MODELLING CONDITIONS FOR QUALITY EGG STORAGE USING RANDOMIZED COMPLETE BLOCK DESIGN

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DECLARATION

This project is my original work and has not been presented elsewhere for a degree or any other award.

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DEDICATION

Special dedication to my treasured dad John, mum Jane and siblings; Maggy, Grace, Joshua, Juliet, Milly, Henry, Francis, Nancy and Kenneth.

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LIST OF ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
AD	Albumen Diameter
AH	Albumen Height
AW	Albumen Weight
BRD	Bovine Respiratory disease
Ch_EW	Change in egg weight
CRD	Complete Randomized Design
Coef	Coefficient of variation of the estimated parameters
CV	Coefficient of variation of the model
EWL	Egg Weight Loss
FEs	Federer's Estimation
FE	Fixed effect
FML	Full Maximum Likelihood
HU	Haugh Unit
ISA	Institut de Sélection Animale
LSD	Least Significant Difference
PCs	Principal Components
PCA	Principal Component Analysis
PRESS	Prediction Sums of Squares
PROC GLM	SAS procedure for General Linear Model
PROC MIXED	SAS Procedure for Mixed Model
RCBD	Randomized Complete Block Design
REML	Restricted Maximum Likelihood

R-sq.	R-Square
R-sq. (pred.)	Predicted R-square
SAS	Statistical Analysis Software
SE	Stein Estimator
SD	Shell Diameter
SW	Shell Weight
VAR	Variance
YD	Yolk Diameter
YH	Yolk Height
YI	Yolk Index
YW	Yolk Weight

LIST OF SYMBOLS

β	beta for model coefficient
ε	epsilon for error
γ	gamma for block effect
Γ_{iq}^{q}	Gamma for a matrix with q columns of the i^{th} treatment effect
λ	lambda for treatment effect
Λ_{ij}^{k}	Lambda for a matrix with k columns of i^{th} treatment and j^{th} block effect
μ	mu for grand mean
Ω_{iq}^{r}	Omega for a matrix with r columns i^{th} treatment effect in column q
T	operation of transposition
ϕ_j	phi for j^{th} block effect
ψ	psi for an interaction effect between lambda and gamma
σ^2	sigma for variance component
Σ	Sigma for the variance-covariance matrix
θ_t	theta for the effect of the t^{th} treatment

OPERATIONAL DEFINITION OF TERMS

An ***estimator:*** is any statistic whose value is used to obtain $u(\theta)$ where $u(\theta)$ is a function of θ and θ is an unknown population parameter (White *et al.*, 2016).

A ***biased estimator:*** any statistics $\hat{\theta} = \hat{\theta}(X_1, X_2, \cdots, X_n)$ is a biased estimator for the unknown parameter if $E(\hat{\theta}) \neq \theta$ (White *et al.,* 2016).

An ***unbiased estimator:*** If the above expectation i.e. $E(\hat{\theta}) = \theta$ such that its bias is zero (White *et al.,* 2016).

A ***parameter:*** is a characteristic of a population (Yang, 2010).

Commercial eggs: Eggs used for income purposes (Pescatore *et al*., 2011).

Fixed model: a model consisting of only fixed component(s) (LeMay & Robinson, 2004).

Haugh unit (HU): is a measure of the quality of protein in the egg based on the height of the albumen (Lee *et al*., 2016).

Mixed model: a model with both fixed and random variables (LeMay & Robinson, 2004).

Model: a mathematical expression embodying a set of statistical assumptions concerning the generation of sample data that can help generate information from the whole population (Yang, 2010).

ABSTRACT

Rearing chicken has contributed positively to global nutrition, especially egg production. This practice attracts both large and small-scale poultry keeping within the world's economy. Egg storage has been a problem due to ineffective methods subjecting many farmers and egg retailers to losses. These methods include various models involving statistical analysis of the storage conditions on the egg quality. However, they do not provide sufficient information. Therefore, confusion persists between the use of fixed and mixed-effect models. The confusion is because some studies analyse randomized complete block design as fixed while others as mixed effect models. Apparent deficiencies of the evidenced information from the randomized complete block design model prompted this study. The quality of the eggs was determined by the physical characterization and changes of both external and internal properties under different temperature conditions and storage duration. The study evaluated the effect of storage temperature at three levels (5 ^oC, 19.5 ^oC and 30 ^oC) and time at four levels (2nd, 12th, 22nd and 32nd) on egg quality using fixed and mixed-effects models. This study used a total of 618 fresh and unfertilized eggs from the ISA (Institut de Sélection Animale) brown layers. Restricted maximum likelihood and analysis of variance methods were used to determine the efficiency of fixed and mixed effect models. Results showed that the physical components of the egg were significantly affected at 5 ^oC, 19.5 ^oC and 30 ^oC $(P < 0.05)$. The effect was more adverse on eggs stored at 30 ^oC for 32 days. However, storage temperatures of 5 ^oC and 19.5 ^oC led to an extensive reduction in the Haugh unit, yolk index, and egg white. Contrariwise, it increased the weight loss, the albumen diameter under storage for 2nd, 12th, 22nd, and 32nd-time intervals. This study recommends a temperature of 5 ^oC for egg quality preservation. The eggs should be reserved in fridge-freezers for 32 days, at 19.5 ^oC for fourteen days, and at 30 ^oC for seven days maximal. The fixed-effect models exhibited smaller components in diameter and height of albumen, yolk index, weight loss, and Haugh unit. This overlapped instances where the fixed-effect models were significantly the same as the mixed-effect models. This study proposes that the fixed effect model is the most appropriate for randomized completely block design experiments. This study obtained G-optimal efficiency of 68% to predict the optimal levels of egg storage for quality maintenance. This study strongly recommends further studies to consider optimization using other classes of storage conditions.

CHAPTER ONE

INTRODUCTION

1.0 Introduction

This chapter comprises the background of information, statement of the problem, justification, objectives of the study, hypotheses, scope, and assumptions of the study.

1.1 Background Information

Globally, there is a tremendous and ongoing human population increase over the past few years. Current geographical and statistical models predict a global population of approximately 10 billion human beings by 2050 (Godfray *et al*., 2010; Kwasek, 2012; Popkin *et al.*, 2012). The world human population was about 8 billion by 2017; this is considered overpopulation (Mottet & Tempio, 2017). Rapid population growth in Africa contributes to at least half of the world's population growing from 1.1 billion to 2.4 billion by 2012 (Alexandratos & Bruinsma., 2012; Latham, 2021). This growth is a result of the densely young generation population that is capable of reproduction. Such an increase implies an intense demand for basic human needs, which the world might find difficult to sustain (Hall *et al*., 2017). Food security and "food for the stomach" are immediate demands for human existence, such as shelter and clothing (Kwasek, 2012). Nevertheless, food security depends on the agricultural sector in several countries worldwide (Osabohien *et al.*, 2018).

Poultry production is a broad area in animal keeping with an acknowledgeable contribution to secure food in several countries (Addo, 2017). Chicken eggs are a significant source of protein for human nutrition and are widely preferred due to their relatively low price (Miller, 2019). Yet, chicken eggs are highly perishable food and may lose quality rapidly if not subjected to proper care from the time of laying, collection, to consumption (Molnár *et al*., 2016). Internal and external structures such as the Haugh unit, which measures freshness, describe egg quality (Lee *et al.*, 2016). The shell does not cover the interior part impeccably and can be easily affected by the storage condition (Molnár *et al*., 2016). Joseph *et al.* (2018) observed a high rate of chicken egg consumption in Sub-Saharan

Africa. The locally produced poultry products experience various challenges in meeting the current demand (Mottet & Tempio, 2017). That situation is not different in Kenya! As the population increases, the need for food also increases (Omondi, 2019).

Various stakeholders have been struggling to put in place measures that could help maintain commercial egg quality from the time laid, collection to consumption. However, the quality has been inferior due to the extensive systems used (Manhique *et al*., 2017). For many farmers and retailers, egg handling and storage have subjected them to significant losses brought about by storage temperatures and storage duration. Egg quality for market value deteriorates with increased storage time and extreme storage temperatures (Lee *et al*., 2016). The storage temperature has a high impact on the yolk characteristics, shell thickness and increases airspace diameter. To a considerable extent, the egg white, a measure of protein composition, also deteriorates drastically (Osabohien *et al*., 2018).

However, the statistical analysis and interpretation of experiments based on commercial egg quality using randomized complete block design may be less critical if there is a failure to sufficiently understand the best model to be fitted. Since 1919, there is a higher rate of development in statistical techniques and their application to design and analyze agricultural experiments. A famous statistician Ronald A. Fisher performed statistical analysis of field and laboratory accumulated experiments as described by (Yang, 2010). He invented Analysis of Variance (ANOVA) and different designs in experiments currently used in field and laboratory experiments. The invention was mainly to accurately determine the estimators of the unknown parameter by eliminating bias and improving precision. Modelling standard randomized complete block design (RCBD) generates a mixed effect model (LeMay & Robinson, 2004).

Conversely, treating blocks as a fixed effect in RCBD experiments is usually the most appropriate choice (Dixon, 2016). Failure to differentiate between fixed versus random effects within an RCBD model has led to significant confusion and uncertainty with mixed model analysis (Yang, 2010). Discourse has risen as to the suitable way to state the hybrid linear model in experimental design (Bremer, 1993). According to Festing (2010), the inability to standardize the nomenclature of RCBD can confuse when teaching the principles of experimental design. Fitting this model gives a standard model, which is a mixed effect model. Therefore, the choice of factors as either fixed or random in a standard RCBD (Equation 1.1) model has a significant consequence in statistical analysis;

$$y_{ijk} = \mu + \lambda_i + \gamma_j + \varepsilon_{ijk} \tag{1.1}$$

where μ is the grand mean, λ_i is the i^{th} treatment effect, γ_j is the j^{th} block effect, and ε_{ijk} residual error which is independent and associated with the observed value y_{ijk}. Whenever both λ_i and γ_j are fixed, then the resulting model is fixed. On the other hand, if either λ_i or γ_j is random and the other fixed, then the resulting model is mixed (Dixon, 2016). This project aims to investigate the effect of storage temperature at three levels (5^0C, 19.5^0C, 30^0C) and storage duration at four levels (2^{nd}, 12^{th}, 22^{nd}, 32^{nd}) days on both the egg's quality determinants using a fixed and mixed-effect model.

1.2 Problem Statement

Most scientists, researchers, and data analysts have embraced several experimental designs such as RCBD, split-plot and completely randomized design (CRD). Studies on comparing mixed and fixed-effect models have concentrated on unbalanced experimental designs. The method of interest of this study was the RCBD which is used widely in both field and laboratory experiments. The specification of factors is not precise. This is because amongst recent researches, some of the data analysts, as well as researchers, consider the fixed effect model Dixon, (2016); Degani *et al.* (2019) and Uyanga *et al.* (2020) for RCBD while others use mixed effect model for the same design (Jjagwe *et al.*, 2020; Cayleigh, 2018; Galindo *et al.*, 2019).

The parameter estimates from this design might be more biased to the unknown parameter depending on the model used, which is a crucial area of poor parameter estimation in statistics from a model worth considering for a study. Improper modelling of the egg storage conditions may subject small or large-scale layer poultry farmers and egg retailers to unexpected losses that are avoidable. Thus, this study sought to scrutinize the desired storage temperatures and duration that enhance egg quality maintenance using RCBD model analysis.

1.3 Justification of the Study

Festing (2010) insisted that the inability to standardize the nomenclature of RCBD can confuse when applying the principles of experimental design. As a result, a study by Dixon (2016) suggested block to be treated as a fixed effect in this design. Moreover, Dixon (2016) used a simulated experimental method to make such a conclusion. However, White *et al.* (2016) claimed that models generated by simulated experimental designs affect the prospect of accurate parameter estimates. Yang (2010) dealt with a comparison of procedure for the general linear model (Proc GLM) and method for mixed model (Proc Mixed). In addition, Yang (2010) further made a comparison of sample data from split-plot design and CRD. Most studies have concentrated on unbalanced designs such as split-plot, split-split plots, and complete randomized designs.

This is significant because of limited research carried out to verify the best model fit for RCBD. Further, chicken eggs, a reliable source of nutritious food and an easily affordable source of income for low-income households in Kenya (Orungo *et al*., 2018). The findings of this study enlighten egg retailers and poultry farmers on the best storage condition to maintain egg quality. It serves as a standardized nomenclature to researchers specializing in the design and analysis of experiments to avoid confusion when applying the principles of experimental design.

It also enlightens different stakeholders such as the technology-based research centres and the government to implement policies on optimal storage conditions for quality egg storage. Such implementation is an achievement of the big four agenda of smallholder productivity for food affordability and nutrition (RoK, 2018). In addition, data analysts can use these findings to avoid poor parameter estimation from a model, thus deriving reliable outputs.

1.4 Objectives

1.4.1 Broad Objectives

To model the effects of storage conditions on the egg quality using a randomized complete block design.

1.4.2 Specific Objectives

The specific objectives were to;

i. Determine the effect of different storage conditions on the egg quality using fixed and mixed effect models.
ii. Evaluate the efficiency of fixed and mixed effect models.
iii. Synthesize optimal levels of storage conditions for quality egg maintenance using the response surface technique.

1.5 Research Hypotheses

This study formulated hypotheses majorly for rejection purposes based on the assignment of a statistical test. Test in statistics is a rule that assigns each possible observation to one of the groups that either has an element of consistency or none to the hypotheses (Massey & Miller, 2006). The following hypotheses guided this study;

i. There is no significant effect of storage conditions on the egg quality.
ii. There is no significant difference between the efficiency of the models.
iii. There are no optimal storage conditions for quality egg maintenance.

1.6 Scope of the Study

This study covered poultry farms lying within a 4KM radius from two major urban centres within the geographical location of Embu County, Kenya; that is, Embu town and Siakago. It experimented on the eggs obtained from ISA brown layers. This study considered ISA Brown layers because they are dominant in the Embu county region. Further, Embu town and Siakago urban centres are major hot spots for poultry products, particularly eggs. The experiment evaluated the effect of different storage duration and temperature conditions on the external and internal features of the commercial egg. The quality of the egg was determined by the height, diameter, weight of yolk, shell weight and diameter, and albumen weight.

1.7 Assumptions of the Study

This study assumed that the effects of confounding factors such as type of feed, layers health, the total number of colonies, different farm managements, and monitoring routines on the quality of egg from the time it is laid to consumption are infinitesimal. Further, only storage duration and temperature affect egg quality from laying, collection to consumption.

CHAPTER TWO

REVIEW OF LITERATURE

2.0 Introduction

This chapter entails the empirical review, the effects of different storage conditions on the physical properties of the egg, model efficiency evaluation, determination of optimal candidate points, and finally, research gap.

2.1 Empirical Review

Poultry production is a broad area in animal keeping with an acknowledgeable contribution to food security in several countries (Addo, 2017). From poultry, chicken eggs are a significant source of animal protein and are highly and widely preferred due to their relatively low price. However, eggs require more careful handling to maintain their freshness and quality (Lee *et al*., 2016). Egg quality is best described in terms of internal and external structure characteristics, i.e. Haugh unit (HU), which measures freshness should be greater than 75 (Lee *et al.*, 2016).

After ovipositing, environmental conditions and maintenance begin affecting both internal and external, e.g. structure. The primary influence on egg quality is storage temperatures; storage time also plays along with temperatures in estimating or measuring egg quality. Characteristics of eggs affected include internal and external properties (Kraus *et al*., 2019; 2020). The quality of the internal structure is indicated mainly by the measurements obtained from the yolk and albumen (Molnár *et al*., 2016). Other features include yolk colour, airspace diameter, and the general odour of the egg albumen. External egg characteristics are summarized by the eggshell, including shell colour, shell thickness, shell weight, and overall egg weight (Sirri *et al*., 2018).

2.2 Effects of Different Storage Conditions on the Physical Properties of the Egg

The egg's physical properties are essential in the design considerations for effective equipment necessary for storage, utilization, transportation, and processing (Bertechini *et al*., 2014). According to Sun *et al*. (2018), the eggshell should be resistant under natural environmental conditions that may lead to cracking for embryo protection until hatching.

The commercial chicken eggs are classified based on their shape. The shape index is majorly used to classify eggs into round, standard, and sharp. This classification of eggs into grades is also based on the shape index, whereby some eggs belonged into Grade –AA or –A (Pescatore *et al*., 2011). The sharp chicken egg should have a shape index $(Shp < 72)$, regular have $(Shp = 72 - 76)$, and round ones have $(Shp > 76)$. These eggs are regarded as Grade AA eggs, and the category is classified as Grade –A or AB eggs respectively (Duman *et al*., 2016).

A study by Balogu & Kolo (2016) shows that egg weight affects some of the physical properties such as length, volume, density, and weight loss but has no effect on the egg width and shape index. Although eggshell is not classified as a quality factor in the egg, the size and thickness affect the overall quality of the egg because its length is inversely proportional to its width. As the commercial hen ages, there is less intestinal calcium uptake, which negatively affects the thickness of an egg, leading to increased egg size and a decrease in production level (An *et al.*, 2016). As per Rayan *et al*. (2010), a rise in the weight of the shell as the hen gets older would be insufficient to serve as an indicator for the weight increase of an egg.

2.3 Evaluation of Model Efficiency Using Variance Component

Computation of variability may depend on the various sources of variation and their conceptualization in a model; different models' formulation and computation yield estimates of variance components that are not similar. Variance component estimation has become an area of statistical interest for approximately more than 50 years. This interest has only been possible since the invention of the ANOVA technique by Fisher. ANOVA methodology assumes unbiasedness in balance data design by equating the error of the mean square to its expectation (El-Hashash, 2017). The ANOVA method has always been recommended over some given techniques of variance component estimation and forgetting the proportion of confidence intervals (Shrout & Fleiss, 1979).

The restricted Maximum Likelihood (REML) estimation procedure was further developed due to the biased nature of variance component estimation through ANOVA in unbalancing data structure (Rodríguez-álvarez *et al*., 2018). REML has been used to estimate variance components to evaluate Bovine Respiratory disease (BRD) (Buchanan *et al*., 2016). It came into existence through estimating variance error in statistical analysis. Mainly obtained by getting the difference between the error of the means square from the model and the observed, then equating to zero. Different studies have used the method of REML to estimate the variance components, for instance, in a genetic and animal breeding program. A comparison between REML and Bayesian approaches of estimating variance components shows that these two methods yield estimates which are close in value (Mandal *et al*., 2020). Estimation of variance component has been applied in ensuring extremely accurate stability in navigation Satellite systems (Li *et al*., 2020).

2.4 Determination of the Optimal Level

Recently, optimal designs have drawn attention to various researchers in the design and analysis of experiments (Yu *et al*., 2018). Different optimality criteria exist to enable the selection of optimal candidate points. These designs include; A- and D- optimality criteria, which deal with high precision estimation of model parameters of the designed matrix. I- and G- optimality criteria are considered widely for predicting the dependent variable with high precision. A-optimality measures seek to minimize the variance of the estimated matrix and coefficients of parameters (Jones *et al*., 2020). According to Herman *et al*. (2020), the A-optimality criterion is most efficient in screening experiments than the D-optimal design. A-optimal design has been used to find the subset of network p_s candidates of censoring point which could select p_t levels.

Further, the A-optimal criterion is used to select the optimal design of candidate points for the Bayesian linear inverse problem. Candidate points represent the inverse of the situation, such that the parameter to be observed maps a f linear and normally distributed function. That is to say, $\min_{s\in[0,1]} tr(B_{post}(s)) + \alpha P(s)$ where $p_s = \sum s$ is the penalty function associated with the Bayes decision (Alexanderian *et al*., 2016). Several studies have used the A-optimal criterion for optimization design. However, very limited studies optimize storage duration and temperature. Thus this study used optimal criteria to determine the optimal storage duration and temperature to maintain egg quality from the time it is laid.

2.5 Research Gap

Studies on comparing mixed and fixed-effect models have concentrated on unbalanced experimental designs such as split-plot, incomplete block design, and many others and have neglected to model RCBD (Harville *et al*., 1984; Yang, 2010; Bell *et al*., 2018). In addition, studies on RCBD modelling have used simulated experimental data set, for instance, an experimental technique used in data analysis, supplementing intuition in mathematical statistics (Bell *et al.*, 2016; Dixon, 2016). Monte Carlo simulation is a scientific tool mainly used for intractable problems analytically and whose experimentation is too costly, not practical, and time-consuming (Cabrera-Llanos *et al*., 2019). Moreover, studies on fixed versus mixed effect models for RCBD have been common in agricultural field experiments like maize breeding. Furthermore, most experiments on egg storage duration and different temperature conditions have been conducted elsewhere in the study area (Giampietro-Ganeco *et al*., 2015).

Very limited research has been done to determine the optimal storage conditions to maintain the quality of the egg from being laid to consumption. Therefore, there is an evidenced need for research-based information which can create awareness to learners, poultry farmers, statisticians, data analysts, and researchers on the model best fit or RCBD arguments based on the variance component estimate. This study thus filled this gap by designing an experiment on RCBD. This study used an investigation to determine the effect of egg storage duration when fresh eggs are subjected to different temperature conditions to formulate both fixed and mixed effect models for comparison purposes.

CHAPTER THREE

MATERIALS AND METHODS

3.0 Introduction

This chapter entails the description of the study site, the sampling technique, study design, and statistical techniques that were used to compute an estimate of variance component for the models such as ANOVA and REML.

3.1 Study Site and Description of Experimental Eggs

The study was conducted in the zoology laboratory at the University of Embu (0^0 35’ 25’’S, 37^0 E 25’ 31’’). This study involved eggs from ISA brown egg-laying hens of two to three years old in approximation. According to Marzec *et al.* (2019), different egg storage conditions have a significant effect on both external and internal egg quality as hens age. The eggs from commercial hen layers of more than 80 weeks are primarily at risk of a faster decrease in rate under storage (Peri *et al.*, 2017). The study exempted any other type of egg layer chicken other than ISA brown. This study excluded eggs laid by ISA brown on any other day other than the collection day. It also disqualified the fresh and unfertilized eggs laid by ISA brown of the specified age, but that had cracks. This study used eggs from the University of Embu, Kirata and Rana poultry farms in Embu County, Kenya.

3.2 Sampling Design

According to Sarmah & Hazarika (2012), a fraction of the population of interest that can be used to make a statistical inference is a sample depending on the type of study. This study purposively sampled three farms based on the intensity of egg production from ISA brown layers at the study time. However, the sampling units for this study were the eggs. This study employed this method to allow each unit included in the sample to experience the pre-assigned probability of inclusion. It is statistically evident that the random sampling procedure gives less biased estimates of the unknown parameter than purposive sampling (Ajay & Micah, 2014). Therefore, within a stratum, this study marked ISA brown with labelled stickers in ascending order. The study generated a random number table of the marked stickers with a sample of interest. Hens under trial were picked and separated from the rest following the random numbers generated. They were kept under laying test a day to the actual egg collection.

3.2.1 Sample Size

According to Tiberious *et al*. (2016), a sample size between 10% and 30% of the population size is adequate for statistical inference. This study used the Tiberious method to obtain a sample of strata. In addition, the Neyman formula, which Yamane proposed, was used to estimate the sample size of fresh eggs under-investigated (Baran & Gokdogan, 2016). Thus, this formula enabled the study to use the principle of proportional allocation. Therefore, obtaining information about the population of interest and its precision is based on stratified sampling. The sample allocation depended on the size of samples within different strata as illustrated by (Pandey & Verma, 2008). Further, the model used for this study consisted of only eggs with a normal shape index (Shp) of 72-76 (Equation 3.1) as documented by (Duman *et al*., 2016). This study excluded the eggs with other shapes. The shape index is characterized by the egg's length (LE) and width (WI) dimensions. These dimensions were measured using a digital Vernier calliper (mm) (Altuntaş & Şekeroğlu, 2008; Ketta & Tůmová, 2018; Galić *et al*., 2019).

$$Shp = \frac{(WI \times 100)}{LE} \tag{3.1}$$

Given that ISA brown has 95% laying rate, this study included 5% of the sample of interest in catering to non-laying rates. Yamane's formula (Equation 3.2) (Johnston *et al*., 2019);

$$n_i = \frac{N_{\text{i1}}}{[1+N_{i1}e^2\ ]} + \frac{N_{\text{i2}}}{[1+N_{i2}e^2\ ]}\ \forall \text{i} = 1,2,3 \tag{3.2}$$

Where; n_i is sample number of ISA brown layers under trial from N_{i1} and N_{i2}. N_{i1} is the estimate of the targeted population size from each poultry farm with 95% laying rate while N_{i2} is the estimate of the targeted population size with 5% non-laying rate from the same poultry farm. $\text{i} = 1,2,3$ represents the University of Embu, Rana, and Karata poultry farms respectively, ℓ = the error limit. At 95% level of confidence and with an error limit of 5%. Thus, the number of layers under trial was $n = n_1 + n_2 + n_3$.

Therefore, from (Equation 3.2), it follows that;

$$n_1 = \left(\frac{520}{[1+520(0.05)^2\ ]} + \frac{26}{[1+26(0.05)^2\ ]} \right) \approx 226 + 24 = 250 \qquad (3.3)$$

$$n_2 = \frac{338}{[1+338(0.05)^2]} + \frac{12}{[1+12(0.05)^2\ ]} \approx 183 + 12 = 195 \qquad (3.4)$$

$$n_3 = \frac{278}{[1+278(0.05)^2]} \ \frac{9}{[1+9(0.05)^2\ ]} \approx 164 + 9 = 173 \qquad (3.5)$$

$$n_1 + n_1 + n_{12} = 618 \text{ eggs} \qquad (3.6)$$

3.3 Materials and Experiments

3.3.1 Experimental Design

This study collected 623 unfertilized fresh eggs from 1183 laying ISA brown chickens. The study used a simple random sampling technique without replacement, as illustrated above. The weight of these fresh eggs was measured and recorded within the same day of laying and collection. However, this study excluded five eggs that cracked from the experimental setup. Therefore, this study ended up with a sample of 618 (Equation 3.6).

This study generated a general complete factorial design with three replicates to assist in experimentation and data collection. Two hundred and six eggs were sampled randomly from the 618 eggs to cater for the experimental control group of room temperature (19.5 ^{o}C). The 19.5 ^{o}C was the average of the mean daily temperature for the whole experimental duration. The room temperature was chosen as a control for this experiment since it is the commonly used temperature by egg retailers and farmers at large. The remaining 412 eggs were placed at random in plastic egg cartons 206 each and stored under different storage temperature conditions (refrigerator 5 ^{o}C and incubator 30 ^{o}C). 5 ^{o}C was the lowest possible temperature for storage while 30 ^{o}C was the highest temperature recorded in the study area.

This study used different temperature conditions as treatments while storage duration acted as blocks.

3.3.2 Experimental and Data Collection Procedures

After storage, a sample of 52 eggs was randomly picked from every storage temperature condition at a time at a periodic interval of days; that is 2^{nd}, 12^{th}, 22^{nd}, and 50 eggs on the 32^{nd} day. Each egg was picked and cracked over a flat surface to reduce the likelihood of shell shards breaking into the egg. The height of yolk and egg white were measured using a spherometer. The yolk, egg weight, and shell weight were measured by electronic sensitive digital weighing balance. This study used Vanier callipers to measure the magnitude of yolk, shell, and white egg diameter. The average of the widest and the narrowest horizontal circumference was measured as the yolk diameter. Moreover, the standard of the broadest flat rim enclosed by the egg white was calculated as the albumen diameter as documented by (Oleforuh-Okoleh & Eze, 2016; Sola-Ojo *et al.,* 2016; Hegab & Hanafy, 2019). Haugh unit (HU) (Equation 3.7) was determined using the following procedure as documented by Haugh (1937) and Tran & Soottawat (2018);

$$HU = 100\ log_{10}\left(H_h - 1.7\ W_w^{0.37} + 7.56\right); \tag{3.7}$$

$HU =$ Haugh unit, $H_h =$ the height of the egg white (mm), and $W_w =$ the weight of the egg (g). The yolk indices were estimated using the following formula (Equation 3.8).

$$YI = {}^{y(height)}\!\big/_{y(width)} \tag{3.8}$$

3.3.3 Detailed Procedure Used

This study used principle methods as documented by Haugh (1937); (Hegab & Hanafy, 2019) Oleforuh-Okoleh & Eze (2016); Sola-Ojo *et al*. (2016); Tran & Soottawat (2018); Hegab & Hanafy (2019), in the determination of both internal and external components of the egg. A petri dish was placed on a digital weighing balance (accurate at 0.01 grams (*g*)) and tared. The eggs were placed on the petri dish one at a time, recording the observed weight for each. Using an electronic Vernier calliper (accuracy of 0.01 millimetre (*mm*)), this study measured the length and breadth of the egg. After breaking the egg in the below internal measurement procedure, the eggshell weight and diameter were also measured using a digital weighing balance and electronic Vernier callipers, respectively.

The internal quality of the egg is determined by the dimensions of the yolk and the albumen. The measurements of the yolk were used to estimate the YI, while those of the egg white was used to find the HU which involves egg weight. An egg was cracked over a flat surface using a spatula, ensuring very little likelihood that the shell shards would break into the egg. Before prising it to open, the egg was held over a petri dish and having another petri dish ready beside it. Two hands were used to break it into two halves. The yolk was slipped back and forth from one half-shell to the other. Tilting it by doing so and letting the white trickle down into the petri dish while hanging on to the yolk.

Once the white trickled in the shells, this study popped the yolk into the other petri dish. Another petri dish was placed on a digital weighing balance (accurate at 0.01 grams) and tared. The yolk and the white were placed on the petri dish separately and then recorded the observed weight. This study measured the diameter of the yolk and albumen using an electronic Vernier calliper (accuracy of 0.01 mm). The height of the albumen and yolk was measured using a spherometer (accuracy of 0.01 mm). A spherometer was first placed gently on a glass slab so that its three legs rest on a flat surface. This study placed an empty petri dish under the screw at the centre. Further, we gently turned the screw downwards till the crew tip touches the surface of the empty petri dish.

This study used a thin rectangular white paper to ensure no gap between the end of the screw and the flat bottom of the empty petri dish. In this case, if the paper can slide easily, it implies a tiny gap between the tip of the screw and the Petri dish's surface. The screw was further turned downwards till there was no gap between the screw tip and the zero mark of the pitch scale. The screw was raised upwards so that the yolk in a petri dish can stay inside it. The screw was further turned downwards till the screw tip touched the upper layer of the yolk. The vertical scale reading, in line with the circular scale reading, was recorded. This procedure was used to measure albumen height.

3.4 Fixed and Mixed Effect Model

3.4.1 Model Diagnostic and Building

This study considered the following RCBD model consisting of treatment effect and block effect (Equation 1.1).

$$y_{ijk} = \mu + \lambda_i + \gamma_j + \varepsilon_{ijk}, \tag{3.9}$$

Where y_{ijk} is the k^{th} observation of the i^{th} treatment effect and the j^{th} block effect, μ is the grand mean, λ_i is the i^{th} treatment effect, γ_j is the j^{th} block effect and ε_{ijk} residual error which is independent and associated with the observed value y_{ijk}. Whenever both λ_i and γ_j are both fixed, then the resulting model is fixed. On the other hand, if either λ_i or γ_j is random and the other fixed, then the resulting model is random. These models could also be associated with interaction effect (Wu *et al.*, 2009);

$$y_{ijk} = \mu + \lambda_i + \gamma_j + (\lambda\gamma)_{ij} + \varepsilon_{ijk}, i = 1, \cdots, w_1 \; j = 1, \cdots, w_2, k = 1, \cdots, w_{ij} \tag{3.10}$$

Where $\mu =$ the grand mean, $\lambda_i, \gamma_j, (\lambda\gamma)_{ij}$ and ε_{ijk} are random variables which are independent and follow a normal distribution with variance as $\sigma^2_\lambda, \sigma^2_\gamma$ and $\sigma^2_{\lambda\gamma}$. The means of these variables are all zero (Khuri, 2000).

The matrix notation of model (equation 3.10) can be expressed as

$$y = A\mu + A_1\lambda + A_2\gamma + A_3\lambda\gamma + \varepsilon \tag{3.11}$$

From Equation (3.11), letting $\lambda\gamma = \psi$, which is the interaction effect between treatment and block, we shall have that;

$$y = A\mu + A_1\lambda + A_2\gamma + A_3\psi + \varepsilon \tag{3.12}$$

Where $A = I_{\sum_i \sum_j w_{ij}}$. Further, given that ε_{ijk} is a random error whose entries are in respect to observed values y, $\varepsilon \sim N(0_{\sum_i \sum_j w_{ij}}, \sigma^2_{\sum_i \sum_j w_{ij}})$ (Stroup & Littell, 2002). For instance, the experiment of this study.

In particular $w_1 = 3$ and $w_2 = 4$

$$A_1 = \begin{bmatrix} \Lambda_{11}^1 \\ \Lambda_{12}^1 \\ \Lambda_{13}^1 \\ \Lambda_{14}^1 \\ \Lambda_{21}^2 \\ \Lambda_{22}^2 \\ \Lambda_{23}^2 \\ \Lambda_{24}^2 \\ \Lambda_{31}^3 \\ \Lambda_{32}^3 \\ \Lambda_{33}^3 \\ \Lambda_{34}^3 \end{bmatrix}, \quad A_2 = \begin{bmatrix} \Gamma_{11}^1 \\ \Gamma_{12}^2 \\ \Gamma_{13}^3 \\ \Gamma_{14}^4 \\ \Gamma_{21}^1 \\ \Gamma_{22}^2 \\ \Gamma_{23}^3 \\ \Gamma_{24}^4 \\ \Gamma_{31}^1 \\ \Gamma_{32}^2 \\ \Gamma_{33}^3 \\ \Gamma_{34}^4 \end{bmatrix}, \quad A_3 = \begin{bmatrix} \Omega_{11}^1 \\ \Omega_{12}^2 \\ \Omega_{13}^3 \\ \Omega_{14}^4 \\ \Omega_{21}^1 \\ \Omega_{22}^2 \\ \Omega_{23}^3 \\ \Omega_{24}^4 \\ \Omega_{31}^1 \\ \Omega_{32}^2 \\ \Omega_{33}^3 \\ \Omega_{34}^4 \end{bmatrix}$$

Thus λ, γ and ψ have mean of, 0_3, 0_4 and 0_{12} respectively and variance-covariance matrix $\sigma_\lambda^2 I_3, \sigma_\gamma^2 I_4$ and $\sigma_\psi^2 I_{12}$ with $\Lambda_{ij}^k, k = 1,2,3$ a $w_{ij} \times 3$ matrix whose column k is a vector of 1's and the rest of 1 is a vector of zeros. $\Gamma_{iq}^q, q = 1,2,3,4$ a $w_{iq} \times 4$ matrix whose column q is a vector of 1's and 1 is a vector of zeros. Ω_{iq}^r, $r = 1,2,\cdots,12$ a $w_{ij} \times 12$ matrix whose column r is a vector of 1's and 1 is a vector of zeros. We can therefore assume that

$$\lambda = \begin{bmatrix} \lambda_1 \\ \lambda_2 \\ \lambda_3 \end{bmatrix} \sim N(0_3, \sigma_\lambda^2 I_3),$$

$$\gamma = \begin{bmatrix} \gamma_1 \\ \gamma_2 \\ \gamma_3 \\ \gamma_4 \end{bmatrix} \sim N(0_4, \sigma_\gamma^2 I_4) \text{ and } \psi = \begin{bmatrix} \psi_1 \\ \psi_2 \\ \vdots \\ \psi_{11} \\ \psi_{12} \end{bmatrix} \sim N(0_{12}, \sigma_\psi^2 I_{12}).$$

It follows that model (Equation 3.12) has a distribution with mean $A\mu$ and a variance-covariance matrix Σ such that;

$$\Sigma(y) = \sigma_{\lambda}^{2} A_1 A_1^T + \sigma_{\gamma}^{2} A_2 A_2^T + \sigma_{\psi}^{2} A_3 A_3^T + \sigma_{\varepsilon}^{2} I_{\sum\sum w_{iq}} ,$$

Where $A_1 A_1^T = \begin{bmatrix} Q_{\sum\limits_{q=1}^{4} n_{1q}} & 0_{\sum\limits_{q=1}^{4} w_{1q} \sum\limits_{q=1}^{4} w_{2q}} \\ 0_{\sum\limits_{j=1}^{4} w_{2j} \sum\limits_{j=1}^{4} w_{1j}} & Q_{\sum\limits_{q=1}^{4} n_{2q}} \end{bmatrix}$ (Kline *et al.*, 2020)

$$A_2 A_2^T = \begin{bmatrix} Q_{w_{11}} & \cdots & 0_{w_{11}w_{14}} & Q_{w_{11}w_{21}} & \cdots & 0_{w_{11}w_{24}} & Q_{w_{11}w_{31}} & \cdots & 0_{w_{11}w_{34}} \\ \vdots & \ddots & \vdots & \vdots & \ddots & \vdots & \vdots & \ddots & \vdots \\ 0_{w_{14}w_{11}} & \cdots & Q_{w_{14}} & 0_{w_{14}w_{21}} & \cdots & Q_{w_{14}w_{24}} & 0_{w_{14}w_{31}} & \cdots & Q_{w_{14}w_{34}} \\ Q_{w_{21}w_{11}} & \cdots & 0_{w_{21}w_{14}} & Q_{w_{21}} & \cdots & 0_{w_{21}w_{24}} & Q_{w_{21}w_{31}} & \cdots & 0_{w_{21}w_{34}} \\ \vdots & \ddots & \vdots & \vdots & \ddots & \vdots & \vdots & \ddots & \vdots \\ 0_{w_{24}w_{11}} & \cdots & Q_{w_{24}w_{14}} & 0_{w_{24}w_{21}} & \cdots & Q_{w_{24}} & 0_{w_{24}w_{31}} & \cdots & Q_{w_{24}w_{34}} \\ Q_{w_{31}w_{11}} & \cdots & 0_{w_{31}w_{14}} & Q_{w_{31}} & \cdots & 0_{w_{31}w_{24}} & Q_{w_{31}} & \cdots & 0_{w_{31}w_{34}} \\ \vdots & \ddots & \vdots & \vdots & \ddots & \vdots & \vdots & \ddots & \vdots \\ 0_{w_{34}w_{11}} & \cdots & Q_{w_{34}w_{14}} & 0_{w_{34}w_{31}} & \cdots & Q_{w_{34}w_{24}} & 0_{w_{34}w_{31}} & \cdots & Q_{w_{34}} \end{bmatrix}$$

Also

$$A_3 A_3^T = \begin{bmatrix} Q_{w_{11}} & \cdots & 0_{w_{11}w_{14}} & 0_{w_{11}w_{21}} & \cdots & 0_{w_{11}w_{24}} & 0_{w_{11}w_{31}} & \cdots & 0_{w_{11}w_{34}} \\ \vdots & \ddots & \vdots & \vdots & \ddots & \vdots & \vdots & \ddots & \vdots \\ 0_{w_{14}w_{11}} & \cdots & Q_{w_{14}} & 0_{w_{14}w_{21}} & \cdots & 0_{w_{14}w_{24}} & 0_{w_{14}w_{31}} & \cdots & 0_{w_{14}w_{34}} \\ 0_{w_{21}w_{11}} & \cdots & 0_{w_{21}w_{14}} & Q_{w_{21}} & \cdots & 0_{w_{21}w_{24}} & 0_{w_{21}w_{31}} & \cdots & 0_{w_{21}w_{34}} \\ \vdots & \ddots & \vdots & \vdots & \ddots & \vdots & \vdots & \ddots & \vdots \\ 0_{w_{24}w_{11}} & \cdots & 0_{w_{24}w_{14}} & 0_{w_{24}w_{21}} & \cdots & Q_{w_{24}} & 0_{w_{24}w_{31}} & \cdots & 0_{w_{24}w_{34}} \\ 0_{w_{31}w_{11}} & \cdots & 0_{w_{31}w_{14}} & 0_{w_{31}} & \cdots & 0_{w_{31}w_{24}} & Q_{w_{31}} & \cdots & 0_{w_{31}w_{34}} \\ \vdots & \ddots & \vdots & \vdots & \ddots & \vdots & \vdots & \ddots & \vdots \\ 0_{w_{34}w_{11}} & \cdots & 0_{w_{34}w_{14}} & 0_{w_{34}w_{31}} & \cdots & 0_{w_{34}w_{24}} & 0_{w_{34}w_{31}} & \cdots & Q_{w_{34}} \end{bmatrix}$$

Variance components are the unknown parameters that are, $\sigma_\lambda^2, \sigma_\gamma^2, \sigma_\psi^2$ and σ_ε^2 *(Silva et al.*, 2015). If $y \in \Re^w$ represents a vector of observed values, the mixed model can be expressed further as;

$$y = X\beta + \sum_{i=1}^{w+1} X_i \beta_i \tag{3.13}$$

Where $X_{w+1} = I_w$, $X_i \in A^{w_i \times r_i}$ and $\beta_i \in \Re^{r_i}$, where $X \in A^{w \times r}$ is a known designed matrix for both block and treatment effect.

3.5 Assessing Model Adequacy of the Models

3.5.1 Assumptions of the Model

The mixed model assumes β_i's are random vectors that are mutually independent. Regarding this assumption, the study considered $\mathrm{E}(\beta_i) = 0_{r_i}$ and $\Sigma(\beta_i) = \sigma_i^2 I_{r_{i_i}}$. This can be expressed further as, $E(y) = X\beta$, $\Sigma y = \sum \sigma_i^2 A_i$, where $A_i = X_i X_i^T$, and $\sigma_1^2, \cdots, \sigma_{r+1}^2$ are positive parameters that are fixed and unknown. This study used these parameters as variance-covariance components implying that $\sigma_i^2 \geq 0. i = 1, \cdots, r$ while $\sigma_{r+1}^2 > 0$. The study assumed that;

$$y \sim N(X\beta, \Sigma), \text{Where } \Sigma = \Sigma(y) = \sum_{i=1}^{r+1} \sigma_i^2 A_i \tag{3.14}$$

This study performed analytical preparation on hypothetically persuasive, remote random, and residual properties on the obtained records through the student zed residual method in R for fixed and mixed effect models.

3.6 Determination Model of Efficiency of the Models

The efficiency of the fixed and mixed-effect model was determined through the estimation of the variance component.

3.6.1 Analysis of Variance Method

According to Corbeil & Searle (1976), analysis of variance procedure was considered for the fixed effect model. In this estimation procedure, a system of linear equations was solved. The formulated structure of the linear equation depended on RCBD.

Let $V_i^2, i = 1,2,\cdots,r+1$ denote the second-order moment error for the i^{th} variation source in the model (Equation 3.8). V_i^2 can be expressed as; $V_i^2 = y^T R_i y,\ R_i \in \Re^r$ in such a way that;

$$X^T R_i X = 0_{rr} \tag{3.15}$$

where $X^T R_i$ is the orthogonal vector to all columns of X and thus,

$$E(V_i^2) = tr(R_i\Sigma) + X\beta^T R_i(X\beta) = tr(\sum_{q=1}^{r+1} \psi_q R_i A_q) = \sum_{q=1}^{r+1} \psi_q tr(X_q^T R_i X_q) \tag{3.16}$$

V_i^2 depends only on the variance component (Heba et al., 2015).

But, $V = \begin{bmatrix} S_1^2 \\ \vdots \\ S_{r+1}^2 \end{bmatrix}$ and $\psi = \begin{bmatrix} \psi_1 \\ \vdots \\ \psi_{r+1} \end{bmatrix}$ $E(S) = Z\psi,\ Z = \begin{bmatrix} tr(X_1^T R_1 X_1) & \cdots & tr(X_{r+1}^T R_1 X_{r+1}) \\ \vdots & \ddots & \vdots \\ tr(X_1^T R_{r+1} X_1) & \cdots & tr(X_{r+1}^T R_{r+1} X_{r+1}) \end{bmatrix}$

3.6.2 Restricted Maximum Likelihood Procedure (REML)

This is a more general method majorly used to estimate a bias-free variance component in a mixed-effect model. Equation (3.12) can be expressed further as;

$y = X\beta + Zb + \varepsilon,$ Where, $y = \begin{bmatrix} y_1 \\ \vdots \\ y_w \end{bmatrix}, X = \begin{bmatrix} X_1 \\ \vdots \\ X_w \end{bmatrix}, Z = \begin{bmatrix} Z_1 & \cdots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \cdots & Z_w \end{bmatrix}, b = \begin{bmatrix} b_1 \\ \vdots \\ b_w \end{bmatrix}$. In this case ε $\sim N(0, u(\theta))$ with $u(\theta) = \begin{bmatrix} u_1(\theta) & \cdots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \cdots & u_w(\theta) \end{bmatrix}$ general covariance matrix parametrized by θ.

If (equation 3.13) is true, then $y^T R_i y$ is the contrast error. It is only possible to come out with at most $w-r$ such vectors which are linearly independent. It follows that $X^T R_i X = 0_{rr}$ and $\{X^T R_i y\} = 0_{rr}$. This can be expressed further as $A^T y = 0,$ where $X^T R_i = A^T$. Let's define a contrast error vector by $c = A^T y(X\beta + Zb + \varepsilon) = A^T \varepsilon \sim N(0, A^T u(\theta))$ which does not contain any element of unknown parameter β and b. The absence of these unknown parameters gives sufficient information about θ when inference is made based on c rather than y (Zhang, 2015). Therefore;

$$L_c = (\theta \mid A^T y) = -\tfrac{1}{2}\log\det u - \tfrac{1}{2}\log\det X^T u^{-1} X - \tfrac{1}{2}(y - X\hat{\beta})^T u^{-1}(y - X\hat{\beta}) \quad (3.17)$$

$$= -\tfrac{1}{2}\log\det\prod_{i=1}^{r} u_i - \tfrac{1}{2}\log\det\prod_{i=1}^{r} X_i^T u_i^{-1} X_i - \tfrac{1}{2}\sum_{1=1}^{r}(y_i - X_i\hat{\beta})^T u_i^{-1}(y_i - X_i\hat{\beta})$$

$$= -\tfrac{1}{2}\sum_{1=1}^{r}\log\det u_i - \tfrac{1}{2}\sum_{i=1}^{r}\log\det X_i^T u_i^{-1} X_i - \tfrac{1}{2}\sum_{1=1}^{r}(y_i - X_i\hat{\beta})^T u_i^{-1}(y_i - X_i\hat{\beta})$$

This study then maximized $L_c = (\theta \mid A^T y)$ for u_i (Equation 3.16). The detailed derivation is found in (Lindstrom & Bates, 1988).

3.6.3 Comparison of Modelling Efficiency

This study compared estimated variance components for a fixed and mixed effect. The model with the smallest variance component estimate was the most accurate. We compared the significant difference of the estimated variance component through LSD.

3.7 Principal Component Analysis

Principle Component Analysis (PCA) was used to obtain cloud representation of points in the change of egg components space with reduced dimensions optimal. PCA is a data reduction process that condenses correlated variables into independent variables called Principal Components (PCs), with each PC having factor loadings referred to as Eigenvalues (Costabile *et al.*, 2009; Jollands *et al.*, 2004; Kourti, 2005; Rabbette & Pilewskie, 2001; Camacho *et al.*, 2010; Bounoua & Bakdi, 2021; Beattie & Esmonde-

White, 2021). The rotation method enhances the loading of a smaller number of highly correlated variables for each principal component (Mairura *et al.*, 2007).

This study included eigenvalues above 0.5 to reduce noise following the assumption that the largest eigenvalues contain the most meaningful information (Wold *et al.*, 1987; Lu *et al.*, 2004; Yiheng *et al.*, 2005; Andrecut, 2009). Factors such as albumen weight that were insignificant in the first objective for both fixed and mixed-effect models were excluded from the PCA. This study searched for the best possible changes of physical characteristics of the egg under storage conditions, enabling excellent visualization of the shape of a cloud with seven dimensions. Sarica *et al.* (2012) used PCA to determine the most actual parameters in genotype. Further, Feddern *et al.* (2017) used PCA to suggest egg quality parameters associated with seasonal layers. This study explored the best possible changes of physical characteristics of the egg under storage conditions, enabling excellent visualization of the shape of a cloud with seven dimensions.

3.8 Response Surface Model

For the response model, this study obtained the averages of observations for replicates as per the designed matrix $3*4*3=36$ runs (Appendix). Even though this study tried its level best to reduce variation by shape index and egg weight, variations were inevitable. Therefore, the study used averages to reduce the margin of error. It fitted a second-order model using the general full factorial design for the response surface of the dependent variables, which allowed for the specification of the two used factors of different levels. This model was used to approximate the response of the corresponding dependent variable. From Equation (3.10);

$$y=\mu+\sum_{i=1}^{3}\lambda_i x_i+\sum_{j=1}^{4}\gamma_j x_j+\sum_{i\neq j}\lambda\gamma x_{ij}+\varepsilon \tag{3.18}$$

3.9 Optimality Criteria

The design prediction criterion of the response surface of the experiment for this model was determined by the G-optimal measure. This criterion was used to minimize the maximum entry in the diagonal of the hat matrix $X(X'X)^{-1}X'$. According to Wong (1992;

Montgomery *et al*. (2002); Zahran *et al*. (2003); Moein & Pan (2016); Atitwa *et al.* (2016), a design is said to be G- optimal if and only if it minimizes the minimum scaled predictive variance over the design region.

$$G-opt = \frac{N\,\mathrm{var}[y(\hat{x})]}{\sigma^2} \tag{3.19}$$

$$G-eff = \frac{k}{\dfrac{\max(N\,\mathrm{var}[y(\hat{x})])}{\sigma^2}} \tag{3.20}$$

Where $y(\hat{x})$ is the predicted response variable, N = the design points and σ^2 = the estimated mean square error of the corresponding response variable.

$$k = \frac{(p+1)(p+2)}{2} \tag{3.21}$$

Such that p = the dimension of the full quadratic polynomial response surface.

CHAPTER FOUR

RESULTS AND INTERPRETATION

4.0 Overview

This chapter presents the findings of this study. The effects of temperature and storage duration on the quality of eggs using mixed and fixed-effect models have been presented. The efficiencies of fixed and hybrid effect models are also demonstrated. In addition, the results of optimal storage conditions using response surfaces are also interpreted in this chapter.

4.1 Effects of Storage Conditions on Egg Quality Components

4.1.1 Egg Weight Loss

Table 4.1.1 displays the effect of different storage durations on egg quality for large (60-64) *g*, extra-large (65-69) *g* and jumbo (70 and above) *g* sizes. R-Square (R-sq.) of the egg weight loss (EWL) indicates that the fixed model was 93%, 85% and 83% for the egg sizes respectively. The large (60-64) *g* egg size is highly explained (Table 4.1.1). The coefficient of variation of EWL was below 30%. The jumbo (70 and above) *g* had the highest coefficient of variation (18%). The effect of storage time on the egg weight was remarkable at 95% confidence intervals with the least significant difference (LSD) 0.34, 0.34 and 0.39, respectively (Table 4.1.1).

The mean weight losses were 1.67 *g*, 1.78 *g*, and 1.99 *g*, with jumbo (70 and above) *g* recording the highest loss. The difference in mean weight loss could be due to different egg sizes. Table 4.1.4 shows the effects of the interaction of storage temperature and duration within the fixed-effect models for every category of egg size. The interaction effect of egg weight loss indicates higher losses on the 32nd day of storage in 5 ^oC, 19.5 ^oC and 30 ^oC for all the egg sizes. This study realized insignificant weight loss of extra-large (65-69) *g* egg size stored at 5 ^oC. However, jumbo (70 and more) *g* had a significant difference on the 32nd day. Table 4.1.2 presents the effect of storage conditions on the physical characterization of the egg with temperature as a fixed effect.

The weight loss increased from low (5 ^{o}C) to high (30 ^{o}C) in large (60-64) *g*, extra-large (65-69) *g* and jumbo (70 and above) *g* sizes. The trend of weight loss was similar in the mixed-effect model. This observation could imply that, EWL increases with egg size. However, EWL increased as the egg size increases. The effect was highly significant in general $(P < 0.05)$ with LSD of 0.352, 0.344 and 0.35, respectively (Table 4.1.2). The temperatures under storage influence a reduction in egg weight. Consequently, eggs subjected to low-level temperature 5 ^{o}C; exhibited a lesser reduction in egg content than room temperature. This observation could be due to a possible low loss of water and other gaseous components on eggs stored under low-level temperature 5 ^{o}C compared to 19.5 ^{o}C similar to Akter *et al*. (2014).

4.1.2 Eggshell Weight

Table 4.1.1 also illustrates the total variation in storage time and temperature for the fixed RCBD model of the shell weight (SW). The results showed R-sq. of 69%, 62% and 67% for large (60-64) *g*, extra-large (65-69) *g* and jumbo (70 and above) *g* sizes, respectively. These models explained over 50% of the SW variation. The slightest variation of the three categories of egg sizes was explained by extra-large (65-69) *g*. The data collected on SW was good at 90%, as defined by the coefficient of variation $< 10\%$ for the egg sizes. The SW model was significant for large (60-64) *g* $P = 0475$ and extra-large (65-69) *g* $P = 0.0382.$

Conversely, the SW model was not significant for jumbo (70 and above) *g* with $P > 0.05.$ Thus, the SW of jumbo (70 and above) *g* size was not significantly affected by the storage duration. We observed the LSDs as 0.6944, 0.6781 and 0.7791, with the highest value in jumbo (70 and above) *g* size. Even though the SW model was insignificant in jumbo (70 and above) *g*, this study realized the highest great SW of 9.079 *g*. The interaction effect on SW in different storage duration was significantly different from 2nd, 12th, 22nd, and 22nd $(P > 0.05)$ in large (60-64) *g*. It was very different on the 32nd $(P < 0.05).$ Similarly, the effect of different storage temperatures was not significantly different from each other at 5 ^{o}C and 19.5 ^{o}C temperatures of storage, as shown in Table 4.1.4. However, we realized a significant difference SW for the extra-large (65-69) *g* and jumbo (70 and above) *g* sizes

$(P < 0.05)$. There was a substantial difference at 30 ^{o}C. So, egg retailers could store eggs at room temperatures or 5 ^{o}C for 22 days with maintained SW. There was a significant difference in SW in the mixed effect model between 19.5 ^{o}C and 30 ^{o}C. Measurements of SW in 30 ^{o}C and 5 ^{o}C also differed significantly $(P < 0.05)$. In contrast, the difference between 5 ^{o}C and 19.5 ^{o}C was infinitesimal.

Table 4.1.1: Effect of Storage Time as a Fixed-Effect in the Fixed-Effect Model

Egg size	Period	EWL	SW	SD	AH	AD	HU	YI
large (60-64) *g*.	2nd	0.57	7.53	0.085	6.6	6.77	79.51	0.3507
	12th	1.11	7.42	0.077	4.06	7.85	56.81	0.3258
	22nd	2.01	7.38	0.073	3.51	8.03	49.82	0.2867
	32nd	3.01	7.17	0.062	1.84	8.85	17.02	0.2296
	Mean	1.67	7.37	0.074	4	7.87	50.79	0.2982
	CV	16.97	8.52	14.53	11.66	13.07	14.14	6.117
	P-Value	0.0002	0.0475	0.0215	0.0195	0.0018	0.0139	0.0002
	LSD	0.340	0.6775	0.0041	1.102	1.050	5.074	0.186
	R-Sq.	0.93	0.69	0.68	0.73	0.62	0.84	0.85
Extra-large (65-69) *g*.	2nd	0.59	8.75	0.0845	6.45	7.88	76.83	0.3409
	12th	1.13	8.64	0.0765	4	9.07	53.46	0.3266
	22nd	2.33	8.61	0.0725	2.65	9.64	31.62	0.278
	32nd	3.07	8.39	0.0619	1.79	10.25	8.32	0.2115
	Mean	1.78	8.59	0.0738	3.72	9.312	42.56	0.2893
	CV	17.34	8.895	14.9	9.95	11.36	12.43	4.41
	P-Value	0.0001	0.0382	0.0112	0.0202	0.002	0.0141	0.0004
	LSD	0.3408	0.6781	0.0047	1.1029	1.0514	5.0753	0.1866
	R-Sq.	0.8542	0.6174	0.7034	0.6699	0.6185	0.7819	0.829
Jumbo (70 and more) *g*.	2nd	0.62	9.23	0.084	6.51	7.96	76.07	0.3325
	12th	1.21	9.12	0.077	4.07	9.38	52.15	0.2865
	22nd	2.75	9.08	0.072	2.62	10.26	27.26	0.2389
	32nd	3.36	8.87	0.061	1.78	12.01	3.99	0.1669
	Mean	1.99	9.079	0.074	3.75	9.9025	39.87	0.2562
	CV	18.14	9.695	15.7	11.48	12.89	13.96	5.933
	P-Value	0.0009	0.0561	0.0522	0.0202	0.0025	0.0146	0.0009
	LSD	0.3918	0.7791	0.0057	1.1039	1.0524	5.0763	0.1876
	R-Sq.	0.8322	0.5954	0.814	0.8619	0.6805	0.7739	0.92

From Table 4.1.2, SW reduced from low (5 ^oC) to high (30 ^oC) in large (60-64) *g*, extra-large (65-69) *g* and jumbo (70 and above) *g* sizes as in the mixed-effect model. This study detected an increase in reduction in SW as the egg size increases. The inter-temperature decrease of SW increases large (60-64) *g* to jumbo (70 and above) *g* sizes. This indicated that the rate of water loss and other vaporous components was higher in larger sizes of eggs. The effect was significant $P < 0.05$ with LSD of 0.558, 0.549, and 0.369, respectively, as shown in Table 4.1.2. Hence, temperature significantly affected the SW

4.1.3 Eggshell Diameter

The R-Sq. of the shell diameter (SD) showed that the fixed model was 68%, 70% and 81% for the large (60-64) *g*, extra-large (65-69) *g* and jumbo (70 and above) *g* egg sizes, respectively (Table 4.1.1). The fixed-effect models were statistically good at explaining the variation during the storage. The variation in shell diameter was highly explained in the jumbo (70 and above) *g* while lower in large (60-64) *g*. The coefficient of variation of SD was below 30%, which showed that the study data was accurate. The study found that jumbo (70 and above) *g* had the highest coefficient of variation (16%). The effect of storage time on the SD was notable at a 5% level of significance with LSD 0.0041, 0.0047 and 0.0061, respectively (Table 4.1.1). It was insignificant in jumbo (70 and above) *g* $P > 0.05$. Which pointed out that shell thickness was not necessarily affected by the storage duration.

The mean SD was 0.074 *cm*, 0.0738 *cm* and 0.074 *cm* with jumbo (70 and above) *g*, which recorded the highest thickness value. From the results in Table 4.1.1 and Table 4.1.2, shell thickness reduces with an increase in egg size. Table 4.1.2 shows the effect of storage temperature on SD. The SD reduced from low (5 oC) to high (30 oC) in large (60-64) g, extra-large (65-69) g and jumbo (70 and above) g sizes similar to the mixed-effect model. The study detected an increase in reduction in SD with an increase in egg size. The inter-temperature decrease of SD increases large (60-64) g to jumbo (70 and above) g sizes revealing that the rate of water loss and other vaporous components was higher in larger sizes of eggs. The effect was significant with LSD of 0.558, 0.549 and 0.369, respectively, as shown in Table 4.1.2, signifying that temperature expressively affected the SD.

Table 4.1.2: Effect of Temperature as Fixed-Effect in the Fixed-Effect Model

Egg size	Temp.	EWL	SW	SD	AH	AD	HU	YI
large (60-64) *g*.	room (19.5℃)	1.77	7.31	0.071	3.28	7.93	51.85	0.2802
	Ref. (5℃)	0.85	7.72	0.091	6.11	7.16	70.67	0.357
	Inc. (30℃)	2.37	7.09	0.059	2.66	8.56	29.84	0.2583
	P-Value	0.0006	0.0018	0.0014	0.0014	0.0086	0.0031	0.0002
	LSD	0.352	0.558	0.0315	0.926	0.881	8.696	0.132
	Var. Estimate	0.1393	0.3792	0.0391	1.108	0.706	67.47	0.0107
Extra-large (65-69) *g*.	room (19.5℃)	1.84	8.67	0.066	3.02	9.38	40.79	0.275
	Ref. (5℃)	0.86	9.75	0.091	5.89	7.7	63.95	0.3546
	Inc. (30℃)	2.64	7.36	0.062	2.25	10.87	22.93	0.2314
	P-Value	0.00083	2.50E-05	0.0016	0.0015	0.0088	0.0033	0.0004
	LSD	0.3435	0.5491	0.02231	0.9169	0.8723	8.6872	0.1234
	Var. Estimate	0.1306	0.3704	0.0303	1.099	0.6972	67.46	0.002
Jumbo (70 and more) *g*.	room (19.5℃)	1.951	8.57	0.064	3.21	9.01	38.48	0.261
	Ref. (5℃)	0.918	12.18	0.099	5.89	7.98	63.17	0.341
	Inc. (30℃)	3.087	6.46	0.057	2.15	12.73	17.95	0.166
	P-Value	0.0009	0.0001	0.0017	0.0017	0.0089	0.0034	0.0005
	LSD	0.35	0.56	0.031	0.93	0.88	8.703	0.1396
	Var. Estimate	0.1293	0.3692	0.02911	1.098	0.696	67.46	0.0007

4.1.4 Egg Albumen Height

Table 4.1.1 also illustrates the total variation explained by the conditions in the fixed RCBD model of the albumen height (AH). The R-Sq. of 73%, 67%, and 86% for large (60-64) *g*, extra-large (65-69) *g*, and jumbo (70 and above) *g* sizes, respectively. The fixed-effect model explained over 50% of the AH variation. The jumbo (70 and above) *g* egg size model explained the highest variation. The measurements on AH were good at 85%, as defined by the coefficient of variation $< 15\%$ for the egg sizes. Storage duration significantly affected the albumen height for all the egg sizes $P < 0.05.$ The LSD was observed as 1.102, 1.103 and 1.103. These LSD values were almost the same as the three egg size classes.

The effect of storage temperature on AH exists in Table 4.1.2. The AH reduced from low (5 ^{o}C) to high (30 ^{o}C) in large (60-64) *g*, extra-large (65-69) *g* and jumbo (70 and above) *g* sizes, similar to the mixed-effect model. This study noticed a general increase in the reduction of AH as the egg size increases. The effect was significant $P < 0.05$ with LSD of 0.926, 0.917, and 0.88, respectively, as shown in Table 4.1.2, indicating that temperature significantly affected the AH. Albumen height decreased from 6.6 *mm* to 1.84 *mm.* The effect of AH on the 2nd and 12th days was remarkably the same, recording the highest peak. It was different from 22nd and 32nd $(P < 0.05).$ The impact at 22nd and 32nd periods were significantly different from those at 30 ^{o}C and 32nd. Jumbo (70 and above) *g* sizes recorded the smallest AH. Further, this study noticed no difference in AH at 5 ^{o}C and 19.5 ^{o}C $(P > 0.05).$ However, there was a significant difference in AH at 30 ^{o}C $(P < 0.05)$ for all the egg sizes. The eggs stored at 5 ^{o}C recorded the highest AH on the 2nd day of storage, as shown in Table 4.1.4.

4.1.5 Egg Albumen Diameter

The R-Sq. of the albumen diameter (AD) pointed out that the fixed models were 62%, 61% and 68% for the egg sizes. The fixed-effect models were statistically good in explaining the variation of the AD during the storage. The jumbo (70 and above) *g* explained the highest value (68%), implying that most variation due to storage conditions was experienced by jumbo (70 and above) *g*. The coefficient of variation of the AD fixed-effect model was below 15%, which showed that the study was precise. They established that jumbo (70 and above) *g* had the highest coefficient of variation (13%) while extra-large (65-69) *g* had the lowest. The effect of time on the AD was highly significant at 95% confidence intervals with LSD, 1.05, as shown in Table 4.1.1.

Table 4.1.3: Effect of TRT as Fixed-Effect and DAY as a Random Effect in the Mixed-Effect Model

	Temp.	EWL	SW	SD	AH	AD	HU	YI
large (60-64) *g.*	room (19.5℃)	1.88	8.21	0.1	4.69	9.37	63.68	0.2717
	Ref. (5℃)	0.67	8.8	0.12	7.27	8.17	83.96	0.3631
	Inc. (30℃)	3.55	7.6	0.1	2.57	9.57	37.15	0.2497
	R.E	1.01	0.094	0.0000	0.31	0.054	32.7	0.0287
	Var. Est.	0.2325	0.3075	0.0004	1.707	0.7424	69.130	0.0542
	P-Value	0.0019	0.0548	0.0091	0.0063	0.0112	0.0167	0.0004
Extra-large (65-69) *g.*	room (19.5℃)	1.908	8.2	0.1	4.69	9.36	61.35	0.2576
	Ref. (5℃)	0.698	8.79	0.12	7.27	8.16	82.52	0.3553
	Inc. (30℃)	3.578	7.59	0.079	2.57	9.56	29.83	0.2466
	R. E	1.02322	0.104	0.0012	0.321	0.064	32.7	0.03
	Var. Est.	0.2213	0.3713	0.0001	1.9074	0.9421	69.415	0.0439
	P-Value	0.0019	0.0548	0.0091	0.0063	0.0112	0.0167	0.0004
Jumbo (70 and more) *g.*	Room (19.5℃)	1.92	8.19	0.097	4.685	9.35	58.96	0.255
	Ref. (5℃)	0.71	8.78	0.122	7.265	8.15	81.07	0.348
	Inc. (30℃)	3.59	7.58	0.076	2.565	9.55	24.74	0.239
	Random effect	1.016	0.103	0.0012	0.321	0.064	32.7	0.0301
	Var. Estimate	0.2284	0.3578	0.0008	1.908	0.992	68.38	0.0445
	P-Value	0.0018	0.0547	0.009	0.0062	0.0111	0.016	0.0003

The means of AD were 7.87 *cm*, 9.31 *cm*, 9.9 *cm*, with jumbo (70 and above) *g* recording the highest thickness value, inferring that the horizontal circumference covered by the albumen generally increased with the egg size. Therefore, a small egg size covered a smaller circumference. From the results in Table 4.1.1 and Table 4.1.2, AD rises with an increase in egg size. Given that a smaller egg size smaller circumference, AD increased from the large (60-64) *g*, extra-large (65-69) *g* and jumbo (70 and above) *g* egg sizes. Table 2 presents the effect of storage temperature on AD.

The AD increases from low (5 ^oC) to high (30 ^oC) in large (60-64) *g*, extra-large (65-69) *g*, and jumbo (70 and above) *g* sizes, similar to the mixed-effect model. This study witnessed an increase in AD as the egg size increases. The inter-temperature decrease of AD increases large (60-64) *g* to jumbo (70 and above) *g* sizes. The rate of water loss and other vaporous components was higher in larger sizes of eggs. The effect was significant $P < 0.05$ with LSD of 0.881, 0.8723, and 0.88 respectively, as shown in Table 4.1.2, demonstrating that temperature significantly affected AD. The interaction effect on HU (large (60-64) *g*) was insignificant at 5 ^oC, 19.5 ^oC, 30 ^oC for the 22nd and 12th time.

4.1.6 Haugh Unit

The effect of different storage periods on Haugh Unit (HU) for large (60-64) *g*, extra-large (65-69) *g*, and jumbo (70 and above) *g* sizes was expressed in Table1. The R-Sq. of the HU shows that the fixed model was 85%, 78%, and 77% for the egg sizes, respectively. These models explained 70% of the effect caused by storage conditions. The large (60-64) *g* expressed the highest variation while the lowest in jumbo (70 and above) *g* sizes had the lowest. The coefficient of variation of HU was below 15%, which shows that the measurements were keenly observed. The large (60-64) *g* had the highest coefficient of variation (14%). The effect of storage time on the HU was significant at 95% confidence intervals with (LSD) of 0.19. The confidence interval was approximately similar for the three classes of egg sizes, as shown in Table 4.1.1.

The mean values of HU were 50.79, 42.56, 39.87, with jumbo (70 and above) *g* recording the lowest value. HU reduced with an increase in the egg size. The observed HU of jumbo on the 12th and 22nd days were different $(P < 0.05)$ at 5 ^oC, 19.5 ^oC, 30 ^oC. A significant difference was observed on eggs stored for 32 and 2 days $(P < 0.05)$. The effect of 2nd-day storage was significantly different from the 12th and 22nd days $(P < 0.05)$. The impact of the 32nd period was also quite different from the 12th and 22nd. The HU reduced steadily from the 2nd to 32nd storage period. Further, this study observed an insignificant difference between the effect of 5 ^{o}C and 19.5 ^{o}C on the HU. The effect of 30 ^{o}C was significantly different at 5 ^{o}C and 19.5 ^{o}C. This study also detected that HU at 5 ^{o}C remained remarkably the same throughout the storage period used for this study.

From the results in Table 4.1.1 and Table 4.1.2, HU decreases with egg size. Table 2 shows the effect of storage temperature on HU. The HU declines from low (5 ^{o}C) to high (30 ^{o}C) in large (60-64) *g*, extra-large (65-69) *g* and jumbo (70 and above) *g* sizes. It signifies that eggs with higher weight recorded lower HU, similar to the mixed-effect model. It demonstrates that the rate of loss of HU was higher in the jumbo (70 and above) *g* egg size than large and extra-large egg size. The rate of water loss and other gaseous components was more elevated in larger size eggs. The effect was significant $P < 0.05$ with LSD of 8.696, 8.687, and 8.703, respectively (Table 4.1.2). Thus, indicating that temperature significantly affected the HU.

4.1.7 Yolk Index

Table 4.1.1 also displays the total variation of the storage conditions in the fixed RCBD model of the YI. The R-Sq. of 85%, 83%, 92% for large (60-64) *g*, extra-large (65-69) *g* and jumbo (70 and above) *g* sizes, respectively. The fixed-effect model explains over 50% of the SW variation. The slightest variation of the three categories of egg sizes was explained by extra-large (65-69) *g*. The obtained values on YI was 93%, as described by the coefficient of variation $< 7\%$ for the egg sizes. The YI model with storage time was highly significant for the three eggs $P < 0.05.$ Which implied that YI was affected by the storage duration. The LSDs observed were 0.186, 0.187, 0.188, with the highest value in jumbo (70 and above) *g* size. The significant differences between YI data points increased with an increase in egg size. The mean values of YI were 0.298, 0.289, 0.256, with large (60-64) *g* size recording the highest. Thus, the yolk index decreases with an increase in egg size.

Table 4.1.4: Effect of Interaction Effect in the Fixed-Effect Model

Egg Size	Temp.	Period (day)	EWL (*g*)	SW (*g*)	SD (*cm*)	AH (*mm*)	AD (*cm*)	HU	YI
Extra-large	19.5℃	2*nd*	0.34^{a}	8.88^{a}	0.11^{a}	8.38^{a}	7.34^{a}	77.84^{a}	0.32^{a}
(65-69) *g*		12*th*	0.91^{a}	7.96^{a}	0.099^{a}	7.61^{a}	8.04^{a}	67.91^{b}	0.308^{a}
		22*nd*	2.12^{b}	7.63^{a}	0.061^{a}	6.12^{b}	9.58^{b}	63.03^{c}	0.27^{b}
		32*nd*	3.02^{c}	6.95^{c}	0.02^{b}	5.22^{c}	12.27^{c}	56.27^{bc}	0.23^{b}
	5℃	2*nd*	0.11^{a}	8.91^{a}	0.13^{a}	8.65^{a}	7.27^{a}	83.63^{d}	0.35^{a}
		12*th*	0.43^{a}	8.41^{a}	0.12^{a}	7.85^{a}	7.65^{a}	80.23^{d}	0.34^{a}
		22*nd*	0.76^{a}	8.01^{a}	0.11^{a}	7.55^{a}	8.19^{a}	76.28^{a}	0.32^{a}
		32*nd*	1.11^{a}	7.91^{a}	0.04^{b}	7.45^{a}	9.11^{b}	64.15^{c}	0.25^{b}
	30℃	2*nd*	1^{a}	8.34^{a}	0.09^{a}	7.45^{a}	7.42^{a}	72.5^{a}	0.27^{b}
		12*th*	1.11^{a}	7.42^{a}	0.054^{a}	6.01^{b}	8.88^{a}	62.33^{c}	0.263^{b}
		22*nd*	2.33^{b}	6.49^{c}	0.03^{b}	5.75^{b}	10.73^{b}	53.68^{bc}	0.24^{b}
		32*nd*	3.89^{d}	4.57^{d}	0.01^{b}	3.62^{d}	12.73^{c}	31.43^{abd}	0.2^{c}
Extra-large	19.5℃	2*nd*	0.38^{a}	8.89^{a}	0.11^{a}	8.24^{a}	7.36^{a}	71.76^{a}	0.31^{a}

(65-69) *g*		12*th*	1.01^{b}	7.63^{a}	0.098^{a}	6.47^{c}	8.12^{a}	65.83^{b}	0.298^{a}
		22*nd*	2.35^{ab}	6.21^{b}	0.06^{b}	5.98^{b}	9.66^{b}	64.95^{b}	0.26^{b}
		32*nd*	3.09^{c}	5.95^{b}	0.02^{b}	5.08^{b}	12.35^{ab}	54.19^{c}	0.22^{bc}
	5℃	2*nd*	0.14^{a}	9.91^{ab}	0.13^{a}	8.52^{a}	7.29^{a}	84.55^{d}	0.35^{d}
		12*th*	0.77^{a}	9.47^{ab}	0.12^{a}	7.71^{a}	7.73^{a}	81.15^{d}	0.33^{a}
		22*nd*	0.97^{b}	8.81^{a}	0.11^{a}	7.42^{ab}	8.27^{a}	77.2^{a}	0.31^{a}
		32*nd*	1.81^{ab}	7.93^{a}	0.04^{b}	7.32^{ab}	9.19^{b}	64.07^{b}	0.24^{b}
	30℃	2*nd*	1.13^{b}	8.34^{a}	0.06^{b}	8.01^{a}	7.44^{a}	70.42^{a}	0.26^{b}
		12*th*	1.7^{ab}	7.22^{a}	0.053^{b}	6.87^{c}	8.96^{a}	60.25^{b}	0.253^{b}
		22*nd*	3.03^{c}	6.62^{b}	0.03^{b}	5.61^{b}	10.81^{b}	52.6^{c}	0.23^{b}
		32*nd*	4.1^{ac}	4.44^{c}	0.01^{c}	3.48^{c}	12.81^{ab}	29.35^{f}	0.2^{c}
Jumbo	19.5℃	2*nd*	0.5^{a}	10.85^{a}	0.11^{a}	8.02^{a}	7.47^{a}	70.55^{a}	0.29^{a}
(70 and more) *g*		12*th*	1^{a}	7.63^{b}	0.09^{a}	7.25^{c}	8.14^{a}	55.62^{b}	0.28^{a}
		22*nd*	2.7^{b}	6.21^{b}	0.06^{a}	5.76^{b}	9.68^{b}	54.74^{b}	0.24^{b}

	32*nd*	3.1^{ab}	5.95^{b}	0.02^{b}	4.86^{b}	12.37^{c}	43.98^{ab}	0.2^{c}
5℃	2*nd*	0.16^{a}	11.47^{a}	0.13^{c}	8.99^{a}	7.37^{a}	85.34^{c}	0.33^{ab}
	12*th*	0.83^{a}	10.56^{a}	0.12^{c}	7.49^{c}	7.75^{a}	79.94^{c}	0.31^{a}
	22*nd*	1.07^{a}	9.38^{a}	0.11^{a}	7.19^{c}	8.29^{a}	71.99^{a}	0.29^{a}
	32*nd*	1.31^{b}	6.93^{b}	0.04^{b}	7.09^{c}	9.21^{b}	60.86^{b}	0.22^{b}
30℃	2*nd*	1.19^{a}	10.22^{a}	0.06^{a}	8.09^{a}	7.52^{a}	68.21^{a}	0.24^{b}
	12*th*	1.9^{b}	8.1^{b}	0.05^{a}	6.65^{c}	8.98^{a}	50.04^{b}	0.24^{b}
	22*nd*	2.73^{b}	4.62^{c}	0.03^{b}	5.11^{b}	10.83^{d}	39.39^{d}	0.21^{c}
	32*nd*	4.4^{c}	3.4^{d}	0.01^{b}	3.26^{d}	12.83^{f}	19.14^{f}	0.17^{d}

Different letters denote significant difference ($P < 0.05$) within different categories of egg size

From the results in Table 4.1.1 and Table 4.1.2, YI reduced with an increase in egg size. Table 2 shows the effect of storage temperature on YI. This study observed a related change of YI in both fixed and mixed-effect models. The YI reduces from low (5 ^oC) to high (30 ^oC) in large (60-64) *g*, extra-large (65-69) *g* and jumbo (70 and above) *g* sizes. The study observed a decrease in YI as the egg size increases. The inter-temperature reduction of YI increased from large (60-64) *g* to jumbo (70 and above) *g* sizes. The effect was significant $P < 0.05$ with LSD of 0.132, 0.1234 and 0.1396, respectively, as shown in Table 2. Validating that temperature significantly affected the YI.

4.2 Comparison of the Model Efficiency

In comparing the model efficiencies, the estimated variance components for the fixed-effect model (EWL) were 0.1393, 0.1306 and 0.1293 in Table 4.1.2. Each of these values was less than 0.2325, 0.2213, 0.2284 of the mixed-effect model in Table 4.1.3. Variance components of the mixed effect models (EWL) were significantly different from fixed-effect models $P < 0.05.$ Further, the estimated variance components for the fixed-effect models (SW) were 0.379, 0.37 and 0.36, while those of the mixed-effect models were 0.3075, 0.3713 and 0.3578 in Table 4.1.3. Each of these values corresponding to the large (60-64) *g*, extra-large (65-69) *g* and jumbo (70 and above) *g* egg sizes was insignificantly different $P > 0.05.$ This was in exception of large (60-64) *g* size, which had a significant difference $P < 0.05.$ The mixed-effect and fixed-effect models were significantly the same $P > 0.05.$

The fixed-effect models (AH) had 1.11, 1.099 and 1.098 estimated variance components, which were significantly lesser than those of the mixed-effect models; 1.707, 1.907 and 1.908 in Table 4.1.3, $(P < 0.05).$ Moreover, the estimated variance components for the fixed-effect models (AD) were 0.706, 0.697 and 0.696. These values were significantly different from those of the mixed-effect model; 0.9424, 0.9421 and 0.9922 in Table 4.1.3. Consequently, the mixed-effect and fixed-effect model was not the same $P < 0.05.$ Fixed effect model posted a small estimated variance component.

Furthermore, the estimated variance components for the fixed-effect models (HU) were 67.47, 67.46 and 67.46. Each of these values was significantly different from those mixed-effect models; 69.13, 69.41 and 68.38 in Table 4.1.3 $(P < 0.05)$. Therefore, mixed and fixed-effect models was not the same. Fixed effect model posted small estimated variance component. Similarly, the estimated variance components for the fixed-effect models (YI) were 0.0107, 0.002 and 0.0007. These values were significantly different from those of the mixed-effect models; 0.0542, 0.0439 and 0.0445 in Table 4.1.3 $P < 0.05$. Hence mixed and fixed-effect models were not the same. Generally, fixed-effect models resulted in smaller estimated variance components hence more efficient. The models were significantly the same in shell thickness $P > 0.05$.

4.3 PCA and Response Surface for Large Egg Size

PCA for large egg size (60-64) *g* was performed as shown in Table 4.3.1 and Table 4.3.2. This made it easy to select interactive variables that were significantly affected by the storage conditions studied.

Table 4.3.1: Eigen Analysis of the Covariance Matrix

Eigenvalue	15.773	4.767	1.187	0.652	0.304	0.039	0.009
Proportion	0.694	0.209	0.052	0.029	0.013	0.002	0.000
Cumulative	0.694	0.904	0.956	0.985	0.998	1.000	1.000

The cumulative proportion was used to establish the amount of variance explained by the principal components. PCs that explained at least 90% were retained for response surface analysis.

Table 4.3.2: Eigenvectors

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
AH	0.103	0.421	-0.396	0.617	0.122	0.406	-0.016
YI	0.515	0.345	0.073	0.607	-0.015	-0.024	-0.442
AD	0.052	-0.351	-0.582	0.282	-0.529	-0.406	-0.106
HU	0.980	-0.427	-0.343	-0.185	0.056	0.007	0.012
SD	0.002	0.402	0.004	-0.001	0.011	-0.014	-0.010
SW	0.586	-0.692	0.440	0.205	-0.654	0.540	0.003
ch_EW	-0.701	-0.535	-0.442	-0.262	0.256	0.598	-0.125

The study confirmed that there were no outliers. All the points were below the reference line. Further, from Table 4.3.2, variables with strong positive-negative correlation coefficients were identified from the first and second principal components. These variables were YI, HU, EWL and SW. They were subjected to further analysis for the response surface model.

4.3.1 Weight Loss of (60-64) *g* Egg Size

Table 4.3.3: Model Summary of Egg Weight Loss (Large)

R-sq.	R-sq. (adj.)	PRESS	R-sq. (pred.)
88.69%	86.80%	8.97094	82.94%

Table 4.3.3 presents a summary of the response surface model of total weight loss for the large egg size. R-square (R-sq.) indicated that this model explained 88% variation in egg weight loss. Sums of squares of prediction (PRESS) were used to validate the EWL model. Since it was 8, the model predictive ability was statistically accepted with a standard error of 0.445. Further, predicted R-square (R-sq. (pred.)) = 86% does not differ so much from adjusted R-square = 82%. This implies that this model fits well the sample.

Table 4.3.4: ANOVA of Egg Weight Loss (Large)

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
Model	5	46.6308	88.69%	46.6308	9.3262	47.05	0.000
Linear	2	41.6125	79.15%	39.8487	19.9244	100.52	0.000
TRT	1	11.6286	22.12%	11.2683	11.2683	56.85	0.000
DAY	1	29.9839	57.03%	28.5805	28.5805	144.19	0.000
Square	2	1.7051	3.24%	1.7051	0.8526	4.30	0.023
TRT*TRT	1	0.1779	0.34%	0.1779	0.1779	0.90	0.051
DAY*DAY	1	1.5273	2.90%	1.5273	1.5273	7.70	0.009
2-Way Interaction	1	3.3131	6.30%	3.3131	3.3131	16.71	0.000
TRT*DAY	1	3.3131	6.30%	3.3131	3.3131	16.71	0.000
Error	30	5.9466	11.31%	5.9466	0.1982		
Lack-of-Fit	6	0.6679	1.27%	0.6679	0.1113	0.51	0.798
Pure Error	24	5.2787	10.04%	5.2787	0.2199		
Total	35	52.5774	100.00%				

Table 4.3.4 presents the ANOVA of the response surface for the egg weight loss (large egg size). Storage duration (DAY), temperature (TRT), second-order factor DAY*DAY and their interaction TRT*DAY were significant. TRT*TRT was insignificant at 95% confidence interval $P = 0.051$ Storage duration contributed a large percentage effect on

the total weight loss (57%). Lack-of-Fit was highly insignificant $P = 0.798,$ indicating that the model was fit for optimization. The regression equation was given as;

$$ch\_EW = 0.211 + 0.0442TRT - 0.0365DAY - 0.00098TRT*TRT + 0.00206DAY*DAY + 0.002647TRT*DAY \tag{4.1}$$

Table 4.3.5: Predictive Variances

Run		$\sigma^2 = 5.9466$	$N = 36$
	$X(X'X)^{-1}X'$	$\sigma^2 X(X'X)^{-1}X'$	$NX(X'X)^{-1}X'$
1	0.2061	1.2256	7.4
⋮	⋮	⋮	⋮
12	0.2061	1.2256	7.4
13	0.1611	0.9581	5.7
⋮	⋮	⋮	⋮
18	0.1611	0.9581	5.7
19	0.125	0.7433	4.5
⋮	⋮	⋮	⋮
30	0.125	0.7433	4.5
31	0.1166	0.6937	
⋮	⋮	⋮	⋮
36	0.1166	0.6937	4.2

Table 4.3.5 presents scaled and unscaled predictive variances of weight loss in large egg sizes. This study observed two design centres. The coded control variables produced predictive variation, the weight loss variance for the large egg size at the point of interest. The contrast of the stationary points was 0.9581 and 0.69377, respectively. Therefore, every moment that was equidistant from the first and second design centres had $\text{var}[\hat{y}_1(x)] = 1.2256$ and $\text{var}[\hat{y}_2(x)] = 0.7433$ respectively. This showed that the design created by this study is rotatable since the variances of the points that were equidistant from the design centres are the same for all non-stationary runs. This study computed scaling Predictive Variance (SPVar) for the comparison of several design runs. The SPVar was used to achieve a measure of the precision of the estimated weight loss for the extra-large egg size at any point in the design space.

A steady SPVar for equidistant and stationary points for the first design centre as 7.4 and 5.7 respectively was obtained. The SPVar for the equidistant and stationary point for the second design centre was also constant. The design had a reproducible sketch of predictive variances with few runs. This implied that the second-order model had had a favourable prediction of the storage conditions of interest. The design criterion prediction of the response surface for the second-order model (EWL for large egg size) was $G-eff = \frac{5}{7.4} = 0.675.$ Thus 68% efficiency was achieved. This meant that the maximum SPVar =7.4 for this design was good.

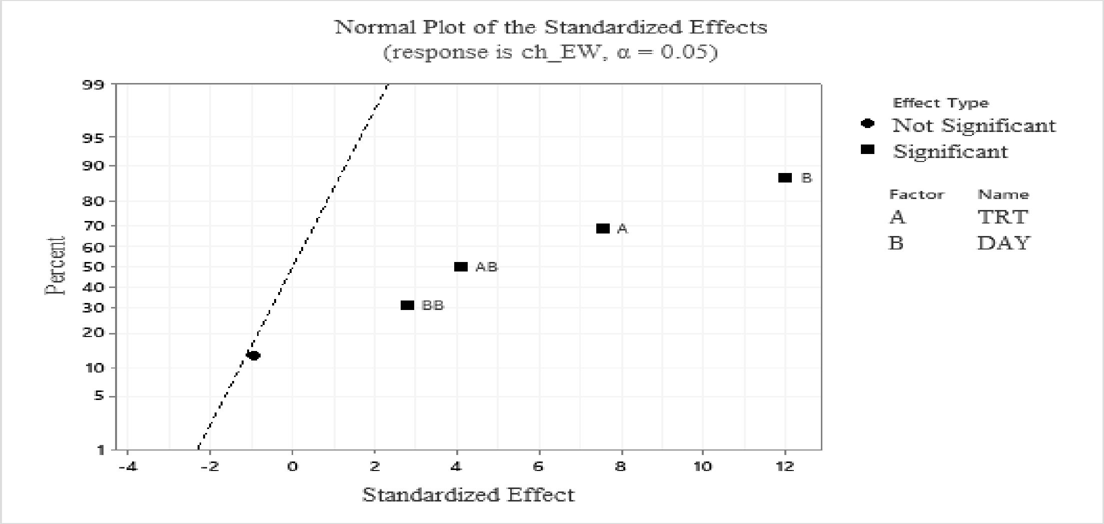

Figure 1: Normal Plot of Standardized Effects of EWL (Large Egg Size)

This study used Fig. 1 above to show the standardized effects of egg weight loss for the large egg size category. From the plot, the direction of significance was seen from the origin of the graph plot. This plot indicates the magnitude of the essential effects on weight loss. Fig. 2 shows residual tests of the EWL. The histogram shows that the data points of weight loss in large egg sizes were normally distributed. The standard probability plot verified the normality assumption. Residuals versus order plots were used to verify the belief that residuals were independent of each other. The plot displayed no trend or pattern in time order. Thus, the pattern verified the independence assumption. Linearity assumption was also satisfied. However, the residual plots seemed to be more concentrated at the initial stages while widely spread at the end stages. The concentration resulted from slight weight loss at 5 ^oC and room temperature. The 30 ^oC had a widely spread weight loss.

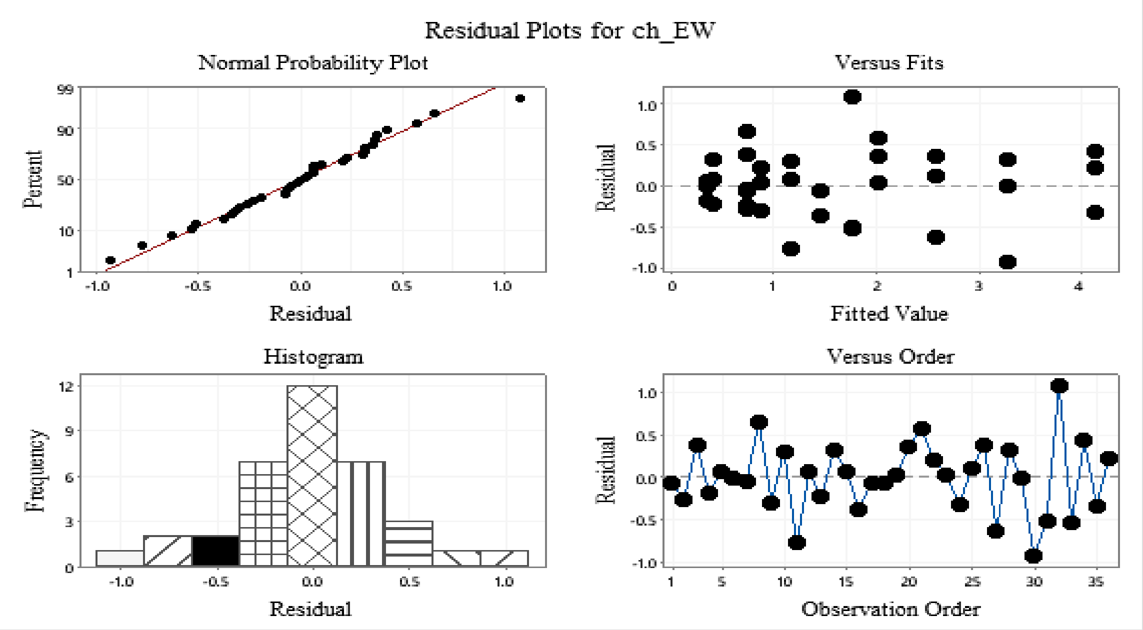

Figure 2: Residual Plot of Ch_EW (Large Egg Size)

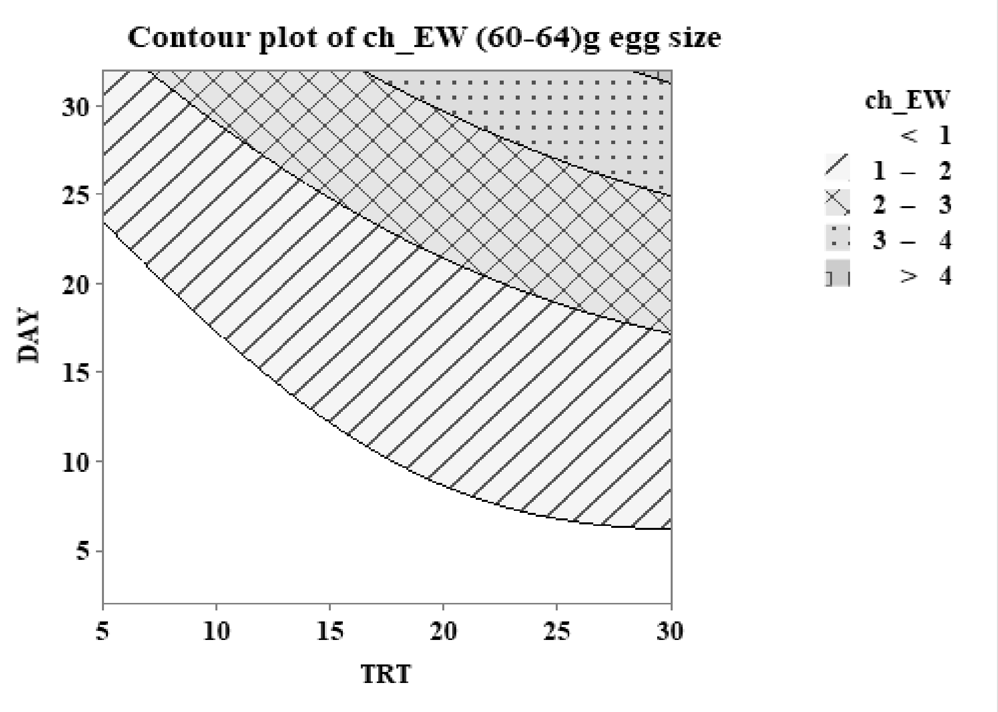

Figure 3: Contour Plots of the effect of storage conditions on EWL (Large)

Fig. 3 determined optimal levels of storage conditions. These levels were significant from the second model in Table 4.3.4. From Fig. 3 the egg's weight loss increases towards the contours' direction. The darker fill contour has the highest weight loss region >4. The less dark area has the most negligible possible weight loss under the given storage conditions <1.

4.3.2 Haugh Unit of (60-64) *g* Egg Size

Table 4.3.6: Model Summary of HU (Large)

R-sq.	R-sq. (adj.)	PRESS	R-sq. (pred.)
93.41%	92.31%	13.63	90.12%

Table 4.3.6 presents a summary of the response surface model of the Haugh Unit for the large egg size. R-sq. indicated that this model explained 93% variation in HU. PRESS validated this model. Since PRESS was 13>3, the predictive model ability was worthy. Further, predicted R-sq. (pred.) = 90% does not differ so much from R-sq. (adj.) = 92%. Therefore, this model fits the sample well.

Table 4.3.7: Analysis of Variance HU (large)

Source	DF	Seq. SS	Contribution	Adj. SS	Adj. MS	F-Value	P-Value
Model	5	13039.9	93.41%	13039.9	2607.98	85.06	0.000
Linear	2	12367.6	88.59%	12209.5	6104.73	199.10	0.000
DAY	1	9584.5	68.66%	9325.1	9325.08	304.13	0.000
TRT	1	2783.1	19.94%	2884.4	2884.38	94.07	0.000
Square	2	370.6	2.65%	370.6	185.28	6.04	0.006
DAY*DAY	1	207.5	1.49%	207.5	207.49	6.77	0.014
TRT*TRT	1	163.1	1.17%	163.1	163.07	5.32	0.028
2-Way Interaction	1	301.7	2.16%	301.7	301.74	9.84	0.004
DAY*TRT	1	301.7	2.16%	301.7	301.74	9.84	0.004
Error	30	919.9	6.59%	919.9	30.66		
Lack-of-Fit	6	170.5	1.22%	170.5	28.42	0.91	0.504
Pure Error	24	749.3	5.37%	749.3	31.22		
Total	35	13959.8	100.00%				

Table 4.3.7 presents the ANOVA of the response surface for the HU (large egg size). Storage duration (DAY), temperature (TRT), second-order factor DAY*DAY, TRT*TRT,

and their interaction TRT*DAY were significant $P < 0.05.$ Lack-of-Fit was highly insignificant $P = 0.504.$ This model was fit for optimization. The regression equation is therefore given as;

$$HU \quad = \quad 95.05 - 1.817\,DAY + 0.595\,TRT + 0.02401\,DAY * DAY - 0.0298\,TRT * TRT - 0.02526\,DAY * TRT \tag{4.2}$$

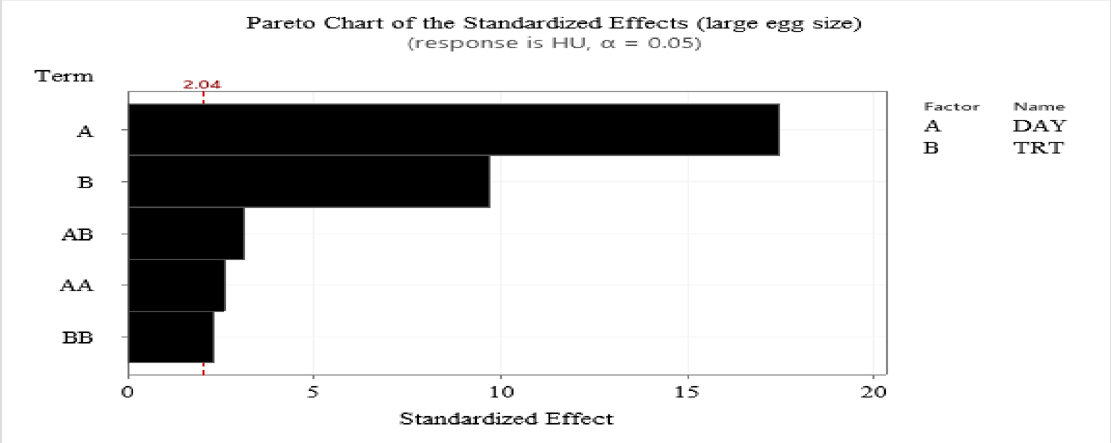

Figure 4: Pareto Chart of the Standardized Effects of HU (Large)

The Pareto chart of HU in Fig. 4 shows that factors A, AA, BB, B, and AB were significant at 95% confidence interval. The effect of A was more robust than the effect of B. Moreover, the interaction effect was resilient than of AA and BB. However, the impact of AB, AA, and BB.

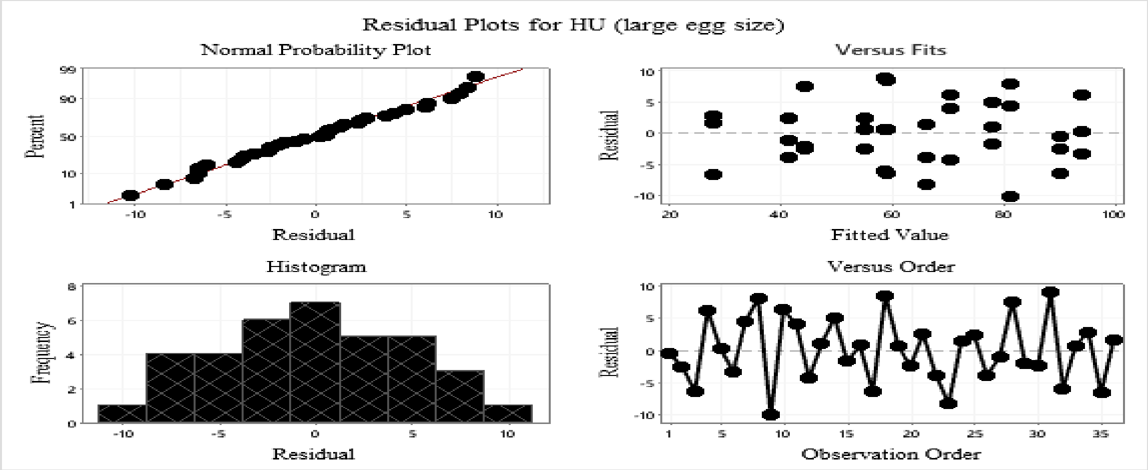

Figure 5: Residual Plots of HU (Large Egg Size)

Residual plots of the HU were shown in Fig 5. The histogram shows that the data points of HU were usually distributed. The standard probability graph verified the normality assumption. Residuals versus order plots were used to verify the hypothesis that residuals were independent of each other. Plots displayed no trend or pattern in time order. Thus, they were linear.

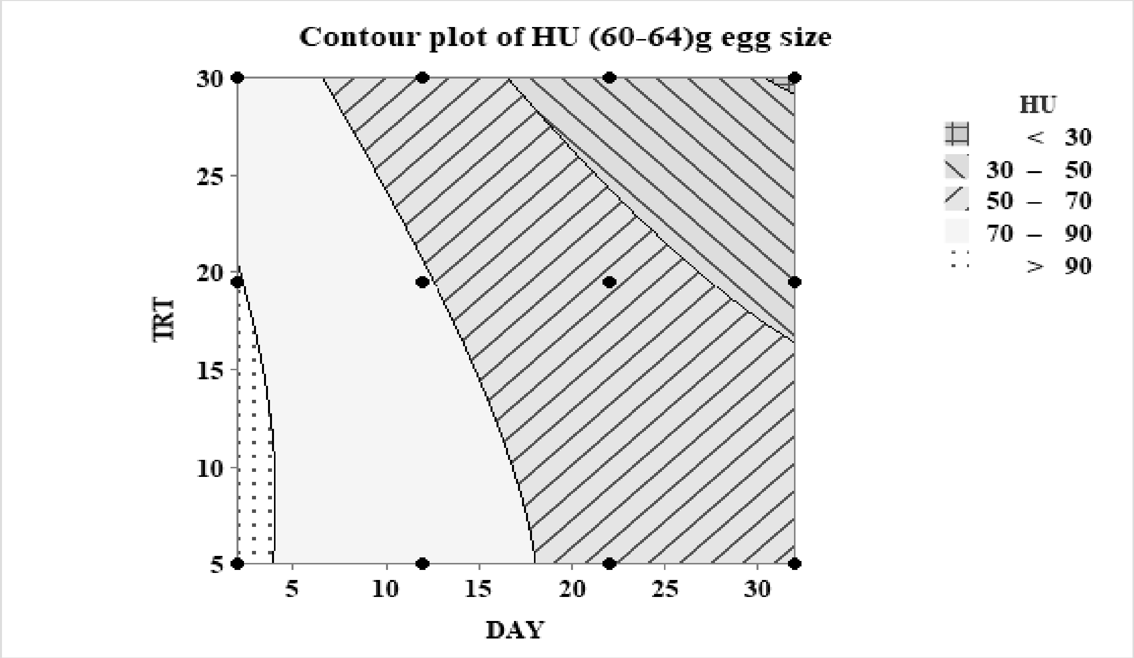

Figure 6: Contour Plot of HU (Large)

Fig. 6 shows the contour plot of the response surface model of HU against storage time (DAY) and temperature (TRT). The direction of the contour plots indicates a reduction in HU from low to high levels of storage conditions. HU was above 90 with storage for less than five days at 5 ^{o}C and room. The eggs stored at room temperature for 12 days had an HU unit between 70-90. However, those stored at room temperature for 32 days had HU between 50-70.

4.3.3 Shell Weight of (60-64) *g* Egg Size

Table 4.3.8: Shell Weight Model Summary (Large)

R-sq.	R-sq. (adj.)	PRESS	R-sq. (pred.)
87.22%	85.08%	19.3479	81.29%

Table 4.3.8 displays a summary of the response surface model of shell weight. R-sq. indicated that this model explained 87.22% variation in the SW, which was statistically

considerable. PRESS authenticated this model. Since it was 19 >3, the predictive model ability was. Further, R-sq. (pred.) = 81% does not differ so much from adjusted R-square = 85%. This model does not overfit the used sample.

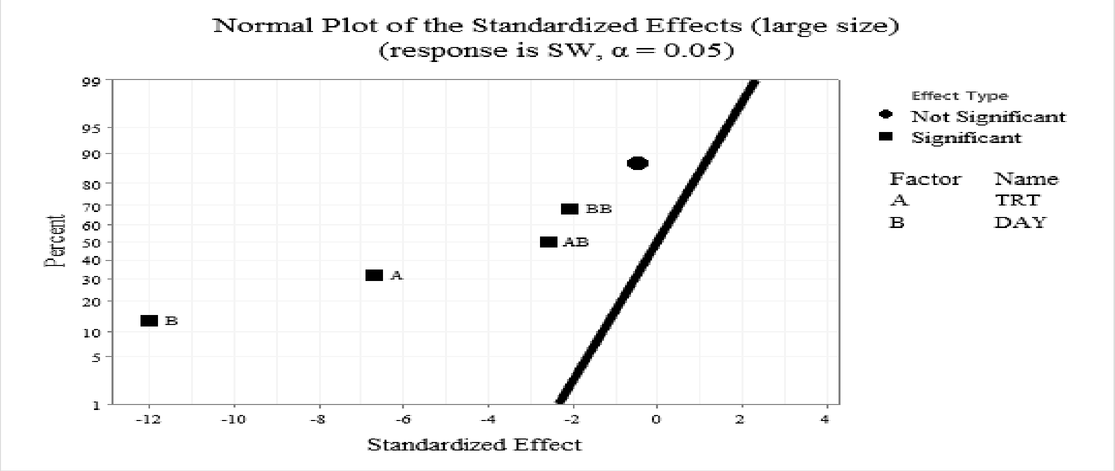

Figure 7: Normal Plot of Standardized Effects of SW (Large)

Fig. 7 shows the magnitude of the standardized effects in the response surface model of SW for the large egg size category. From the plot, the direction of significance from the origin of the property. This plot indicates the magnitude of the significant effects on the shell weight. Storage duration represented by B had the highest considerable impact in this study. Fig. 8 shows residual properties of the SW. The histogram shows that the data points of SW were approaching normal distribution. The standard probability plot verified the linearity assumption. Residuals versus order plots were used to verify the hypothesis that residuals were independent of each other. Fig. 8 displayed no trend or pattern in time order.

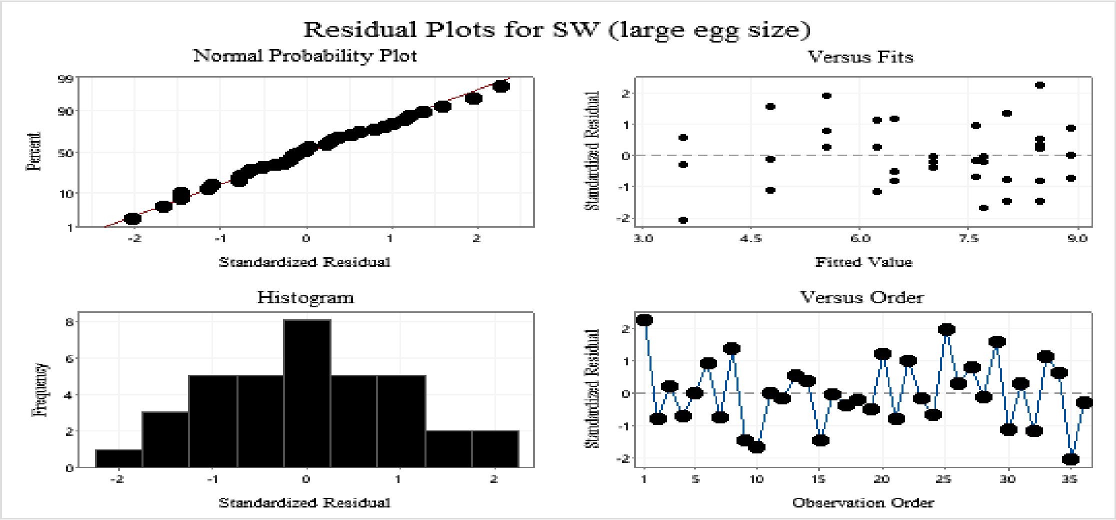

Figure 8: Residual Plots of SW(Large Egg Size)

Table 4.3.9: ANOVA of SW for the Large Egg Size

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
Model	5	88.894	87.61%	88.8938	17.7788	42.42	0.000
Linear	2	84.003	82.79%	82.0062	41.0031	97.84	0.000
TRT	1	18.846	18.57%	18.8594	18.8594	45.00	0.000
DAY	1	65.157	64.22%	63.1468	63.1468	150.68	0.000
Square	2	2.096	2.07%	2.0960	1.0480	2.50	0.099
TRT*TRT	1	0.047	0.05%	0.0471	0.0471	0.11	0.740
DAY*DAY	1	2.049	2.02%	2.0490	2.0490	4.89	0.035
2-Way Interaction	1	2.794	2.75%	2.7944	2.7944	6.67	0.015
TRT*DAY	1	2.794	2.75%	2.7944	2.7944	6.67	0.015
Error	30	12.573	12.39%	12.5727	0.4191		
Lack-of-Fit	6	2.379	2.34%	2.3787	0.3965	0.93	0.489
Pure Error	24	10.194	10.05%	10.1939	0.4247		
Total	35	101.467	100.00%				

Table 4.3.9 shows the ANOVA of the response surface for the egg weight loss (large egg size). Storage duration (DAY), temperature (TRT), second-order factor DAY*DAY and their interaction TRT*DAY were significant $P < 0.05.$ TRT*TRT was insignificant at 95% confidence interval $P > 0.05.$ This study observed that lack-of-Fit was highly insignificant $P = 0.489.$ Which implied model fitness for optimization. The regression equation is therefore expressed as;

$$SW = 8.975 - 0.0119TRT + 0.0050DAY - 0.00051TRT*TRT - 0.00239DAY*DAY - 0.002431TRT*DAY$$

(4.3)

Fig. 9 shows the contour plot of the response surface model of SW against storage time (DAY) and temperature (TRT). The direction of the contour plots indicates a reduction in HU from low to high levels of storage conditions. Storage conditions increase from the origin. SW was above 7.4 *g* when stored for less than 16 days at 5 ^{o}C.

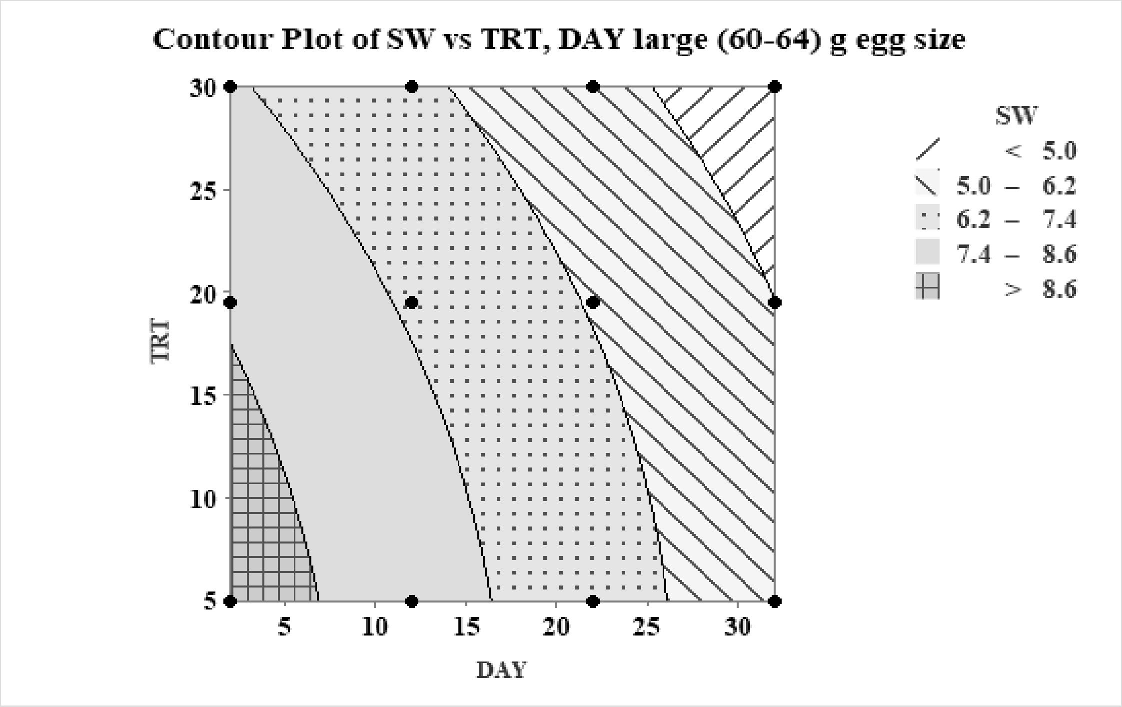

Figure 9: Contour Plot of SW (Large Egg Size)

4.3.4 Yolk Index (60-64) *g* Egg Size

Table 4.3.10: Coded Coefficients of YI (Large)

Term	Coef	SE Coef	95% CI	T-Value	P-Value	VIF
Constant	27.68	1.19	(25.25, 30.10)	23.32	0.000	
TRT	-2.418	0.666	(-3.778, -1.058)	-3.63	0.001	1.01
DAY	-5.653	0.731	(-7.146, -4.160)	-7.73	0.000	1.00
TRT*TRT	-1.47	1.19	(-3.90, 0.96)	-1.24	0.025	1.01
DAY*DAY	0.79	1.22	(-1.70, 3.29)	0.65	0.021	1.00
TRT*DAY	-1.415	0.890	(-3.232, 0.403)	-1.59	0.032	1.00

From Table 4.3.10, temperature (TRT) and DAY had a significant effect on the yolk index $(P < 0.05)$. TRT*TRT, DAY*DAY and their interaction TRT*DAY was insignificant

$(P > 0.05)$. DAY, TRT, DAY*DAY and TRT*DAY negative coefficients (Coef.) of $-5.653, -2.418, -1.415$ and -1.47 respectively. DAY*DAY had a positive coefficient and the T-Value of 0.79. TRT had the lowest standard error of the coefficient 0.666. $VIF = 1$ and 1.01, indicated no correlation between temperature and period of storage. Therefore, this study proceeded to decide on which level factors are responsible for the change in the YI.

Table 4.3.11: Model Summary of YI (Large)

R-sq.	R-sq.(adj.)	PRESS	R-sq.(pred.)
72.37%	67.77%	4.55	60.63%

Table 4.3.11 presents a summary of the response surface model of the percentage yolk index. R-sq. indicated that this model explained 85.26% variation in egg weight loss. We further used PRESS to test the validity of this model. Since it was >3, the predictive model ability was good. Further, R-sq. (pred.) = 60% does not differ so much from adjusted R-sq. = 67% implies that this model fits the sample well.

Table 4.3.12: ANOVA of YI (Large)

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
Model	5	836.78	72.37%	836.780	167.356	15.72	0.000
Linear	2	789.06	68.25%	776.735	388.367	36.47	0.000
TRT	1	132.79	11.49%	140.336	140.336	13.18	0.001
DAY	1	656.27	56.76%	636.399	636.399	59.77	0.000
Square	2	20.81	1.80%	20.806	10.403	0.98	0.388
TRT*TRT	1	16.32	1.41%	16.318	16.318	1.53	0.025
DAY*DAY	1	4.49	0.39%	4.488	4.488	0.42	0.021
2-Way Interaction	1	26.91	2.33%	26.915	26.915	2.53	0.022
TRT*DAY	1	26.91	2.33%	26.915	26.915	2.53	0.032
Error	30	319.43	27.63%	319.433	10.648		
Lack-of-Fit	6	54.32	4.70%	54.323	9.054	0.82	0.566
Pure Error	24	265.11	22.93%	265.109	11.046		
Total	35	1156.21	100.00%				

Table 4.3.12 exhibits the ANOVA of the response surface for the yolk index. DAY, TRT, second-order factor TRT*TRT, DAY*DAY, and interaction TRT*DAY were significant. Storage duration contributed a large percentage effect on the yolk index (56%). Lack-of-

Fit was insignificant $P = 0.566,$ inferring this model was fit for optimization. The regression equation is therefore expressed as;

$$YI = 33.36 + 0.265TRT - 0.365DAY - 0.00942TRT*TRT + 0.00353DAY*DAY - 0.00755TRT*DAY \tag{4.4}$$

Residual plots of the yolk index are shown in Fig. 10. The histogram shows that the data points of the YI were usually distributed. Typical probability plot verified the normality assumption. Residuals versus order plots were used to verify the belief that residuals were independent of each other. The scatter plot displayed no trend or pattern in time, suggesting that they were independent. Linearity assumption was also satisfied.

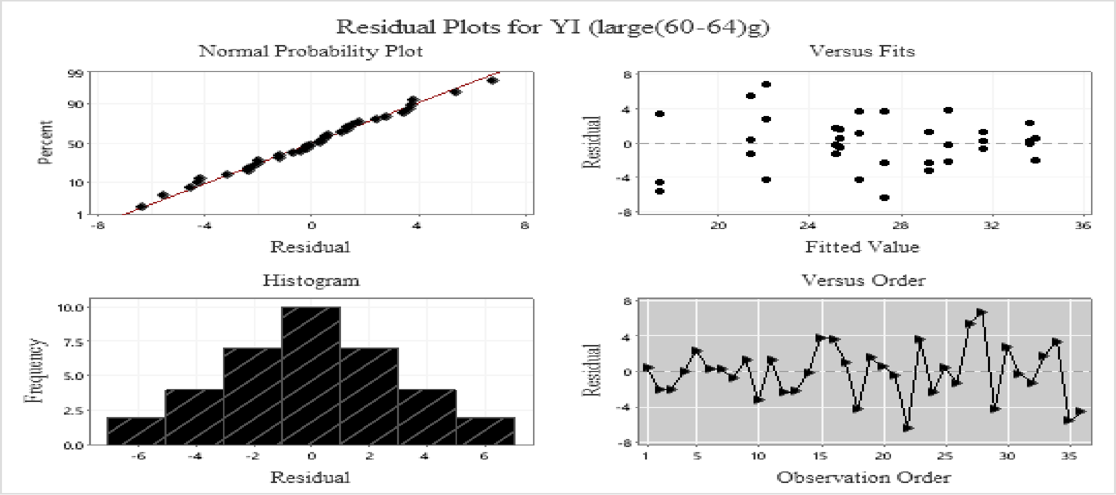

Figure 10: Residual Plots for YI (Large Egg Size)

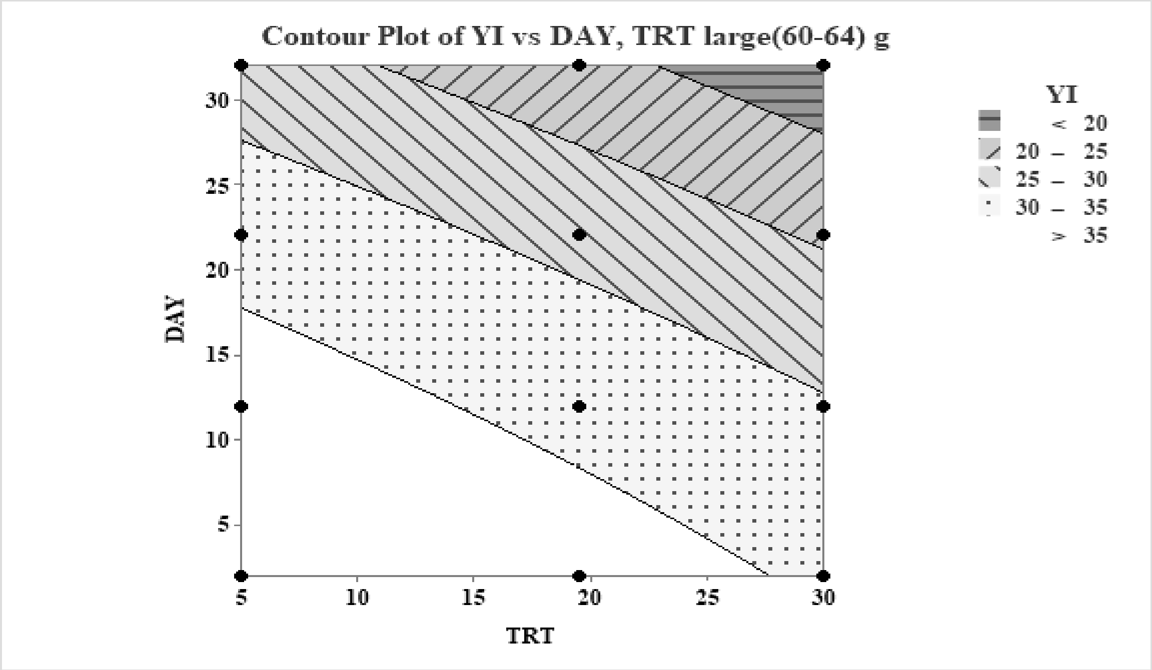

Figure 11: Contour Plot of YI (Large Egg Size)

From the contour plot of the YI of the large egg size against the storage time and temperature, the yolk index decreases towards the direction of the contours (Fig. 11). The darker fill contour has the highest yolk index region $> 0.32.$ The less dark area has the smallest possible yolk index considered for this study $< 20\%.$

4.4 PCA and Response Surface for Extra-Large Egg Size

Table 4.4.1: Eigen Analysis of the Covariance Matrix (Extra-Large)

Eigenvalue	31.65	12.39	1.22	0.82	0.36	0.00	0.00
Proportion	0.988	0.006	0.003	0.002	0.001	0.000	0.000
Cumulative	0.988	0.994	0.997	0.999	1.000	1.000	1.000

The Eigen analysis of the covariance matrix (extra-large) was presented in Table 4.4.1. The cumulative proportion was used to establish the amount of variance explained by the principal components. The Eigenvalues > 1 explained up to $99.7\%.$ However, this study had an interest in main features that explained at least 90% of the original data. Therefore, we only retained the first principle component for response surface analysis in the extra-large egg size.

Table 4.4.2: Eigenvectors

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
ch_EW	-0.752	-0.160	-0.047	-0.426	0.976	0.017	-0.000
SW	0.004	0.101	0.629	0.757	0.144	-0.011	0.005
SD	0.001	0.007	0.004	0.003	0.001	0.050	-0.999
AD	-0.352	-0.458	0.716	-0.513	-0.110	0.003	-0.002
AH	0.040	0.866	0.299	-0.384	0.109	-0.006	0.006
HU	0.996	-0.067	0.021	-0.021	0.040	-0.001	0.000
YI	0.552	0.410	0.007	0.009	-0.014	0.999	0.050

This study identified variables with a high correlation coefficient from the first principle component. These variables were YI, HU, and EWL. They were subjected to further analysis for the response surface model.

4.4.1 Response Surface Model of Ch_EW

Table 4.4.3: Coded Coefficients of EWL (Extra-Large Egg Size)

Term	Coef	SE Coef	95% CI	T-Value	P-Value	VIF
Constant	1.437	0.183	(1.064, 1.810)	7.87	0.000	
DAY	1.148	0.112	(0.919, 1.378)	10.21	0.000	1.00
TRT	0.648	0.102	(0.438, 0.857)	6.32	0.000	1.01
DAY*DAY	0.582	0.188	(0.198, 0.967)	3.09	0.004	1.00
TRT*TRT	-0.241	0.183	(-0.615, 0.133)	-1.32	0.198	1.01
DAY*TRT	0.459	0.137	(0.179, 0.738)	3.35	0.002	1.00

From the above Table 4.4.3, TRT, DAY and their interaction TRT*DAY had a significant effect on the egg weight loss $(P < 0.05)$. TRT*TRT was insignificant $(P > 0.05)$. The temperature had a moderate Coef. of 0.648. Their interaction had the Coef. of 0.459 while the storage period had the highest Coef. 1.148. $VIF = 1/1 - R_i^2 = 1$, indicating that temperature and period of storage were not correlated. Therefore, this study proceeded to decide on which factor is responsible for the ch_EW.

Table 4.4.4: Model Summary of EWL (Extra-Large Egg Size)

R-sq.	R-sq.(adj.)	PRESS	R-sq.(pred.)
85.26%	82.80%	11.2399	78.09%

Table 4.4.4 presents a summary of the response surface model of EWL. R-sq. indicated that this model explained 85.26% variation in egg weight loss. Similarly, PRESS was used to validate this model. Since it was $11 > 3$, the model productivity ability was good. Further, R-sq. (pred.) does not differ so much from R-sq., purporting that this model fits well the sample.

Table 4.4.5 presents the ANOVA of the response surface for the egg weight loss. DAY, TRT, second-order factor DAY*DAY and their interaction TRT*DAY were significant. However, TRT*TRT was insignificant to the response model of EWL. It was also clear that lack-of-fit was negligible, which inferred that this model was fit for optimization. The regression equation is therefore expressed as;

$$ch\_EW = 0.232 - 0.0542\,DAY + 0.0642\,TRT + 0.002588\,DAY * DAY - 0.00154\,TRT * TRT + 0.002447\,DAY * TRT \tag{4.5}$$

Table 4.4.5: ANOVA of the Response Surface of EWL (Extra-Large)

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
Model	5	46.9253	88.74%	46.9253	9.3851	47.27	0.000
Linear	2	41.9071	79.25%	40.1357	20.0679	101.07	0.000
TRT	1	11.6286	21.99%	11.2683	11.2683	56.75	0.000
DAY	1	30.2785	57.26%	28.8675	28.8675	145.39	0.000
Square	2	1.7051	3.22%	1.7051	0.8526	4.29	0.023
TRT*TRT	1	0.1779	0.34%	0.1779	0.1779	0.90	0.351
DAY*DAY	1	1.5273	2.89%	1.5273	1.5273	7.69	0.009
2-Way Interaction	1	3.3131	6.27%	3.3131	3.3131	16.69	0.000
TRT*DAY	1	3.3131	6.27%	3.3131	3.3131	16.69	0.000
Error	30	5.9567	11.26%	5.9567	0.1986		
Lack-of-Fit	6	0.6780	1.28%	0.6780	0.1130	0.51	0.792
Pure Error	24	5.2787	9.98%	5.2787	0.2199		
Total	35	52.8820	100.00%				

Figure 12: Normal Plot of the Standardized Effects for EWL in Extra-Large Egg Size

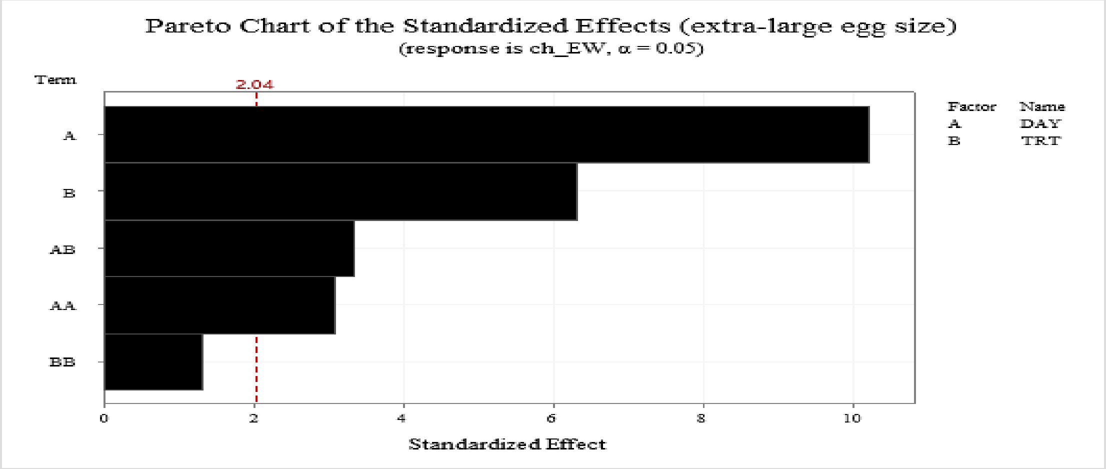

Figure 13: Pareto Chart of the Standardized Effects for EWL in Extra-Large Size

From the usual plot of the standardized effects (Fig. 12), DAY(A), TRT(B), AA and AB were statistically significant with BB in exclusion. e Pareto chart of the student zed effects shows the magnitude of the factors' effects (Fig. 13). Elements were necessary for standardized results beyond the dotted line at 2.04 (A, B, AB, AA). Only (BB) remains at the left-hand side of the dotted line, hence, insignificant.

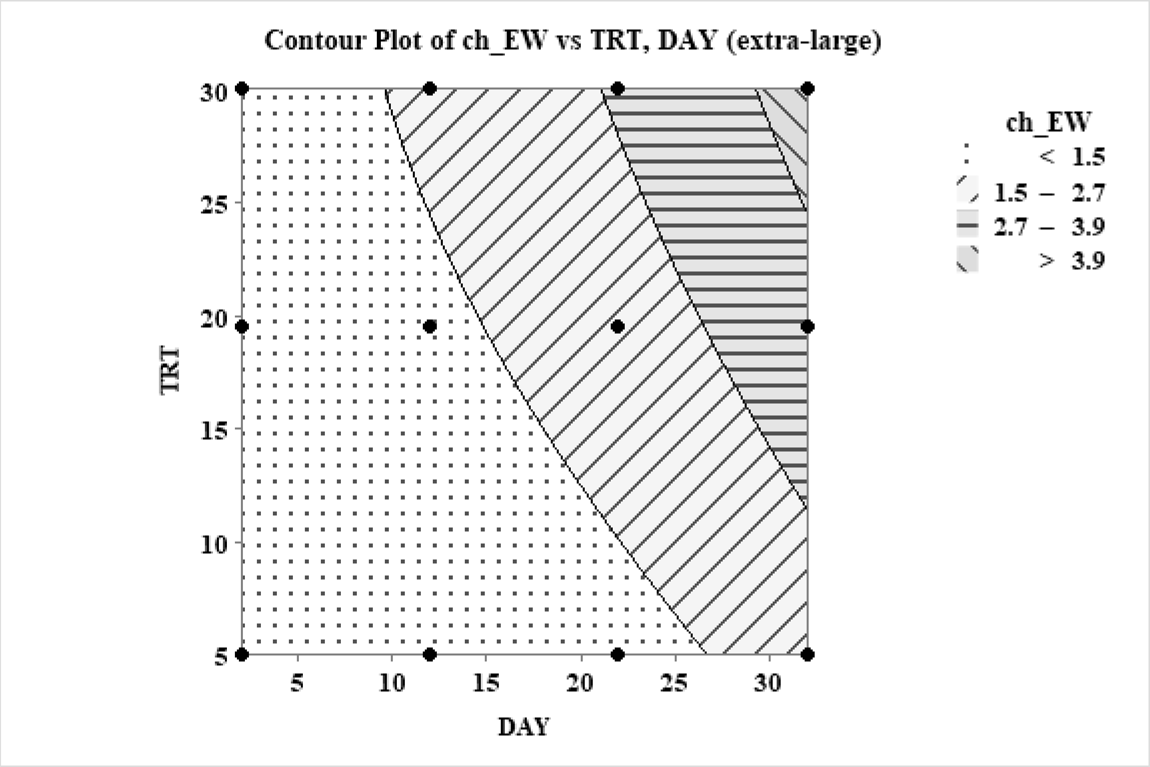

Figure 14: Contour Plot of EWL vs Storage Conditions (Extra-Large Egg Size)

From Fig. 14, EWL increases towards the direction of the contours. The darker fill contour has the highest weight loss region > 4. The less dark part has the most negligible possible weight loss under the given storage conditions < 1. The optimal level for minimum loss is 20 days of storage at 8.5 ^{o}C for 14 days at 13 ^{o}C and 7 days at 30 ^{o}C.

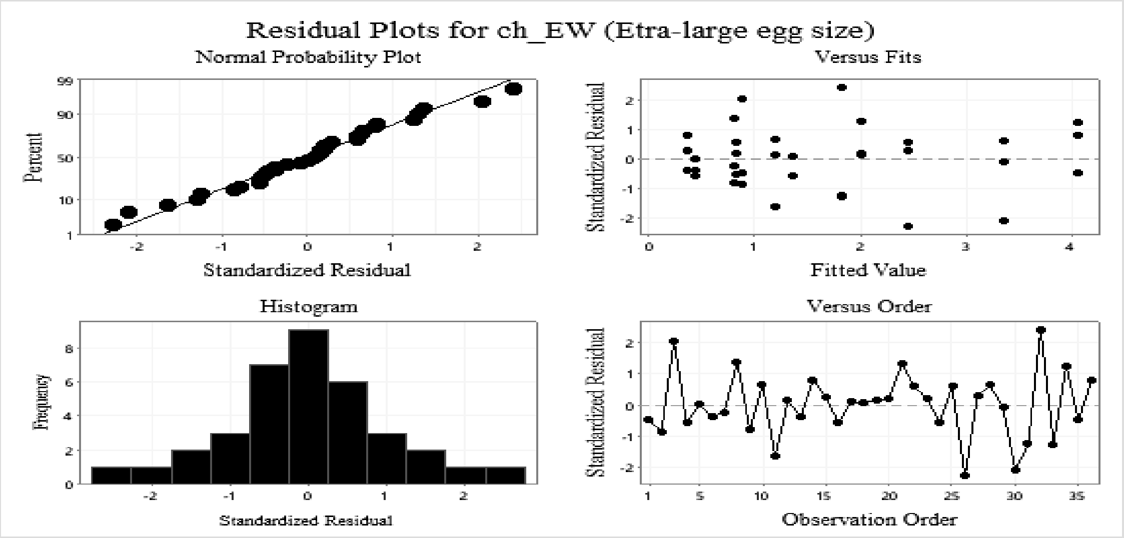

Figure 15: Residual Plots for the Change of Egg Weight

Fig. 15 presents residual plots of EWL in the extra-large egg size. Even though the histogram of the egg weight loss might not be accurate for the normality of this response surface model, the standard probability plot verified the normality assumption. Residuals versus order plots were used to verify the hypothesis that residuals were independent of each other. Further, scatter plots displayed no trend or pattern in time order. Thus, they were autonomous.

4.4.2 Response Surface Model of Haugh Unit (Extra-Large)

Table 4.4.6: Model Summary of HU (Extra-Large)

R-sq.	R-sq.(adj.)	PRESS	R-sq.(pred.)
94.85%	93.99%	10.88	92.39%

Table 4.4.6 presents a summary of the response surface model of the Haugh Unit for the extra-large egg size. R-sq. indicated that this model explained 94% variation in HU. PRESS was used to validate this model. Since it was $10 > 3$, the predictive model ability was good. Further, R-sq. (pred.) $= 92\%$ does not differ so much from R-sq. (adj.) $= 93\%$, meaning that this model fits well the sample.

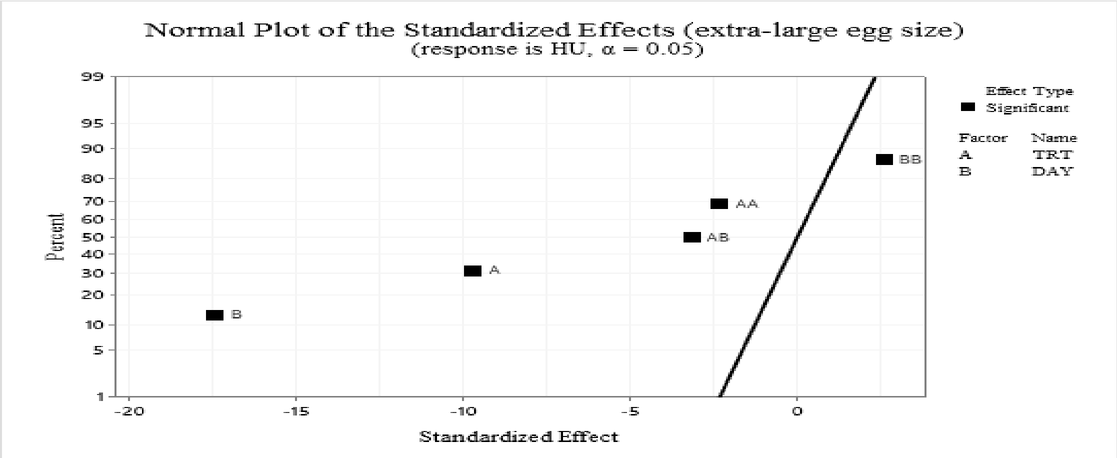

Figure 16: Normal Plot of the Standardized Effects of HU (Extra-Large)

Fig. 16 shows the magnitude of the standardized effects in the HU response surface model for the extra-large egg size category. From the plot, the direction of significance was seen from the origin of the plot. Factors approaching the distribution line had minimal effect. Storage duration represented by B had the most decisive significant influence in this study. Factor BB goes beyond the distribution line with a minimal impact on the HU; however, it was noteworthy at 95% confidence interval.

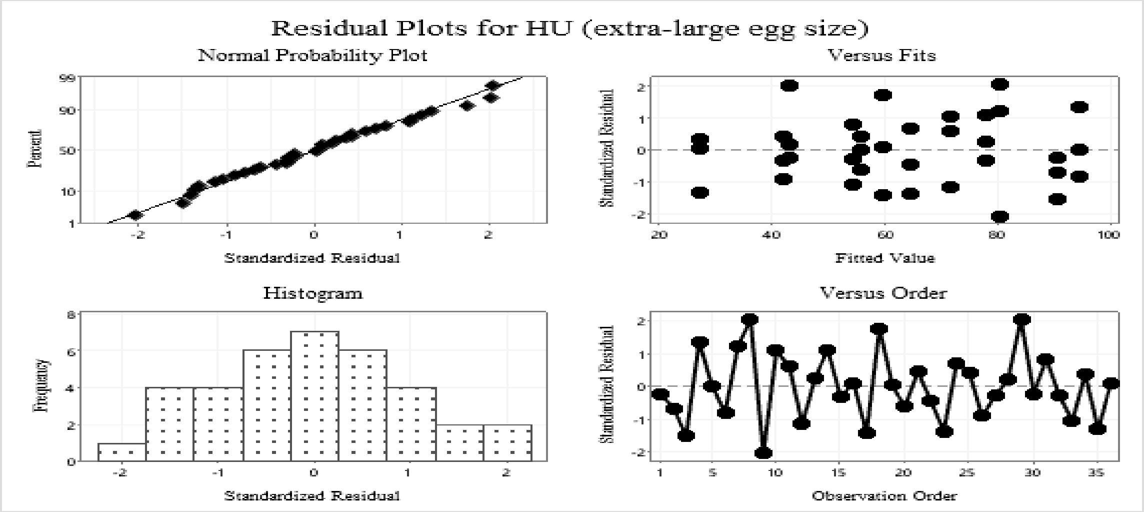

Figure 17: Residual Plots of HU (Extra-Large)

Residual plots of the HU were shown in Fig. 17. The histogram shows that the data points of HU were typically approaching distribution. The standard probability plot with the line of fit verified the linearity assumption. Residuals versus order plots were used to verify the belief that residuals were independent of each other. The dotted properties displayed no trend or pattern in time order. Thus, they were individualistic.

Table 4.4.7: ANOVA of HU (Extra-Large)

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
Model	5	13564.6	94.85%	13564.6	2712.9	110.44	0.000
Linear	2	13112.6	91.69%	12966.4	6483.2	263.92	0.000
TRT	1	2540.3	17.76%	2627.2	2627.2	106.95	0.000
DAY	1	10572.2	73.92%	10339.2	10339.2	420.89	0.000
Square	2	250.0	1.75%	250.0	125.0	5.09	0.013
TRT*TRT	1	134.7	0.94%	134.7	134.7	5.48	0.026
DAY*DAY	1	115.3	0.81%	115.3	115.3	4.69	0.038
2-Way Interaction	1	202.0	1.41%	202.0	202.0	8.22	0.007
TRY*DAY	1	202.0	1.41%	202.0	202.0	8.22	0.007
Error	30	737.0	5.15%	737.0	24.6		
Lack-of-Fit	6	107.2	0.75%	107.2	17.9	0.68	0.667
Pure Error	24	629.8	4.40%	629.8	26.2		
Total	35	14301.5	100.00%				

Table 4.4.7 presents the ANOVA of the response surface for the HU (large egg size). DAY, TRT, second-order factor DAY*DAY, TRT*TRT and their interaction TRT*DAY were significant at 95% confidence interval $P < 0.05.$ Lack-of-Fit was highly insignificant $P = 0.667$ suggesting that this model was fit for optimization. The regression equation is therefore expressed as;

$$HU_{extr} \quad = \quad 95.92 + 0.462\,TRT - 1.766\,DAY - 0.0271\,TRT*TRT + 0.01789\,DAY*DAY - 0.02067\,TRT*DAY \tag{4.6}$$

Fig. 18 presents the contour plot of the response surface model of HU against DAY and TRT. The direction of the contour plots indicates a reduction in HU from low to high levels of storage conditions. HU was above 90 when eggs we stored for less than five days at 5 ^{o}C and room. Past 12th day of storage, HU reduced from 70 to 50 at room temperature. The eggs stored at room temperature for 12 days less had an HU unit above 70. However, those stored at room temperature for 25 days had HU between less than 50. The eggs stored at 5 ^{o}C had an HU of 50 and above for the whole study duration.

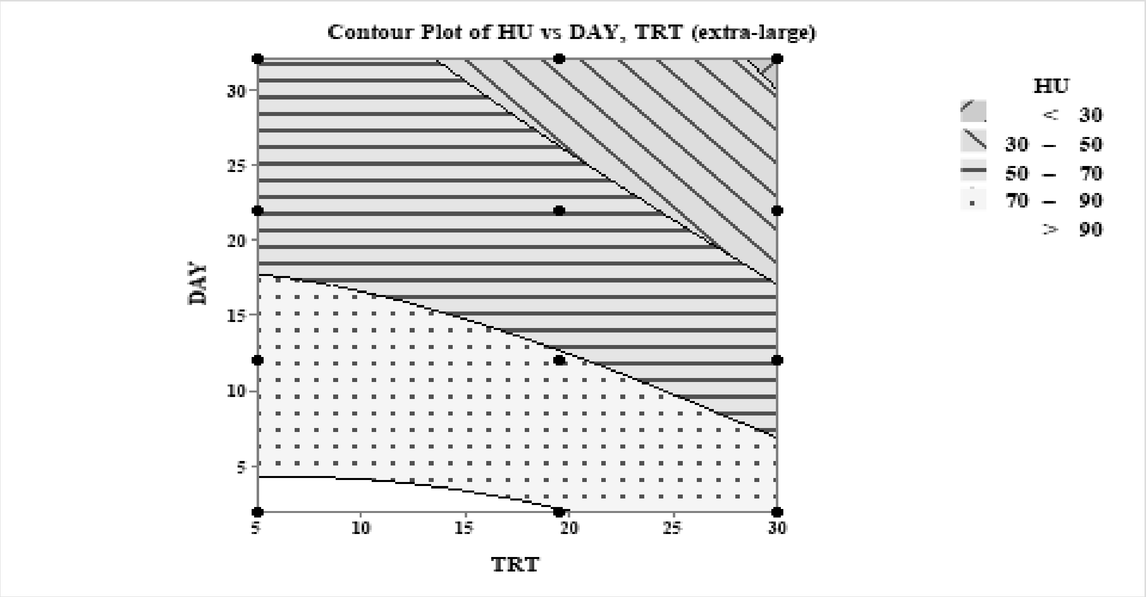

Figure 18: Contour Plot of HU (Extra-Large)

4.4.3 Response Surface Model of YI (Extra-Large)

Table 4.4.8: Model Summary of YI (extra-large)

R-sq.	R-sq. (adj.)	PRESS	R-sq. (pred.)
95.78%	95.07%	9.490	93.73%

Table 4.4.8 presents a summary of the response surface model of the yolk index for the extra-large egg size. R-sq. indicated that this model explained 95% variation in YI. PRESS was used to validate this model. Since it was $9 > 3$, the predictive model ability was good. Further, R-sq. (pred.) $= 93\%$ does not differ much from R-sq. (adj.)$= 94\%$; accordingly, this model fits the sample well.

Table 4.4.9: ANOVA of YI (Extra-Large)

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
Model	5	1444.78	95.78%	1444.78	288.96	136.08	0.000
Linear	2	1406.99	93.27%	1391.08	695.54	327.55	0.000
TRT	1	280.31	18.58%	287.26	287.26	135.28	0.000
DAY	1	1126.69	74.69%	1103.82	1103.82	519.82	0.000
Square	2	20.28	1.34%	20.28	10.14	4.78	0.016
TRT*TRT	1	9.10	0.60%	9.10	9.10	4.28	0.047
DAY*DAY	1	11.18	0.74%	11.18	11.18	5.27	0.029
2-Way Interaction	1	17.51	1.16%	17.51	17.51	8.25	0.007
TRT*DAY	1	17.51	1.16%	17.51	17.51	8.25	0.007
Error	30	63.70	4.22%	63.70	2.12		
Lack-of-Fit	6	8.59	0.57%	8.59	1.43	0.62	0.710
Pure Error	24	55.12	3.65%	55.12	2.30		
Total	35	1508.49	100.00%				

Table 4.4.9 presents the ANOVA of the response surface for the YI (extra-large egg size). DAY, TRT, second-order factor TRT*TRT, DAY*DAY and their interaction TRT*DAY were significant at 95% confidence interval $P < 0.05.$ Lack-of-Fit was highly insignificant $P = 0.710$ showing that this model was fit for optimization. The regression equation is therefore expressed as;

$$YI_{extra-large} = 39.00 + 0.073TRT - 0.2003DAY - 0.00703TRT*TRT - 0.00557DAY*DAY - 0.00609TRT*DAY \tag{4.7}$$

Fig. 19 presents the contour plot of the response surface model of percentage YI against DAY and TRT. The direction of the contour plots indicates a reduction in YI from low to high levels of storage conditions. The medium value of the YI was 30%. Yolk index above 30% was considered suitable for this study. Further, 20 days of storage duration at room temperature demonstrated a medium value of YI. However, eggs stored at 5 ^{o}C expressed 30 (YI) and above for 25 days of storage duration.

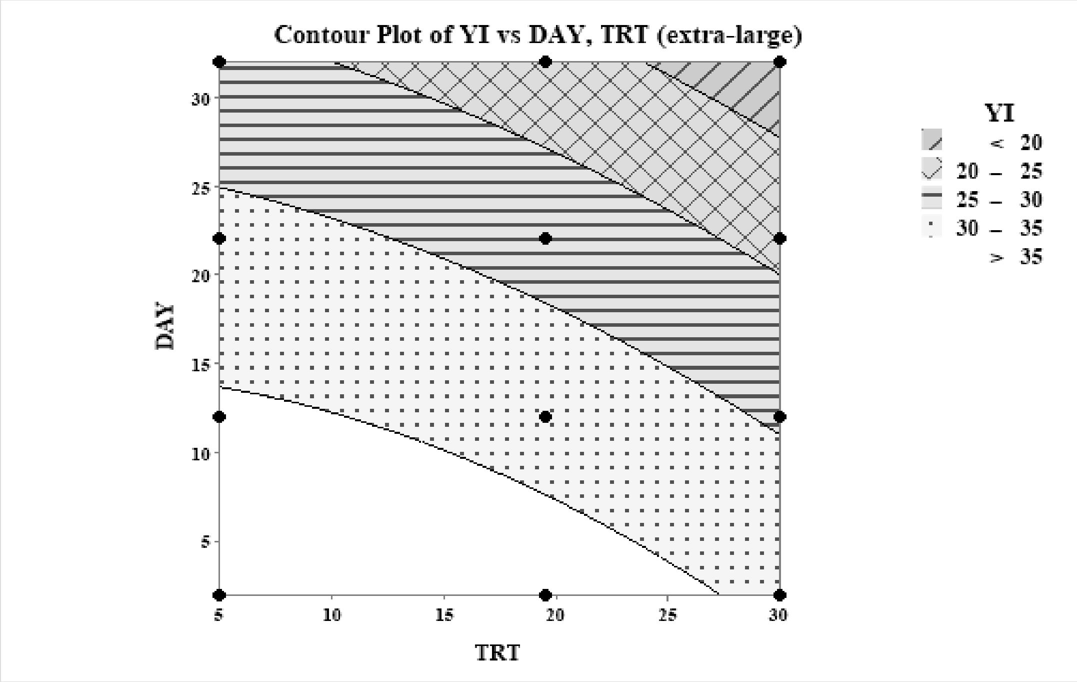

Figure 19: Contour Plot of YI (Extra-Large)

4.5 PCA and Response Surface Model for (Jumbo Egg Size)

Table 4.5.1: Eigen analysis of the covariance matrix (jumbo)

Eigenvalue	35.90	21.38	8.97	1.20	0.54	0.07	0.00
Proportion	0.815	0.163	0.018	0.002	0.001	0.000	0.000
Cumulative	0.815	0.978	0.996	0.999	1.000	1.000	1.000

The Eigen analysis of the covariance matrix (jumbo) was presented in Table 4.5.1. This study used cumulative proportion to determine the amount of variance explained by the principal components. The Eigenvalues >1 explained up to 99.9% from the first to the fourth PC. Nevertheless, this study had an interest in principal components that explained at least 90%. Therefore, only the first and second PCs were reserved for response surface analysis in the jumbo egg size, as shown from Table 4.5.1. The total variance explained by the two principal components is 97.8% of the original variables.

This study identified variables with strong positive or negative correlation coefficients from the first and second PCs. These variables were YI, HU and EWL. They were subjected to further analysis for the response surface model (Table 4.5.2).

Table 4.5.2: Eigenvectors (Jumbo)

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
AH	0.346	0.490	0.109	-0.054	-0.406	-0.585	0.014
SD	-0.004	0.405	0.110	0.123	0.380	0.576	-0.013
ch_EW	-0.151	0.696	-0.028	0.211	0.791	-0.571	-0.013
YI	0.662	0.001	0.001	-0.006	-0.021	-0.008	-1.000
HU	-0.991	-0.046	0.089	0.076	0.047	-0.001	0.000
SW	0.103	0.156	-0.976	-0.112	0.010	0.013	-0.000
AD	-0.052	-0.031	-0.123	0.959	-0.249	0.020	-0.001

4.5.1 Response Surface Model of YI

Table 4.5.3: Model summary of YI

R-sq.	R-sq.(adj.)	PRESS	R-sq.(pred.)
78.42%	72.32%	17.561	69.72%

Table 4.5.3 summarises the response surface model of the percentage YI. R-sq. indicated that this model explained 78% variation in the YI. PRESS was used to validate the YI model. Since it was 17 > 3, the predictive model ability was good. Further, R-sq.(pred.) = 69% does not differ much from R-sq.(adj.) = 72%; consequently, this model fits the sample well.

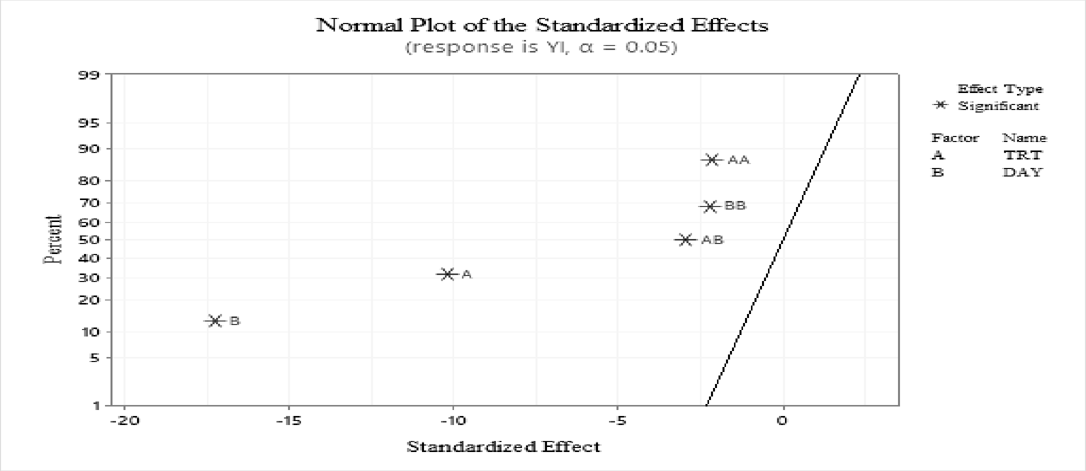

Figure 20: Normal Plot of the Standardized Effects of YI (Jumbo)

Fig. 20 was used to show the direction of significant effects of the second-order model. From the plot, the order of significance was seen from zero towards the left. Factors approaching the distribution line had minimal impact. Storage duration represented by B had the highest significant effect in this study. Factor AB goes beyond the distribution line with a minimal impact on the YI. However, it was necessary at 95% confidence interval. The effect of AA and that of BB were almost the same.

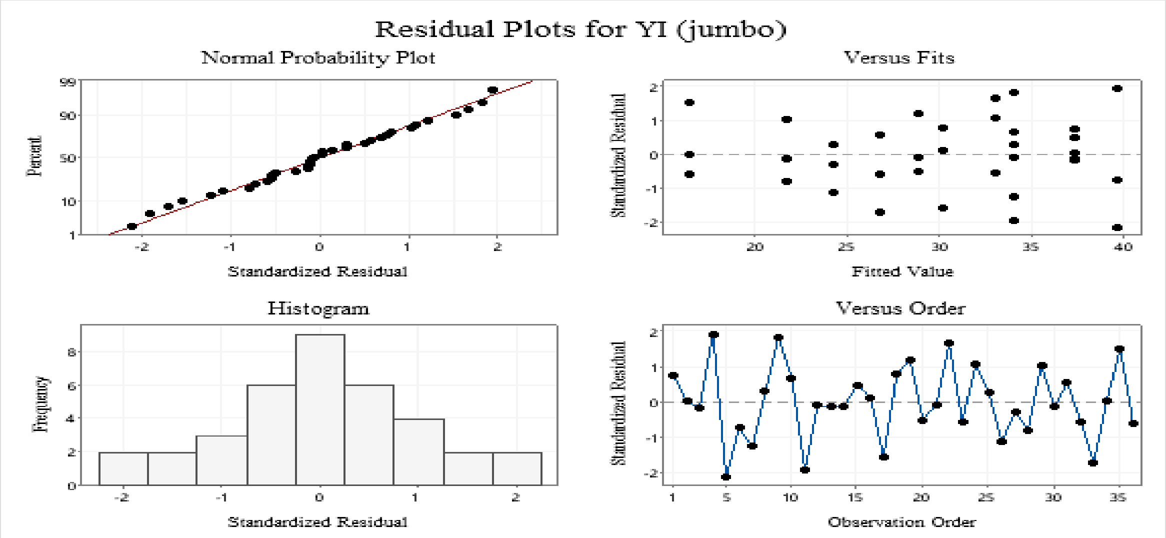

Figure 21: Residual Plots of YI for the Jumbo Egg Size

Residual plots of the YI were shown in Fig. 21. The histogram showed that the data points of YI were not skewed to either left or right. It was clear from the standard probability plot with the line of fit that the verified the linearity assumption. Residuals versus order plots were used to verify the homogeneity of variance assumption. From the residuals versus observation, the dots displayed no systematic effect in time order.

Table 4.5.4: ANOVA of YI (Jumbo)

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
Model	5	1595.68	93.42%	1595.68	319.14	85.12	0.000
Linear	2	1528.66	89.49%	1510.77	755.39	201.47	0.000
TRT	1	380.65	22.28%	392.30	392.30	104.63	0.000
DAY	1	1148.01	67.21%	1118.47	1118.47	298.32	0.000
Square	2	35.01	2.05%	35.01	17.51	4.67	0.017
TRT*TRT	1	16.98	0.99%	16.98	16.98	4.53	0.042
DAY*DAY	1	18.03	1.06%	18.03	18.03	4.81	0.036
2-Way Interaction	1	32.02	1.87%	32.02	32.02	8.54	0.007
TRT*DAY	1	32.02	1.87%	32.02	32.02	8.54	0.007
Error	30	112.48	6.58%	112.48	3.75		
Lack-of-Fit	6	8.30	0.49%	8.30	1.38	0.32	0.921
Pure Error	24	104.18	6.10%	104.18	4.34		
Total	35	1708.16	100.00%				

Table 4.5.4 presents the ANOVA of the response surface for the YI (jumbo egg size). DAY, TRT, second-order factor TRT*TRT, DAY*DAY and their interaction TRT*DAY were significant at 95% confidence interval $P < 0.05.$ Lack-of-Fit was highly insignificant $P = 0.921$ indicating the model was fit for optimization. The regression equation is therefore expressed as;

$$YI_{jumbo} = 39.02 + 0.153TRT - 0.115DAY - 0.00961TRT*TRT - 0.00708DAY*DAY - 0.00823TRT*DAY \tag{4.8}$$

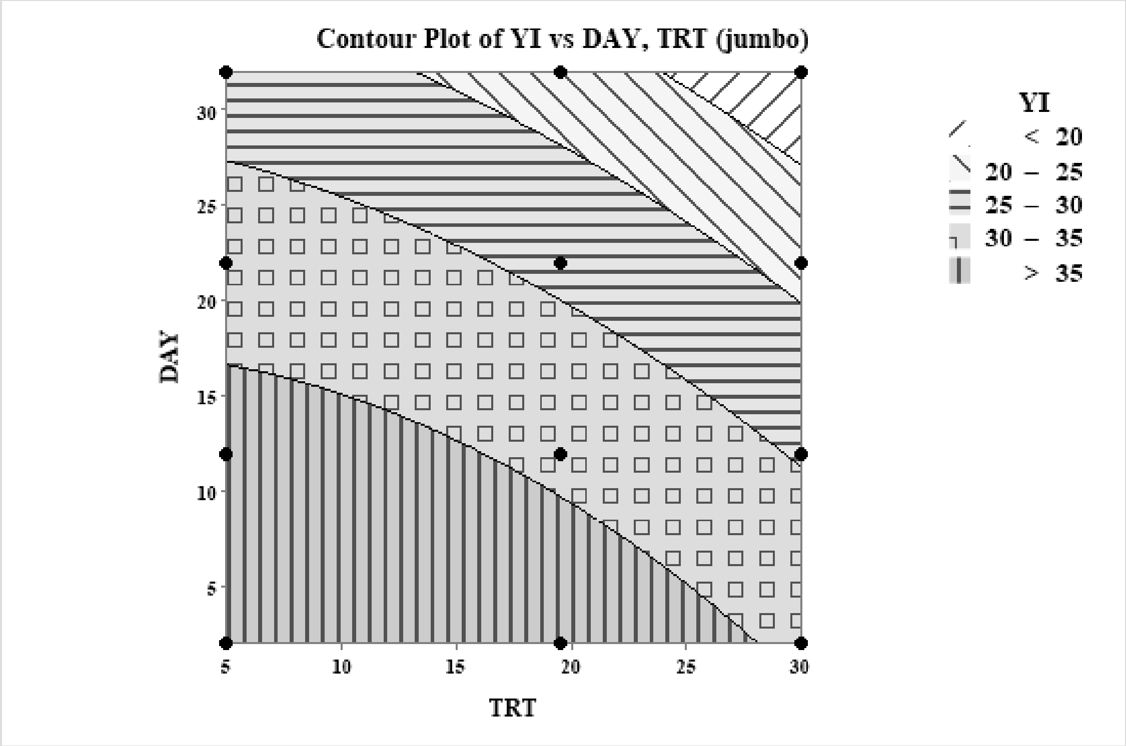

Figure 22: Contour Plots of YI (Jumbo)

Fig. 22 presents the contour plot of the response surface model of percentage YI against DAY and TRT. The direction of the contour plots indicates a reduction in YI from low to high levels of storage conditions. The medium value of the YI was 25% to 30%. However, eggs stored at 5 ^oC expressed 30% and above for 27 days of storage duration.

4.5.2 Response Surface Model of Ch_EW (Jumbo)

Table 4.5.5: Model summary of egg weight loss

R-sq.	R-sq. (adj.)	PRESS	R-sq. (pred.)
90.73%	89.19%	7.31163	86.24%

Table 4.5.5 presents a summary of the response surface model of EWL. R-sq. indicated that this model explained 90% variation in egg weight loss. PRESS validated ch_EW model. Since it was 7 > 3, the model productivity ability was considerable. Further, R-sq. (pred.) does not differ so much from R-sq., which implies that this model fits the sample well.

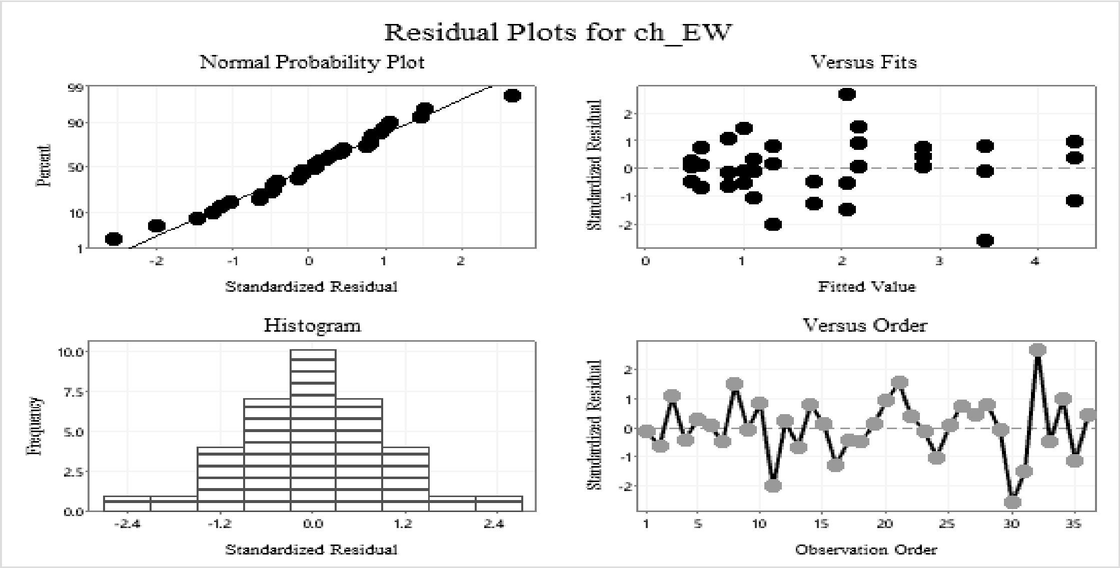

Figure 23: Residual Plot of Egg Weight Loss (Jumbo)

Residual plots of the weight loss are shown in Fig. 23. The histogram shows the normal distribution of data points of weight loss distributed. It was clear from the standard probability plot displays verification of the normality assumption. Residuals versus order plots were used to verify the belief that residuals were independent of each other. There was no trend or pattern. Linearity assumption was also satisfied.

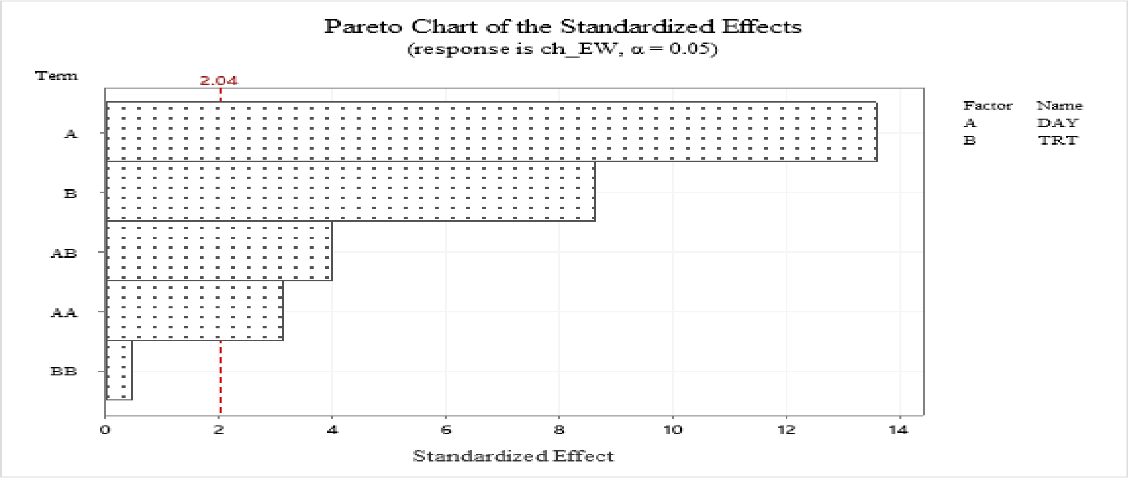

Figure 24: Pareto Chart of the Standardized Effects of EWL (Jumbo)

The Pareto chart of the student zed effects (Fig. 24) clearly illustrates the magnitude of the second-order factors. Factors significant for standardized results were beyond the dotted line at 2.04 (A, B, AB, AA). Only (BB) remains at the left-hand side of the dotted line; hence, insignificant

Table 4.5.6: ANOVA of EWL (Jumbo)

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
Model	5	48.2098	90.73%	48.2098	9.6420	58.73	0.000
Linear	2	43.9923	82.79%	42.4744	21.2372	129.36	0.000
DAY	1	31.5993	59.47%	30.3049	30.3049	184.59	0.000
TRT	1	12.3930	23.32%	12.1695	12.1695	74.13	0.000
Square	2	1.6258	3.06%	1.6258	0.8129	4.95	0.014
DAY*DAY	1	1.5918	3.00%	1.5918	1.5918	9.70	0.004
TRT*TRT	1	0.0340	0.06%	0.0340	0.0340	0.21	0.652
2-Way Interaction	1	2.5916	4.88%	2.5916	2.5916	15.79	0.000
DAY*TRT	1	2.5916	4.88%	2.5916	2.5916	15.79	0.000
Error	30	4.9252	9.27%	4.9252	0.1642		
Lack-of-Fit	6	0.9168	1.73%	0.9168	0.1528	0.91	0.501
Pure Error	24	4.0084	7.54%	4.0084	0.1670		
Total	35	53.1350	100.00%				

Table 4.5.6 displays the ANOVA of the response surface for the change in the egg weight (jumbo egg size). DAY, TRT, second-order factor TRT*TRT and their interaction TRT*DAY were significant at 95% confidence interval $P < 0.05.$ DAY*DAY was insignificant $P > 0.05.$ Lack-of-Fit was highly insignificant $P = 0.501,$ implying that this model was fit for optimization. The regression equation is therefore expressed as;

$$ch\_EW_{jumbo} = 0.341 - 0.0302\,DAY + 0.0322\,TRT + 0.002103\,DAY * DAY - 0.000430\,TRT * TRT + 0.002341\,DAY * TRT \tag{4.9}$$

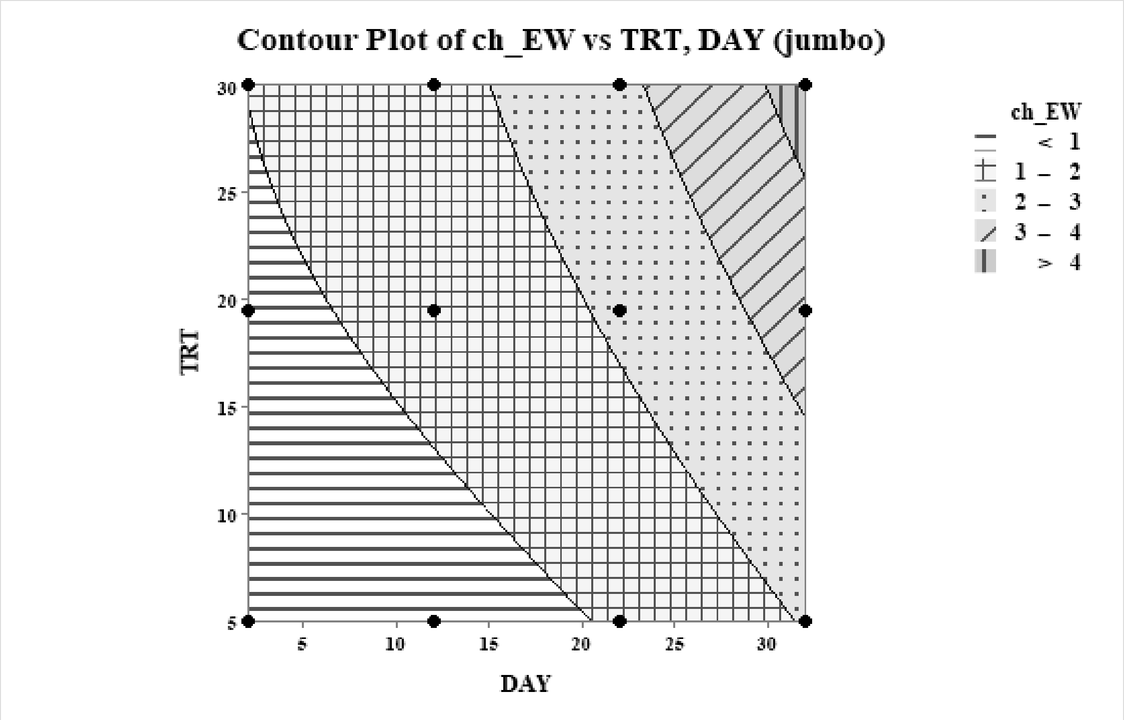

Figure 25: Contour Plot of EWL (Jumbo)

Fig. 25 illustrates the contour plot of the response surface model (EWL) corresponding to DAY and TRT. The direction of the contour plots indicates a reduction in EWL from low to high levels of storage conditions. Darker regions indicate higher weight loss. From this study, 2 *g* was observed as moderate weight loss. EWL was less than 2 *g* from the plot when stored for the 32 days at five 5 ^oC.

4.5.3 Response Surface Model of HU (Jumbo)

Table 4.5.7: Regression Estimates for the Second-Order Model (HU)

Term	Est.	Std. error	95% CI	T-Value	P-Value	VIF
Constant	65.03	1.71	(61.53, 68.53)	37.94	0.000	
TRT	-9.963	0.962	(-11.927, -7.998)	-10.36	0.000	1.01
DAY	-22.41	1.06	(-24.57, -20.26)	-21.23	0.000	1.00
TRT*TRT	-4.75	1.72	(-8.25, -1.24)	-2.76	0.010	1.01
DAY*DAY	4.09	1.77	(0.48, 7.70)	2.31	0.028	1.00
TRT*DAY	-4.26	1.28	(-6.88, -1.64)	-3.32	0.002	1.00

From Table 4.5.7, TRT and DAY had a significant effect on the HU $(P < 0.05)$. TRT*TRT, DAY*DAY and their interaction TRT*DAY was insignificant $(P > 0.05)$. DAY, TRT, TRT*TRT and TRT*DAY negative coef. of $-22.41, -9.963, -4.75$ and -4.26 respectively. DAY*DAY had a positive coefficient and T-Value of 4.09. TRT had the lowest standard error of the coefficient of 0.962. $VIF = 1$ and 1.01. This indicated that temperature and period of storage were not correlated. Therefore, this study proceeded to decide on which level factors are responsible for the change in HU.

Table 4.5.8: Model Summary of HU (Jumbo)

R-sq.	R-sq.(adj.)	PRESS	R-sq.(pred.)
95.15%	94.34%	9.477	92.88%

R-sq. indicated that this model explained 95% variation in HU. PRESS was used to validate the HU model. Since it was 9 > 3, the predictive model ability was good. Further, R-sq. (pred.) = 92% does not differ so much from R-sq. (adj.) = 94%, implying that this model fits the sample well. The model adequacy was verified and confirmed to be good (Table 4.5.8). DAY, TRT, second-order factor TRT*TRT, DAY*DAY and their interaction TRT*DAY were significant at 95% confidence interval. Lack-of-Fit was highly insignificant, implied that this model was fit for optimization (Table 4.5.9). The regression equation is therefore expressed as;

$$HU = 93.57 + 0.652TRT - 1.714DAY - 0.0304TRT*TRT + 0.01817DAY*DAY - 0.02272TRT*DAY \tag{4.10}$$

Table 4.5.9: ANOVA of HU (Jumbo)

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
Model	5	13071.3	95.15%	13071.3	2614.3	117.77	0.000
Linear	2	12538.7	91.28%	12387.8	6193.9	279.03	0.000
TRT	1	2285.9	16.64%	2382.1	2382.1	107.31	0.000
DAY	1	10252.8	74.64%	10005.7	10005.7	450.75	0.000
Square	2	288.5	2.10%	288.5	144.2	6.50	0.005
TRT*TRT	1	169.6	1.23%	169.6	169.6	7.64	0.010
DAY*DAY	1	118.9	0.87%	118.9	118.9	5.36	0.028
2-Way Interaction	1	244.1	1.78%	244.1	244.1	11.00	0.002
TRY*DAY	1	244.1	1.78%	244.1	244.1	11.00	0.002
Error	30	665.9	4.85%	665.9	22.2		
Lack-of-Fit	6	66.8	0.49%	66.8	11.1	0.45	0.841
Pure Error	24	599.1	4.36%	599.1	25.0		
Total	35	13737.2	100.00%				

The direction of the contour plots shows a reduction in HU from low to high levels of storage conditions. Darker regions specify higher HU. The study observed a medium HU of 60 (Fig. 26).

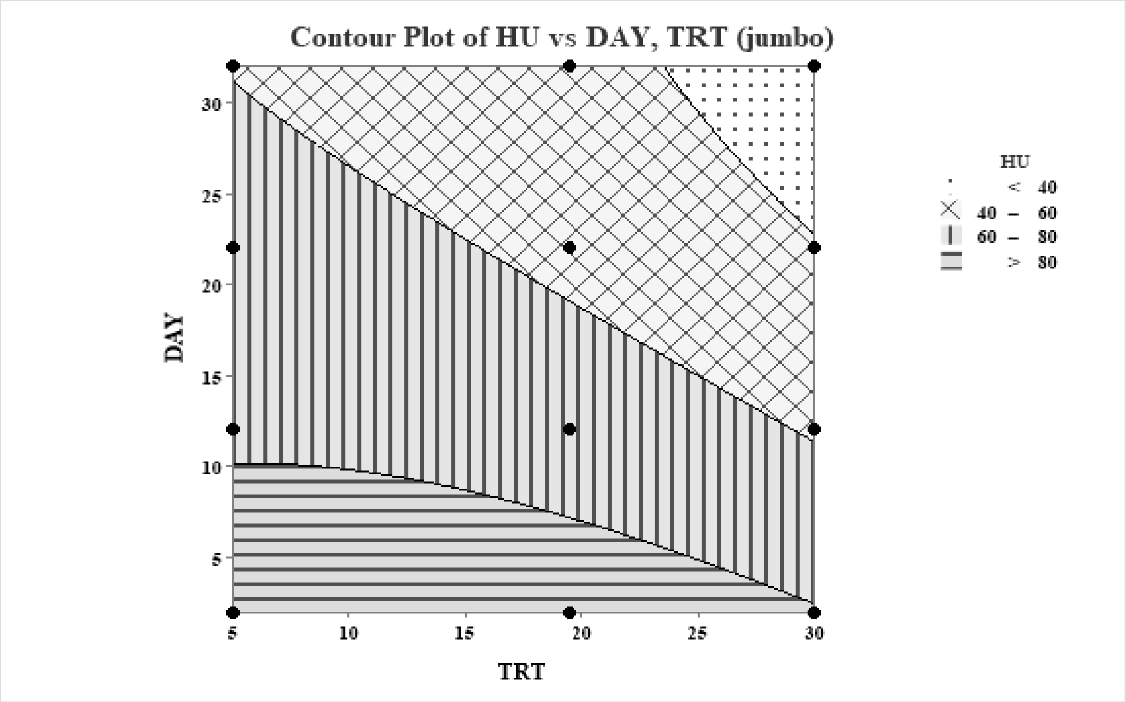

Figure 26: Contour Plot of HU (Jumbo)

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.0 Introduction

This chapter discusses results in comparison with the available literature. A conclusion has also been made based on the findings and recommendations given afterwards.

5.1 Discussion

5.1.1 Effect of Storage Conditions on Egg Quality Parameters Using Fixed and Mixed Effect Models

The interaction effect of egg weight loss indicated higher losses on the 32nd day of storage in 5 ^{o}C, 19.5 ^{o}C and 30 ^{o}C for all the egg sizes. These findings are in agreement with Fasenko *et al.* (2001); Hassan *et al.* (2005); Reijrink *et al.* (2010); Alsobayel & Albadry (2011); Akter *et al*. (2014) and Yimenu *et al*. (2017), who attested that total egg weight reduces with prolonged storage duration. The temperatures under storage influence a reduction in egg weight. Consequently, eggs subjected to low-level temperature 5 ^{o}C; exhibited an expressively lesser reduction in egg content than room temperature. Thus the loss of water and other gaseous components was diminutive on eggs stored under low-level temperature 5 ^{o}C compared to those under 19.5 ^{o}C. These outcomes support Samli *et al*. (2005) and Hasan & Alylin (2014), who observed a decline in the egg's weight at 29 ^{o}C with the 10th day of storage.

The SW model was significant for the large egg size (60-64) *g* $P = 0475$ and extra-large (65-69) *g* $P = 0.0382.$ The finding on significant levels was in line with Samli *et al*. (2005), who encountered a considerable effect of storage duration and storage temperature on eggshell weight. Conversely, this model was not significant for jumbo (70 and more) *g* $P > 0.05.$ The insignificant impact of the storage for this study was also in line with Akter *et al*. (2014), who reported that there is no significant relationship between shell weight, temperature and time of storage. The study further detected an increase in reduction of SD as the egg size increases in the mixed-effect model. The effect was significant $P < 0.05.$

The finding in the mixed effect model contradicts Saleh *et al*. (2020) and Akter *et al.* (2014), who found an insignificant effect on SD. Further, this study observed a significant interaction effect in the fixed-effect model.

This study noted that AD increases with an increase in egg size. This finding was in agreement with Altuntaş & Şekeroğlu (2008), who argues that the egg internal components increases with an increase in egg size. It increased albumen diameter from the large (60-64) *g*, extra-large (65-69) *g* and jumbo (70 and more) *g* egg sizes. HU decreased with an increase in egg size. This finding contradicts Emsley *et al*. (1977), who established that HU increases with an increase in egg size. Nevertheless, Kinney *et al*. (1970), Van Tijen & Kuit (1970) and Iposu *et al*. (1994) findings conformed with the current study. The HU declined from high (5 ^oC) to low (30 ^oC) in large (60-64) *g*, extra-large (65-69) *g* and jumbo (70 and more) *g* sizes. Eggs with more considerable weight recorded lower HU in both fixed and mixed-effect models. Storage duration was highly significant for the three egg sizes $P < 0.05.$ YI was affected by the storage duration. This finding was in line with Yimenu *et al*. (2011), who realized a decrease in YI as storage duration increases.

5.1.2 Evaluation of Models' Efficiencies

From the results in section 4.2, it was clear that the fixed-effect model exhibited a minor variance component in yolk index, egg weight loss, Haugh unit, albumen diameter and albumen height. This overlapped instance where the fixed effect model was significantly the same as the mixed-effect model. Therefore, this study proposes that treating blocks as a static effect in the RCBD experiment is appropriate. This finding was in line with a survey carried out by Dixon (2016), who suggested that the blocking effect in RCBD should be treated as a fixed effect. Conversely, our study disputes recommendations by LeMay & Robinson (2004); Festing (2010), who stressed that RCBD experiments should be analysed as a mixed effect model.

5.1.3 Response Surface Modelling

The PCA used suggested the first and the second principal components, which explained a cumulative variance of 90%. The variables from the main features were YI, HU, egg weight loss and shell weight with a correlation coefficient of $|0.5|$ and above. The findings on PCA for the three egg sizes were in line with Feddern *et al*. (2017), who reported that HU was among the factors highly associated with better egg quality. This study observed that two PCs are sufficient in explaining the interdependency in the original eggs traits for the jumbo and large egg sizes. Such finding contradicts a study by Ukwu *et al*. (2017), who claimed that three PCs explains over 90% cumulative frequency of the original egg quality components. The G-optimality used for prediction was 68% efficient with replicated design points. This G-eff was almost similar to Moein & Pan (2016), who obtained 67% G-efficiency for predictive variance with two factors.

The prediction efficiency for this study was higher than Montgomery *et al*. (2002), who achieved a G-efficiency of 28.8%. However, a study by Atitwa *et al*. (2016) performed a G-efficiency of 89% which was higher than this study. This study predicts that HU can be above 90 when eggs are stored for less than 5 days at 5 ^{o}C and 19.5 ^{o}C. Past 12*th* day of storage, HU reduces from 70 to 50 at 19.5 ^{o}C. This finding was in line with Samli *et al*. (2005), who realized that the HU unit decreases from approximately 90 to 70 at 5 ^{o}C. The eggs stored at room temperature for 12 days less had an HU unit above 70. However, those stored at room temperature for 25 days had HU between less than 50. The eggs stored at 5 ^{o}C had an HU of 50 and above for the whole study duration.

5.2 Conclusions

This study established that the determinants of egg quality, physical components of the egg, were significantly affected at 5 ^{o}C, 19.5 ^{o}C and 30 ^{o}C. The effect was more adverse on eggs stored at 30 ^{o}C within 32 days of storage. Results on model efficiency showed that the fixed effect model was the most suitable for RCBD experiments. Given that most egg retailers use room temperature in the study area, for the sake of cost-effectiveness, eggs should be stored at 19.5 ^{o}C for 14 days and at 30 ^{o}C for seven days maximal. The eggs can be stored at room temperature for ten days with very minimal weight loss. However, keeping an egg at a lower temperature of 5 ^{o}C maintains its weight for 22 days or more. For the extra-large egg size, the optimal level for minimum loss is 20 days of storage 8.5 ^{o}C, 14 days at 13 ^{o}C and seven days at 30 ^{o}C. From this study, 2 *g* was observed as moderate weight loss. EWL was less than 2 *g* for the 32 days at 5 ^{o}C. However, the storage period was 22 days for eggs kept at room temperature. Storage duration contributed a significant percentage effect on the HU (68%).

For extra-large, HU was above 90 for less than 5 days at 5 ^{o}C and 19.5 ^{o}C. Past 12th day of storage, HU reduced from 70 to 50 at 19.5 ^{o}C (Fig. 18). The eggs stored at room temperature for 12 days less had an HU unit above 70. However, those stored at 19.5 ^{o}C for 25 days had HU between less than 50. The eggs stored at 5 ^{o}C had an HU of 50 and above for the whole study duration. The egg stored at 19.5 ^{o}C for 20 days expressed an HU of 60 and above. The study realized that eggs stored at 5 ^{o}C had (50) a medium HU unit and above for 26 days of storage. Those stored at 30 ^{o}C only stayed for 12 days expressing medium.

The eggs stored at room temperature for 22 days had an SW unit between (6-7) *g*. SW below 5 *g* was considered low by this study. Eggs stored at 19.5 ^{o}C had low SW after 25 days (Fig. 9). Those kept at higher level temperature had low SW after the 19th day. The optimal level for the highest YI was five days of storage at 19.5 ^{o}C and 5 ^{o}C (Fig. 11). The study classified YI as fresh and regular. The fresh yolk was observed for 16 days at room temperature. YI above 30% was considered suitable for this study. Twenty days of storage duration at room temperature demonstrated a medium value of YI. However, eggs stored at 5 ^{o}C expressed 30 ^{o}C and above for 25 days of storage duration (Fig. 22).

5.3 Recommendations

The study recommended a 5 ^{o}C storage temperature for the excellent quality of egg maintenance since it enables eggs to be stored for more days before they are consumed or purchased. Such practice would enhance adequate revenue generated by egg retailers and poultry farmers. This study recommends that eggs retailers should store eggs in fridge-freezers for 32 days. However, this study devoutly suggests future studies consider some other optimization criteria in experimentation.

Further, different levels of storage conditions other than the ones used by this study can also be of much interest in concluding. This study has its limitation as the study design was

RCBD which could only accommodate two factors. The effects of confounding factors resulting from different farm managements such as the total number of colonies, types of feeds and feeding habits of layers were considered trivial. The assumption of this study includes that freshness of different egg sizes from collection to consumption was majorly affected by storage temperature and time. Therefore, further studies should consider determining the effect of confounding factors on egg quality from laying, collection to consumption.

REFERENCES

Addo, A. (2017). *Impact of egg storage duration and storage temperature on egg quality, fertility, hatchability and chick quality, of naked neck chickens' egg* [Doctoral dissertation, Kwame Nkrumah University of Science and Technology]. http://ir.knust.edu.gh/handle/123456789/9957.

Ajay, S., & Micah, B. (2014). Sampling techniques and determination of sample size in applied statistics research: an overview. *International Journal of Economics, Commerce and Management*, *II*(11), 1–22. https://doi.org/Available from: http://ijecm.co.uk/wp-content/uploads/2014/11/21131.pdf.

Akter, Y., Kasim, A., Omar, H., & Sazili, A. Q. (2014). Effect of storage time and temperature on the quality characteristics of chicken eggs. *Journal of Food, Agriculture and Environment*, *12*(2), 87–92.

Alexanderian, A., Petra, N., Stadler, G., & Ghattas, O. (2016). A fast and scalable method for A-optimal design of experiments for infinite-dimensional Bayesian non-linear inverse problems. *Journal of Scientific Computing*, *38*(1), 243–272. https://doi.org/10.1137/140992564.

Alexandratos, N., & Bruinsma., J. (2012). World agriculture towards 2030/2050: the 2012 revision. In *ESA Working paper*. https://doi.org/10.1002/jso.2930300113.

Alsobayel, A. A., & Albadry, M. A. (2011). Effect of storage period and strain of layer on internal and external quality characteristics of eggs marketed in Riyadh area. *Journal of the Saudi Society of Agricultural Sciences*, *10*(1), 41–45. https://doi.org/10.1016/j.jssas.2010.04.001.

Altuntaş, E., & Şekeroğlu, A. (2008). Effect of egg shape index on mechanical properties of chicken eggs. *Journal of Food Engineering*, *85*(4), 606–612. https://doi.org/10.1016/j.jfoodeng.2007.08.022.

An, S. H., Kim, D. W., & An, B. K. (2016). Effects of dietary calcium levels on productive performance, eggshell quality and overall calcium status in aged laying hens. *Asian-Australasian Journal of Animal Sciences*, *29*(10), 1477–1482. https://doi.org/10.5713/ajas.15.0655.

Andrecut, M. (2009). Parallel GPU implementation of iterative PCA algorithms. *Journal of Computational Biology : A Journal of Computational Molecular Cell Biology*, *16*(11), 1593–1599. https://doi.org/10.1089/cmb.2008.0221.

Atitwa, E. B., Koske, J. K., & Mutiso, J. M. (2016). The optimum settings of culture conditions for optimum growth of kefir grains for nutrition and health using RSM with BBD. *International Journal of Statistics and Applied Mathematics, 1*(4), 24–33.

Balogu, D. O., & Kolo, S. I. (2016). Physical properties of hen's egg. *Journal of Foods, Natural and Life Sciences*, *1*, 16–23.

Baran, M. F., & Gokdogan, O. (2016). Determination of energy balance of sugar beet production in Turkey : a case study of Kirklareli Province. *Journal of Energy*

Efficiency, *9*(2), 487–494. https://doi.org/10.1007/s12053-015-9375-x.

Beattie, J. R., & Esmonde-White, F. W. L. (2021). Exploration of principal component analysis: deriving principal component analysis visually using spectra. *Applied Spectroscopy*, *75*(4), 361–375. https://doi.org/10.1177/0003702820987847.

Bell, A., Fairbrother, M., & Jones, K. (2016). Fixed and random effects : making an informed choice. *March) [Google Scholar]*. http://www.researchgate.net/publication/299604336.

Bell, A., Fairbrother, M., & Jones, K. (2018). Fixed and random effects models: making an informed choice. *Quality and Quantity*, *53*(2), 1051–1074. https://doi.org/10.1007/s11135-018-0802-x.

Bertechini, A. G., Mazzuco, H., Rodrigues, E. C., & Ramos, E. M. (2014). Study of the utilization of light egg-type males: A proposal for the sustainability of the egg industry. *Poultry Science*, *93*(3), 755–761. https://doi.org/10.3382/ps.2013-03462.

Bounoua, W., & Bakdi, A. (2021). Fault detection and diagnosis of nonlinear dynamical processes through correlation dimension and fractal analysis based dynamic kernel PCA. *Chemical Engineering Science*, *229*, 116099. https://doi.org/10.1016/j.ces.2020.116099.

Bremer, R. H. (1993). Choosing and modelling your mixed linear model. *Communications in Statistics - Theory and Methods*, *22*(12), 3491–3521. https://doi.org/10.1080/03610929308831229.

Buchanan, J. W., Macneil, M. D., & Van Eenennaam, A. L. (2016). Rapid communication: variance component estimates for Charolais-sired fed cattle and relative economic impact of bovine respiratory disease. *Journal of American Society of Animal Science*, *94*(12), 5456–5460. https://doi.org/10.2527/jas2016-1001.

Cabrera-Llanos, A. I., Ortiz-Arango, F., & Cruz-Aranda, F. (2019). A model for minimizing maintenance costs of medical equipment using fuzzy logic. *Revista Mexicana de Economía y Finanzas*, *14*(3), 379–396. https://doi.org/10.21919/remef.v14i3.410.

Camacho, J., Picó, J., & Ferrer, A. (2010). Data understanding with PCA: structural and variance information plots. *Chemometrics and Intelligent Laboratory Systems*, *100*(1), 48–56. https://doi.org/10.1016/j.chemolab.2009.10.005.

Cayleigh, H. (2018). *The influence of garden treatment on the nutrition profile and agronomic performance of a dark green leafy vegetable grown in peri-urban setting* [Doctoral dissertation, University of KwaZulu-Natal]. https://researchspace.ukzn.ac.za/handle/10413/16717.

Corbeil, R. R., & Searle, S. R. (1976). Restricted maximum likelihood (REML) estimation of variance components in the mixed model. *Technometrics*, *18*(1), 31–38. https://doi.org/10.2307/1267913.

Costabile, F., Birmili, W., Klose, S., Tuch, T., Wehner, B., Wiedensohler, A., Franck, U., Konig, K., & Sonntag, A. (2009). Spatio-temporal variability and principal components of the particle number size distribution in an urban atmosphere. *Atmospheric Chemistry and Physics*, *9*(9), 3163–3195. https://doi.org/10.5194/acp-9-3163-2009.

Degani, E., Leigh, S. G., Barber, H. M., Jones, H. E., Lukac, M., Sutton, P., & Potts, S. G. (2019). Crop rotations in a climate change scenario: short-term effects of crop diversity on resilience and ecosystem service provision under drought. *Agriculture, Ecosystems and Environment*, *285*, 106625. https://doi.org/10.1016/j.agee.2019.106625.

Dixon, P. (2016). Should Blocks Be Fixed or Random? *Conference on Applied Statistics in Agriculture*, 23–39. https://doi.org/10.4148/2475-7772.1474.

Duman, M., Şekeroğlu, A., Yıldırım, A., Eleroğlu, H., & Camcı. (2016). Zusammenhang zwischen Formindex des eies und eiqualitätsmerkmalen. *European Poultry Science*, *80*, 1–9. https://doi.org/10.1399/eps.2016.117.

El-Hashash, E. F. (2017). Comparison of variance components methods for one-way random effects model in cotton. *Asian Journal of Advances in Agricultural Research*, *3*(1), 1–9. https://doi.org/10.9734/AJAAR/2017/36955.

Emsley, A., Dickerson, G. E., & Kashyap, T. S. (1977). Genetic parameters in progeny-test selection for field performance of strain-cross layers. *Poultry Science*, *56*(1), 121–146. https://doi.org/10.3382/ps.0560121.

Fasenko, G. M., Robinson, F. E., Whelan, A. I., Kremeniuk, K. M., & Walker, J. A. (2001). Prestorage incubation of long-term stored broiler breeder eggs: 1. Effects on hatchability. *Poultry Science*, *80*(10), 1406–1411. https://doi.org/10.1093/ps/80.10.1406.

Feddern, V., Prá, M. C. De, Mores, R., Nicoloso, R. da S., Coldebella, A., & Abreu, P. G. de. (2017). Egg quality assessment at different storage conditions, seasons and laying hen strains. *Ciência e Agrotecnologia*, *41*(3), 322–333. https://doi.org/10.1590/1413-70542017413002317.

Festing, M. F. W. (2010). A design by any other name. *Journal of Statistics Education*, *18*(1), 1–4. https://doi.org/10.1080/10691898.2010.11889485.

Galić, A., Filipović, D., Pliestić, S., Janječić, Z., Bedeković, D., Kovačev, I., Čopec, K., & Koronc, Z. (2019). The comparison of quality characteristics of Pekin duck and Cherry Valley duck eggs from the free-range raising system. *Journal of Central European Agriculture*, *20*(4), 1099–1110. https://doi.org//10.5513/JCEA01/20.4.2432.

Galindo, F. S., Teixeira Filho, M. C. M., Buzetti, S., Pagliari, P. H., Santini, J. M. K., Alves, C. J., Megda, M. M., Nogueira, T. A. R., Andreotti, M., & Arf, O. (2019). Maize yield response to nitrogen rates and sources associated with Azospirillum brasilense. *Agronomy Journal*, *111*(4), 1985–1997. https://doi.org/10.2134/agronj2018.07.0481.

Gene, M., Mandal, A., Baneh, H., Koloi, S., & Bhakat, C. (2020). Estimation of variance components and genetic parameters for lactation persistency indices in crossbred cattle using Bayesian and REML methods. *Meta Gene*, *26*(2020), 100780. https://doi.org/10.1016/j.mgene.2020.100780.

Giampietro-Ganeco, A., Scatolini-Silva, A. M., Boiago, M. M., Souza, P. A. de, & Mello, J. L. M. de. (2015). Quality assessment of eggs packed under modified atmosphere. *Scientific Electronic Library Online*, *39*(1), 82–88. hhttps://doi.org/10.1590/S1413-70542015000100010.

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., Pretty, J., Robinson, S., Thomas, S. M., & Toulmin, C. (2010). Food security: The challenge of feeding 9 billion people. *Science*, *327*(5967), 812–818. https://doi.org/10.1126/science.1185383.

Hall, C., Dawson, T. P., Macdiarmid, J. I., Matthews, R. B., Smith, P., Dawson, T. P., Macdiarmid, J. I., Matthews, R. B., Smith, P., & Hall, C. (2017). The impact of population growth and climate change on food security in Africa : looking ahead to 2050. *International Journal of Agricultural Sustainability*, *15*(2), 124–135. https://doi.org/10.1080/14735903.2017.1293929.

Hasan, A., & Alylin, A. O. (2014). Effect of hen age, storage duration and temperature on egg quality in laying hens. *International Journal of Poultry Science*, *13*(11), 634–636. https://doi.org/10.3923/ijps.2014.634.636.

Hassan, S. M., Siam, A. A., Mady, M. E., & Cartwright, A. L. (2005). Egg storage period and weight effects on hatchability of ostrich (Struthio camelus) eggs. *Poultry Science*, *84*(12), 1908–1912. https://doi.org/10.1093/ps/84.12.1908.

Haugh, R. R. (1937). The Haugh unit for measuring egg quality. *United States Egg and Poultrymagazine*, *43*, 522–555. https://ci.nii.ac.jp/naid/10019768224/.

Heba, A. E. L., Zakaria, A. A. W., & Mohamed, S. A. (2015). On the non-negative estimation of variance components in mixed linear models. *Journal of Advanced Research*, *7*(1), 59–68. https://doi.org/10.1016/j.jare.2015.02.001.

Hegab, I. M., & Hanafy, A. M. (2019). Effect of egg weight on external and internal qualities, physiological and hatching success of Japanese quail eggs (Coturnix coturnix Japonica). *Revista Brasileira de Ciencia Avicola*, *21*(3). https://doi.org/10.1590/1806-9061-2018-0777.

Herman, E., Alexanderian, A., & Saibaba, A. K. (2020). Randomization and reweighted \ell_1- minimization for A-optimal design of linear inverse problems. *Journal on Scientific Computing*, *42*(3), 2352–2380. https://doi.org/10.1137/19M1267362.

Iposu, S. O., Onwuka, C. F. I., & Eruvbetine, D. (1994). The relationship between selected egg quality traits and egg size. *Journal of Animal Production*, *21*, 156–160.

Jjagwe, J., Chelimo, K., Karungi, J., Komakech, A. J., & Lederer., J. (2020). Comparative performance of organic fertilizers in maize (Zea mays L.) growth, yield and economic results. *Journal of Agronomy*, *10*(1), 69. https://doi.org/10.3390/agronomy10010069.

Johnston, K. M., Lakzadeh, P., Donato, B. M. K., & Szabo, S. M. (2019). Methods of sample size calculation in the descriptive retrospective burden of illness studies. *BMC*

Medical Research Methodology, *19*(1), 1–7. https://doi.org/10.1186/s12874-018-0657-9.

Jollands, N., Lermit, J., & Patterson, M. (2004). Aggregate eco-efficiency indices for New Zealand - A principal components analysis. *Journal of Environmental Management*, *73*(4), 293–305. https://doi.org/10.1016/j.jenvman.2004.07.002.

Jones, B., Allen-Moyer, K., & Goos, P. (2020). A-optimal versus D-optimal design of screening experiments. *Journal of Quality Technology*, 1–14. https://doi.org/10.1080/00224065.2020.1757391.

Joseph, A., Matthew, F., & Adeleke, A. (2018). Diagnosis and control of chicken coccidiosis : a recent update. *Journal of Parasitic Diseases*, *42*(4), 483–493. https://doi.org/10.1007/s12639-018-1048-1.

Kackar, R. N., & David, A. H. (1984). Approximations for standard errors of estimators of fixed and random effects in mixed linear models. *Journal of the American Statistical Association*, *79*(388), 853–862. https://doi.org/10.1080/01621459.1984.10477102.

Ketta, M., & Tůmová, E. (2018). Relationship between eggshell thickness and other eggshell measurements in eggs from litter and cages. *Italian Journal of Animal Science*, *17*(1), 234–239. https://doi.org/10.1080/1828051X.2017.1344935.

Khuri, A. I. (2000). Designs for variance components estimation : past and present. *International Statistics Institute*, *68*(3), 311–322. https://doi.org/10.1111/j.1751-5823.2000.tb00333.x.

Kinney, T. B., Bohren, B. B., Craig, J. V., & Lowe, P. C. (1970). Responses to individual, family or index selection for short term rate of egg production in chickens. *Poultry Science*, *49*(4), 1052–1064. https://doi.org/10.3382/ps.0491052.

Kline, P., Saggio, R., & Sølvsten, M. (2020). Leave-out estimation of variance components. *Econometrica*, *88*(5), 1859–1858. http://www.nber.org/papers/w26244.

Kourti, T. (2005). Application of latent variable methods to process control and multivariate statistical process control in the industry. *International Journal of Adaptive Control and Signal Processing*, *19*(4), 213–246. https://doi.org/10.1002/acs.859.

Kraus, A., Zita, L., & Krunt, O. (2019). The effect of different housing systems on quality parameters of eggs with the age in brown egg-laying hens. *Bulgarian Journal of Agricultural Science*, *25*(6), 1246–1253. http://agrojournal.org/25/06-23.pdf.

Kraus, A., Zita, L., Krunt, O., Volek, Z., Tyller, M., & Anderle, V. (2020). Comparison of basic internal and external egg quality traits of brown and white egg-laying hens with their age. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, *68*(1), 49–56. https://doi.org/10.11118/actaun202068010049.

Kwasek, M. (2012). Threats to food security and common agricultural policy. *Economics of Agriculture*, *59*(4), 701–713. https://agris.fao.org/agris-search/search.do?recordID=RS201300344.

Latham, J. (2021). The myth of a food crisis. In *The Bioscience Resource Project* (pp. 93–111). Elsevier Inc. https://doi.org/10.1016/b978-0-12-816410-5.00005-0.

Lee, M. H., Cho, E. J., Choi, E. S., Bang, M. H., & Sohn, S. H. (2016). The effect of hen age on egg quality in the commercial layer. *Korean Journal of Poultry Science*, *43*(4), 253–261. http://dx.doi.org/10.5536/KJPS.2016.43.4.253.

LeMay, V. M., & Robinson, A. P. (2004). Design, performance, and evaluation of experiments. In *Encyclopedia of Forest Sciences* (pp. 1–7). Elsevier. http://biometrics.sites.olt.ubc.ca/files/2013/06/exp_design_encyl_2004.pdf.

Li, M., Nie, W., Xu, T., Rovira-garcia, A., & Fang, Z. (2020). Helmert variance component estimation for multi- GNSS relative positioning. *Sensors*, *20*(3), 699. https://doi.org/10.3390/s20030669.

Lindstrom, M. J., & Bates, D. M. (1988). Newton—Raphson and EM algorithms for linear mixed-effects models for repeated-measures data. *Journal of the American Statistical Association*, *83*(404), 1014–1022. https://doi.org/10.1080/01621459.1988.10478693.

Lu, N., Gao, F., Yang, Y., & Wang, F. (2004). PCA-based modelling and online monitoring strategy for uneven-length batch processes. *Industrial and Engineering Chemistry Research*, *43*(13), 3343–3352. https://doi.org/10.1021/ie030736f.

Mairura, F. S., Mugendi, D. N., Mwanje, J. I., Ramisch, J. J., Mbugua, P. K., & Chianu, J. N. (2007). Integrating scientific and farmers' evaluation of soil quality indicators in Central Kenya. *Geoderma*, *139*(1–2), 134–143. https://doi.org/10.1016/j.geoderma.2007.01.019.

Manhique, A. J., King'ori, A. M., & Wachira, A. M. (2017). Effect of ground matures Prosopis (Prosopis juliflora) pods inclusion in layer diets on performance of improved indigenous chicken in Kenya. *Livestock Research for Rural Development*, *29*(1).

Marzec, A., Damaziak, K., Kowalska, H., Riedel, J., Michalczuk, M., Koczyw, E., Cisneros, F., Lenart, A., & Niemiec, J. (2019). Effect of hens age and storage time on functional and physicochemical properties of eggs. *Journal of Applied Poultry Research*, *28*, 290–300. https://doi.org/10.3382/japr/pfy069.

Massey, A., & Miller, S. J. (2006). Tests of hypotheses using statistics. *Brown University, Mathematics Department, Providence, RI 02912*, 1–32. https://web.williams.edu/Mathematics/sjmiller/public_html/BrownClasses/162/Handouts/StatsTests04.pdf.

Miller, M. R. (2019). Knowledge, policy, action in the decade of nutrition. *World Nutrition*, *10*(2), 4–7. https://wphna.org/worldnutritionjournal/index.php/wn/article/download/643/562.

Moein, S., & Pan, R. (2016). A clustering-based coordinate exchange algorithm for generating G-optimal experimental designs. *Journal of Statistical Computation and Simulation*, *86*(8), 1582–1604. https://doi.org/10.1080/00949655.2015.1077252.

Molnár, A., Maertens, L., Ampe, B., Buyse, J., Kempen, I., Zoons, J., & Delezie, E. (2016). Changes in egg quality traits during the last phase of production: is there potential for an extended laying cycle? *British Poultry Science*, *57*(6), 842–847. https://doi.org/10.1080/00071668.2016.1209738.

Montgomery, D. C., Loredo, E. N., Jearkpaporn, D., & Testik, M. C. (2002). Experimental designs for constrained regions. *Quality Engineering*, *14*(4), 587–601. https://doi.org/10.1081/QEN-120003561.

Mottet, A., & Tempio, G. (2017). Reviews global poultry production : current state and future outlook and challenges. *World's Poultry Science Journal*, *73*(2), 245–256. https://doi.org/10.1017/S0043933917000071.

Oleforuh-Okoleh, V. U., & Eze, J. (2016). Effect of storage period and method on internal egg quality traits of the Nigerian native chicken. *Livestock Research for Rural Development*, *28*(6). http://www.lrrd.org/lrrd28/6/news2806.htm.

Omondi, S. O. (2019). Economic analysis of small-scale poultry production in Kenyan medium-sized cities of Kisumu and Thika. *6th African Conference of Agricultural Economists*, 1–19. https://ageconsearch.umn.edu/record/277360.

Orungo, O. J., Alarcon, P., Karani, M., Muinde, P., Miser, J., Maud, C., Fevre, E. M., Häsler, B., & Rushton, J. (2018). Identification of production challenges and benefits using value chain mapping of egg food systems in Nairobi, Kenya. *Agricultural Systems*, *159*, 1–8. https://doi.org/10.1016/j.agsy.2017.10.001.

Osabohien, R., Osabuohien, E., & Urhie, E. (2018). Food security, institutional framework and technology: examining the nexus in Nigeria using ARDL approach. *Current Nutrition & Food Science*, *14*(2), 154–163. https://doi.org/10.2174/1573401313666170525133853.

Pandey, R., & Verma, M. R. (2008). Samples allocation in different strata for impact. *Rev. Bras. Biom*, *26*(4), 103–112. http://jaguar.fcav.unesp.br.

Peri, L., Mirjana, Đ., & Bjedov, S. (2017). The effect of storage and age of hens on the quality of table eggs. *Advanced Research in Life Science*, *1*(1), 64–67. https://doi.org/10.1515/arls-2017-0011.

Pescatore, T., Jacob, J., & Cantor, A. (2011). Grading table eggs. In *University of Kentucky College of Agriculture*. http://www2.ca.uky.edu/afspoultry-files/pubs/Grading_table_eggs.pdf.

Popkin, B. M., Adair, L. S., & Ng, S. W. (2012). Global nutrition transition and the pandemic of obesity in developing countries. *Nutrition Reviews*, *70*(1), 3–21. https://doi.org/10.1111/j.1753-4887.2011.00456.x.

Rabbette, M., & Pilewskie, P. (2001). Multivariate analysis of solar spectral irradiance measurements. *Journal of Geophysical Research-Atmospheres*, *106*(D9), 9685–9696. https://doi.org/10.1029/2000JD900582.

Rayan, G. N., Galal, A., Fathi, M. M., & El-Attar, A. H. (2010). Impact of layer breeder flock age and strain on mechanical and ultrastructural properties of eggshell in chicken. *International Journal of Poultry Science*, *9*(2), 139–147. https://doi.org/10.3923/ijps.2010.139.147.

Reijrink, I. A. M., Berghmans, D., Meijerhof, R., Kemp, B., & van den Brand, H. (2010). Influence of egg storage time and preincubation warming profile on embryonic development, hatchability, and chick quality. *Poultry Science*, *89*(6), 1225–1238. https://doi.org/10.3382/ps.2009-00182.

Rodríguez-álvarez, M. X., Durban, M., & Paul, D. L. (2018). On the estimation of variance parameters in non-standard generalised linear mixed models : application to penalised smoothing. *Statistics and Computing*. https://doi.org/10.1007/s11222-018-9818-2.

RoK. (2018). Eye on the ' 'big four agenda. *Parliamentary Service Commission*, *11*, 1–65. www.parliament.go.ke.

Saleh, G., El Darra, N., Kharroubi, S., & Farran, M. T. (2020). Influence of storage conditions on quality and safety of eggs collected from Lebanese farms. *Food Control*, *111*, 107058. https://doi.org/10.1016/j.foodcont.2019.107058.

Samli, H. E., Agma, A., & Senkoylu, N. (2005). Effects of storage time and temperature on egg quality in old laying hens. *Journal of Applied Poultry Research*, *14*(3), 548–553. https://doi.org/10.1093/japr/14.3.548.

Sarica, M., Onder, H., & Yamak, U. S. (2012). Determining the most effective variables for egg quality traits of five hen genotypes. *International Journal of Agriculture and Biology*, *14*(2), 235–240.

Sarmah, H. K., & Hazarika, B. B. (2012). Importance of the size of the sample and its determination in the context of data related to the schools of greater Guwahati. *Bull. Guwahati University Mathematics. Association*, *12*, 55–75. https://www.researchgate.net.

Shrout, P. E., & Fleiss, J. L. (1979). Intraclass correlations: uses in assessing rater reliability.1. Shrout PE, Fleiss JL: Intraclass correlations: uses in assessing rater reliability. Psychol Bull 1979, 86:420–8. *Psychological Bulletin*, *86*(2), 420–428. http://www.ncbi.nlm.nih.gov/pubmed/18839484.

Silva, A., Fonseca, M., & Mexia J.Verde. (2015). Variance components estimation in the mixed linear model-the sub-diagonalization method. *International Conference on Matrix Analysis and Its Applications*, *192*, 317–341. https://doi.org/10.1007/978-3-319-49984-0.

Sirri, F., Zampiga, M., Berardinelli, A., & Meluzzi, A. (2018). Variability and interaction of some egg physical and eggshell quality attributes during the entire laying hen cycle. *Poultry Science*, *97*(5), 1818–1823. https://doi.org/10.3382/ps/pex456.

Sola-Ojo, F. E., Adeyina, O. A., Opowoye, I. O., & Yusuf, A. O. (2016). Performance and egg quality traits of Issa brown layer chicken fed: Moriga oleifera leaf meal(MOLM) as supplemented diets. *African Journal of Natural Sciences*, *19*, 1119–1104.

Stroup, W., & Littell, R. (2002). Impact of variance component estimates on fixed effect inference in unbalanced linear mixed models. *Conference on Applied Statistics in Agriculture*, 32–48. https://doi.org/10.4148/2475-7772.1198.

Sun, K., Zhang, W., Pan, L., & Tu, K. (2018). Recognition of a cracked hen egg image using a sequenced wave signal extraction and identification algorithm. *Food Analytical Methods*, *11*(4), 1223–1233. https://doi.org/10.1007/s12161-017-1105-x.

Tiberious, M., Mwania, J. M., & Mwinzi, J. (2016). The influence of financial resources on the integration of the national goals of education. *International Journal of Education and Research*, *4*(9), 51–62. www.ijern.com.

Tran, H. Q., & Soottawat, B. (2018). Quality, protease inhibitor and gelling property of duck egg albumen as affected by storage conditions. *Journal of Food Science and Technology*, *55*(2), 513–522. https://doi.org/10.1007/s13197-017-2960-6.

Ukwu, H. O., Abari, P. O., & Kuusu, D. J. (2017). Principal Component Analysis of Egg Quality Characteristics of Isa Brown Layer Chickens in Nigeria. *World Scientific News*, *70*(2), 304–311. www.worldscientificnews.com.

Uyanga, V. A., Onagbesan, O. M., Oke, O. E., Abiona, J. A., & Egbeyale, L. T. (2020). Influence of age of broiler breeders and storage duration on egg quality and blastoderm of Marshall broiler breeders. *Journal of Applied Poultry Research*, *29*(3), 535–544. https://doi.org/10.1016/j.japr.2020.03.001.

Van Tijen, W. F., & Kuit, A. R. (1970). The heritability of characteristics of egg quality, their mutual correlation and the relationship with productivity. *Arch Geflugel*, *34*, 201–210.

White, A., Tolman, M., Thames, H. D., Withers, H. R., Mason, A., & Transtrum, M. K. (2016). The limitations of model-based experimental design and parameter estimation in sloppy systems. *PLoS Computational Biology*, *12*(12), 1–26. https://doi.org/10.1371/journal.pcbi.1005227.

Wold, S., Esbensen, K., & Geladi, P. (1987). Principal component analysis. *Chemometrics and Intelligent Laboratory Systems*, *2*(1–3), 37–52. https://doi.org/10.1016/0169-7439(87)80084-9.

Wong, W. K. (1992). A unified approach to the construction of minimax designs. *Biometrika*, *79*(3), 611–619. https://doi.org/10.1093/biomet/79.3.611.

Wu, M., Yu, K., & Liu, A. (2009). Estimation of variance components in the mixed-effects models : A comparison between analysis of variance and spectral decomposition. *Journal of Statistical Planning and Inference*, *139*(12), 3962–3973. https://doi.org/10.1016/j.jspi.2009.03.014.

Yang, R. C. (2010). Towards understanding and use of mixed-model analysis of agricultural experiments. *Canadian Journal of Plant Science*, *90*(5), 605–627. https://doi.org/10.4141/CJPS10049.

Yiheng, Z., Brooks, D. H., Franceschini, M. A., & Boas, D. A. (2005). Eigenvector-based spatial filtering for reduction of physiological interference in diffuse optical imaging. *Journal of Biomedical Optics*, *10*(1), 011014. https://doi.org/10.1117/1.1852552.

Yimenu, S. M., Kim, J. Y., Koo, J., & Kim, B. S. (2011). Predictive modelling for monitoring egg freshness during variable temperature storage conditions. *Poultry Science*, *96*(8), 2811–2819. https://doi.org/10.3382/ps/pex038.

Yu, J., Zavala, V. M., & Anitescu, M. (2018). A scalable design of experiments framework for optimal sensor placement. *Journal of Process Control*, *67*, 44–55. https://doi.org/10.1016/j.jprocont.2017.03.011.

Zahran, A., Anderson-Cook, C. M., Myers, R. H., & Smith, E. P. (2003). Modifying 22 factorial designs to accommodate a restricted design space. *Journal of Quality Technology*, *35*(4), 387–392. https://doi.org/10.1080/00224065.2003.11980236.

Zhang, X. (2015). A tutorial on restricted maximum likelihood estimation in linear regression and linear mixed-effects model. *Biometrica*, *58*(1), 1–11. https://people.csail.mit.edu/xiuming/docs/tutorials/reml.pdf.

APPENDIX

Appendix 1: Design Matrix for this Study

b0	A	B	A2	B2	AB
1	1	-1	1	1	-1
1	1	-1	1	1	-1
1	1	-1	1	1	-1
1	1	2	1	4	2
1	1	2	1	4	2
1	1	2	1	4	2
1	-1	2	1	4	-2
1	-1	2	1	4	-2
1	-1	2	1	4	-2
1	-1	-1	1	1	1
1	-1	-1	1	1	1
1	-1	-1	1	1	1
1	0	-1	0	1	0
1	0	-1	0	1	0
1	0	-1	0	1	0
1	0	2	0	4	0
1	0	2	0	4	0
1	0	2	0	4	0
1	-1	0	1	0	0
1	-1	0	1	0	0
1	-1	0	1	0	0
1	1	0	1	0	0
1	1	0	1	0	0
1	1	0	1	0	0
1	-1	1	1	1	-1
1	-1	1	1	1	-1
1	-1	1	1	1	-1
1	1	1	1	1	1
1	1	1	1	1	1
1	1	1	1	1	1
1	0	1	0	1	0
1	0	1	0	1	0
1	0	1	0	1	0
1	0	0	0	0	0
1	0	0	0	0	0
1	0	0	0	0	0