a change in before parameters at constant conditions will elicit a direct change in the after quality of the storage sample. In spite of the fact that, there was statistical significance in the Means of before and after data at 95 % CI, the initial TM did not affect the quality of the dry cocoa beans after storage. The system maintained the levels of mould present before storage within the acceptable margin for grade I ($0 \leq X \leq 3$) specification (Details of Standard mean for grade specification in Appendix B-4). Having a system that prevents increase in fungi population during storage is important to avoid OTA producing fungi from developing (Amézqueta et al., 2008). Guehi et al., (2007) affirms the presence of fungi in high levels from dry cocoa beans that are stored poorly in their work on “Enumeration and Identification of Main Fungal Isolates and Evaluation of Fermentation’s Degree of Ivorian Raw Cocoa Beans”. The storage system used therefore had performed as expected and fit for its purpose on the count of TM Below is Figure 4.5 showing linear regression for TMB and TMA.

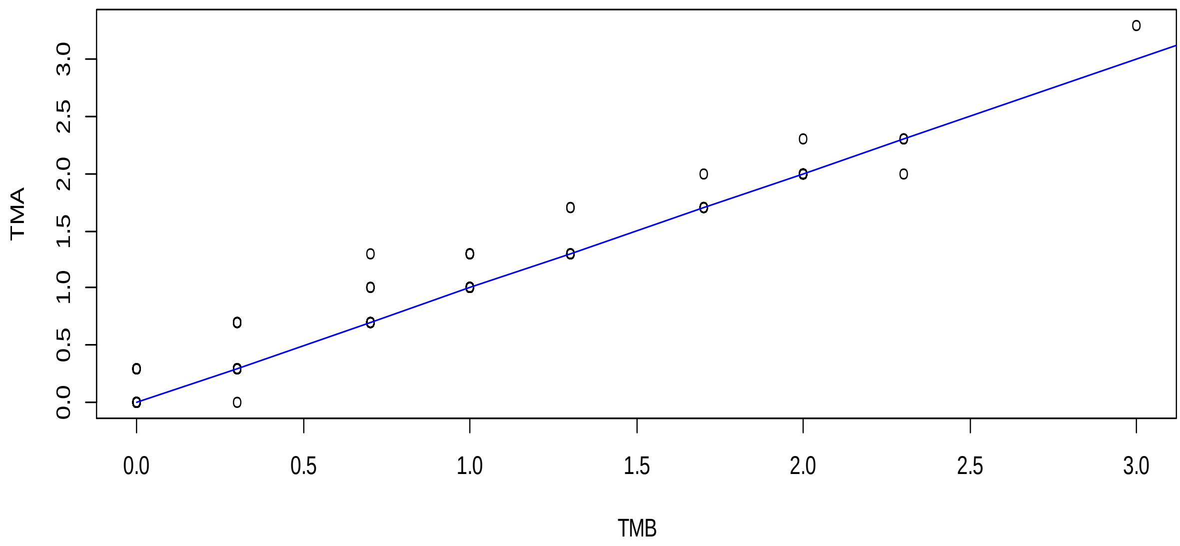

Figure 4.5: A linear Regression for TMB and TMA

4.4 Performance data for All Other Defect (AOD)

A paired-samples t-test was run on 201 before and after storage samples to determine whether there was a statistically significant mean difference in the AOD before and AOD after data. The mean AOD was higher in the before storage (1.7721 % ± 1.1494) than after storage (1.1338 % ± 1.0044); Mean AOD for before and after data was statistically significant (95% CI, 0.5247, 0.7519) AOD, t (200) = 11.08, p < .000. Below is Table 4.8 showing Paired T for (AOD) Before – After.

Table 4.8: Paired T for (AOD) Before – After

	N	MEAN	StDev	SE Mean
Before	201	1.7721	1.1494	0.0811
After	201	1.1338	1.0044	0.0708
Difference	201	0.6383	0.8170	0.0576

95% CI for mean difference: (0.5247, 0.7519)

T-Test of mean difference = 0 (vs not = 0): T-Value = 11.08   P-Value = 0.000

A linear regression established that the after storage statistically significantly predicted before storage, $F(1, 199) = 214.25$, $p < .000$, and the after storage accounted for 51.8% of the explained variability in the before storage. The regression equation was: (AOD) Before = 0.838 + 0.824 (AOD) After. Below is Table 4.9 and Table 4.10 showing Regression Analysis and Analysis of Variance respectively for AOD Before versus After.

Table 4.9: Regression Analysis: (AOD) Before versus After

Predictor	Coef	SE Coef	T	P
Constant	0.83786	0.08518	9.84	0.000
After	0.82401	0.05629	14.64	0.000
S = 0.799640 R-Sq. = 51.8% R-Sq. (adj) = 51.6%				

Table 4.10: Analysis of Variance: (AOD) Before versus After

Source	DF	SS	MS	F	P
Regression	1	137.00	137.00	214.25	0.000
Residual Error	199	127.25	0.64		
Total	200	264.24			

A Pearson's product-moment correlation was run to assess the relationship between before and after AOD. There was a weak positive correlation between before and after data, $r(199) = 0.720$, $p < .000$

4.4.1 Benchmarking Performance data for AOD

All other defects comprise of germinated, insect damage and other defects that includes flat beans and any other defect. This quality attribute of cocoa beans identified through the cut test was key to determine the grade of the samples analysed. The research design of this study was such that the uncontrolled situation of hidden infestation could only be a determining factor for explained variability only when detectable insect live forms was excluded from the sample during sample preparation before bottling and storage. Samples were therefore selected from fumigated stacks that were ready for shipment. By default, all fumigated stacks are free from detectable insect life forms. With this approach, even if any surviving eggs persist in the sample during storage, the eggs could be constraint in an attempt to growth and develop because of delimiting resources of oxygen but not food present in the closed system. The generation times for major stored insect pest of cocoa beans include *Ephestia cautella, Tribolium castenuem, Lasioderma serricone* all have their generation times at favorable conditions to be 29-31 days, 30-137 days, 25-120 days (Melorose, Perroy, & Careas, 2015b) respectively and *Corcyra cephalonica;* 91-121 days (N.B Osman, n.d.). In the work of Woods & Hill, (2004), on "Temperature-dependent oxygen limitation in insect eggs", the authors argued that the requirement of insect eggs to undergo metabolism meant the need for oxygen from the environment. This requirement could also be satisfied at lower temperatures and hence warmer conditions would limit the availability of the oxygen to the egg tissues for metabolic activity thereby causing. Their work found out that, "Metabolic rates, measured as rates of CO2 emission, were virtually insensitive to hypo- and hyperoxia at 22 degrees Celsius". Their data showed that at practical temperatures of 32-37 Degree Celsius, eggs were oxygen limited.

Since the conditions in the closed system was limiting for oxygen flow, the temperatures in the storage environment (20-25oC) could still encourage the eggs to undergo metabolism according to Woods & Hill, (2004). This study however did not measure the infestation index of the samples before storage and hence could not attribute reason to the adult forms of the insects encountered after storage. The 48.2% of the unexplained variability in the data was hence attributed to the presence of hidden infestation as reported by Kendra et al., (2011) and Lu & Ariana, (2013). The rationale behind this generalization is based on the exclusion of all detectable insect live forms before the closed system storage of the dried cocoa beans. Stubbs et al., (1985) also confirms the vulnerability of cocoa beans and possible variation in the before and after attributes because of the elusive nature and difficulty in early detection of hidden infestation in stored products. During the cut test analysis after storage, insect damage cocoa beans were observed indicating feeding action of insects (FAO, 2001). The larvae that had matured into adult forms had fed on the cocoa samples. Tettey, (2014) describes the larval forms of stored product pest as the most destructive life forms of the insects' life cycle. Germinated and Other defects in the samples analyzed reduced in the after data from 4.72% to 4.17%, a 0.55% difference. This however did not affect the after storage quality and can be attributed to random errors in the system. The weak positive correlation, 48.2% unexplained variability, statistically significant mean difference of 0.6383 still could not change the quality of the initial dry Cocoa bean sample. Even though some samples might have changed quality from one to the other, a snapshot generalization of the entire population analysed showed that the initial quality on the count of AOD had remained within acceptable levels. Below is Figure 4.6 of a linear Regression showing the relationship between Before AOD and After AOD.

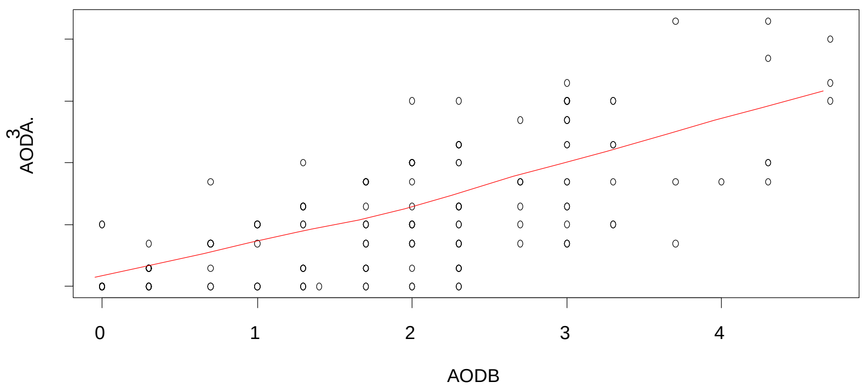

Figure 4.6: Linear Regression for AODB and AODA

4.5 Performance data for Bean Weight (BW)

A paired-sample t-test was run on 201 samples before and after storage to determine whether there was a statistically significant mean difference in the before and after storage data. The Mean bean weight was higher for after storage (1.17679 % ± 0.05794) than before storage (1.17321 % ± 0.06072); with a statistically significant mean increase of 0.003580 (95% CI, -0.005206 to -0.001954) bean weight, t (200) = -4.34, $p < .000$. Below is Table 4.11 showing Paired T for (BW) Before – After.

Table 4.11: Paired T for (BW) Before – After

	N	MEAN	StDev	SE Mean
Before	201	1.17321	0.06072	0.00428
After	201	1.17679	0.05794	0.00409
Difference	201	-0.003580	0.011691	0.000825

95% CI for mean difference: (-0.005206, -0.001954)

T-Test of mean difference = 0 (vs not = 0): T-Value = -4.34 P-Value = 0.000

A linear regression established that the after storage statistically significantly predicted before storage, F (1, 199) = 5281.72, $p < .000$, and the after storage accounted for 96.4% of the explained variability in before storage. The regression equation was (BW) Before = - 0.0376 +

1.03 (BW) After. Below is Table 4.12 and Table 4.13 showing Regression Analysis and Analysis of Variance respectively for BW Before versus After.

Table 4.12: Regression Analysis: (BW) Before versus After

Predictor	**Coef**	**SE Coef**	**T**	**P**
Constant	-0.03759	0.01668	-2.25	0.025
After	1.02890	0.01416	72.68	0.000
S = 0.0115997 R-Sq. = 96.4% R-Sq. (adj) = 96.4%				

Table 4.13: Analysis of Variance: (BW) Before versus After

Source	**DF**	**SS**	**MS**	**F**	**P**
Regression	1	0.71067	0.71067	5281.72	0.000
Residual Error	199	0.02678	0.00013		
Total	**200**	**0.73745**			

A Pearson's product-moment correlation was run to assess the relationship between before and after bean weight. There was a strong positive correlation between before and after data, r (199) = 0.982, p < .000. Below is Figure 4.7 of a linear Regression showing the relationship between Before BW and After BW.

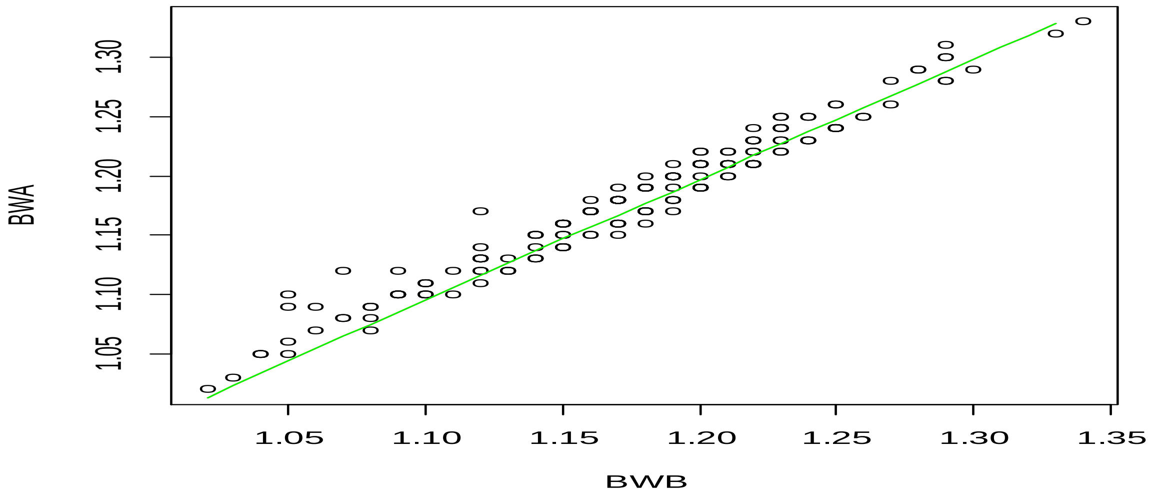

Figure 4.7 A linear Regression for BWB and BWA

4.5.1 Benchmarking Performance data for BW

The bean weight was determined from the weight of the whole bean and count of the whole bean in 100g ("Quality Certification for Dry Cocoa beans," n.d.). In the Cocoa industry, a higher bean weight is preferable since the weight of the parcel is used in the pricing of the commodity (Food et al., 2013). A higher bean weight is hence commercially good for the business. The expectation was for the bean weight of each sample to remain constant. Since the bean count in 100g is inversely proportional to the weight of the whole bean, an increase in the weight was going to cause a drop in the count in 100g. At a statistically significant Mean increase of 0.003580, the bean weight of the sample before storage (1.174) and the Mean bean weight after storage (1.177) remained within acceptable bean weight specification of where X ≥ 1 and X is bean weight (TIS, 2016). A strong positive correlation indicates that, an initial bean weight of the sample will determine the bean weight after storage at standard conditions of storage. An increase of 0.01161 for Mean moisture content and 0.003580 for Mean bean weight suggested relationship between MC and BW.

CHAPTER FIVE

CONCLUSION, SUMMARY OF FINDINGS AND RECOMMENDATION

5.1 Conclusion

The storage system design and testing showed that the system provided results as intended for its design with the mean frequency counts for TM, TS, BW, AOD and MC all within acceptable levels when the before and after data were compared. Ninety-two (92.04%) of the total samples observed maintained their initial quality (Appendix A-5). Samples that had a change of grade was as a result of ‘‘borderline ripples’’ from sampling error and reduction of purple defect after storage as documented by Kenten, (1965) in his work on “Loss of anthocyanins during the storage of dry fermented Cocoa”. The laboratory test methods used to observe the system proved to be efficient and reliable. The data analysis performed however did not segregate individual samples from the population and hence inferences based on samples was difficult to conclude.

The Mean frequency count of before and after data for TM, TS, AOD, MC and BW differed significantly. The correlation of the frequency count for before and after storage for TM, TS, MC and BW were robust whereas for the variable AOD, the correlation of frequency count for before and after storage data was weak. There were however some unexplained observations of variability in AOD for before and after data collected. The results from this study represented the entire population and not individual members of the population even though each individual member within the population was monitored separately from the lot. This enabled a snapshot generalization to apply to the performance of the entire system. The increase and/or decrease in the Mean frequency counts could not cause the quality to change because the change in the after mean frequency counts remained in acceptable range. The assumption of the presence of hidden infestation could not engender a change in quality of dry cocoa beans stored in a sterilized, well-sealed and temperature controlled environment even though it led to 48.2% of unexplained variability in the performance data for the variable AOD before and after storage.

The storage system for dry cocoa beans was fit to perform its intended purpose of maintaining the quality integrity of retention samples. The work agreed with proponents such as Dano et al., (2013), who supports the theory of quality maintenance of stored agro products linked to minimal or zero infestation and good environmental conditions of storage. Predictive models from Regression analysis for individual variables under study could serve as bench mark for future verification of storage system performance. Results from sample testing in this storage system after system testing and validation can serve as evidence in the event of quality arbitration in the Cocoa trade since a validated storage system in the end is reliable and assure the provision of valid results for retention sample analyses. At controlled conditions of temperature and an uncontrolled situation of hidden infestation, the quality of dry cocoa beans in a sterilized food storage container maintained its quality even though there was a statistically significant difference in the variables observed.

5.2 Summary of Findings

There was a strong positive relationship between the before and after quality of raw cocoa beans on the count of TM, TS, MC and BW. Differences in the mean frequency counts before and after storage statistically was significant for the observed variables but practically were within initial range of industry accepted standard. The null hypothesis hence was true for observed variables TM, TS, MC and BW.

The null hypothesis was rejected for the observed variable AOD because of weak correlation in the frequency count of the before and after data. There was 48.2% unexplained variability in the AOD data which likely caused the null hypothesis to be rejected. The limitations within the design approach accounted for the difficulty in making strong inference of causality. A snapshot generalization on the performance of the entire system's capacity to maintain the initial quality of raw cocoa beans stored in Kilner jars for a period of Four (4) months (121 days) concludes a high-performance criterion for the storage system.

System Verification, Sensitivity analysis and Precision on the storage system can employ Regression models for MC: [(MC)Before = - 0.0376 + 1.03 (MC)After], TM: [(TM)Before = - 0.0566 + 0.988 (TM)After], AOD: [(AOD) Before = 0.838 + 0.824 (AOD)] and BW: [(BW) Before = - 0.0376 + 1.03 (BW)] as benchmark models for system validation checks.

5.3 Recommendation

The study design was to enable a snapshot generalization of the quality relationship before and after storage between variables investigated. The following suggestions are given to improve this study;

1. Experimental designs should be used to test raw cocoa bean quality before and after storage a) on the count of AOD b) incorporating dehumidifier to control the microclimate of the closed system.
2. Performance of other storage systems should be compared to Kilner jar for dry cocoa beans.
3. Research co-operation among different testing laboratories using similar systems is encouraged in this area of food storage system validation geared at reference material development for routine systems validity checks.
4. The sensitivity, reproducibility, robustness and precision of the storage system for raw cocoa beans retention samples needs to be investigated in future studies.

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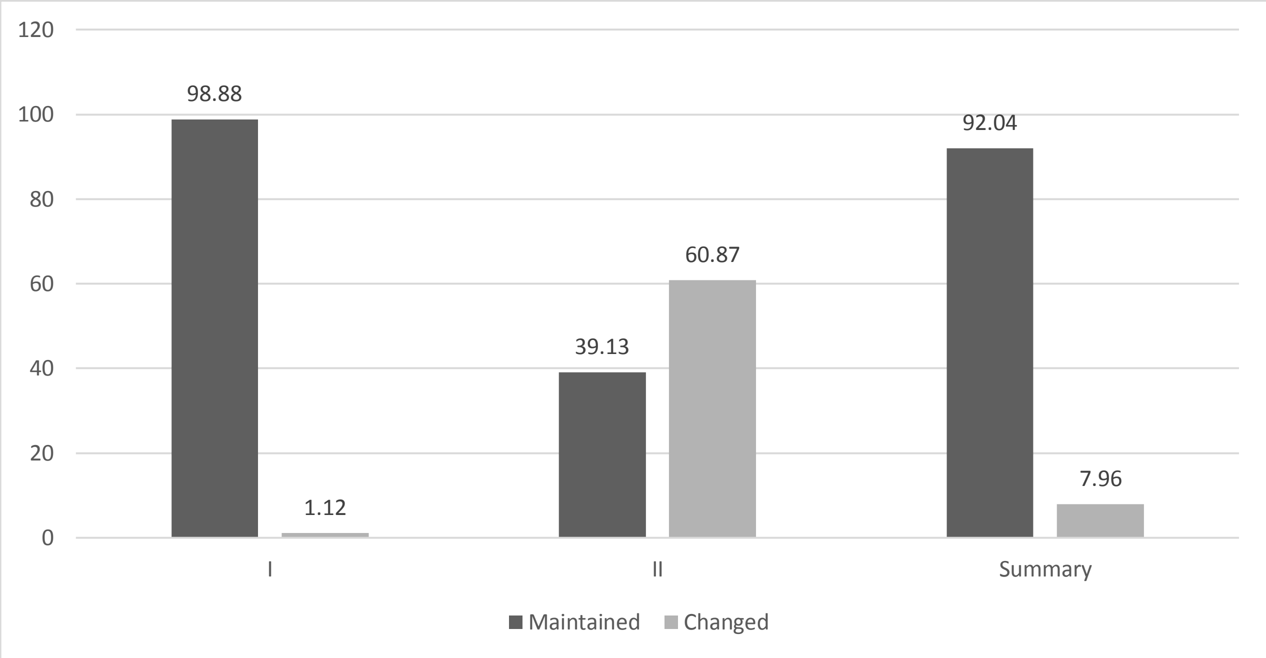
APPENDICES

Appendix A-5: Grade Status after Storage



Appendix B-4: Standard mean values of variables under study

Maximum Defect Levels			
GRADE	TM	TS	AOD
Grade I	3%	3%	3%
Grade II	4%	8%	6%

Quality Specification for Ghana Cocoa (COCOBOD)