EVALUATION OF MICROBIAL COMMUNITY, MODELLING AND OPTIMIZATION OF PROCESSING PARAMETERS OF A MAIZE-COWPEA BLEND WEANING FORMULA.

BY

OTUNEME, ONYINYECHI. FLORENCE (B.TECH)

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CERTIFICATION

This is to certify that this research work "EVALUATION OF MICROBIAL COMMUNITY, MODELLING AND OPTIMIZATION OF PROCESSING PARAMETERS OF A MAIZE-COWPEA BLEND WEANING FORMULA" was carried out by **OTUNEME, ONYINYECHI FLORENCE,** (Registration Number: **20194215708)** in the Department of Microbiology, School of Biological Sciences, Federal University of Technology, Owerri, in partial fulfillment for the award of Master of Science (M.Sc) degree in Food and Industrial Microbiology.

.....
PROF. WESLEY BRAIDE
(Supervisor)

20-02-2024
.....
Date

.....
DR (MRS). N.U. NWOGWUGWU
(Co-Supervisor)

20-02-2024
.....
Date

.....
PROF I. E. ADIEZE
(HOD, Microbiology)

20/02/2024
.....
Date

.....
PROF. C.S ALISI
(Dean School of Biological Sciences)

20/02/24
.....
Date

.....
PROF B. O. ESONU
(Dean, Postgraduate School, FUTO)

.....
Date

.....
PROF T.G SOKARI
EXTERNAL EXAMINER

20th February, 2024
.....
Date

DEDICATION

This work is dedicated to God Almighty for his faithfulness from the beginning to the end of this project work. To Him be all the glory. Amen.

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I am grateful to God for his constant love, guidance and protection throughout this period that led to the success of this work.

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ABSTRACT

Protein deficiency and malnutrition in locally processed infant formula is known to cause stunted growth and preventable diseases in infants. This study was carried out to determine the ideal processing conditions and microorganisms involved in the production of a weaning food made from maize and cowpea blend. A Box-Behnken Design method under Response Surface Methodology (RSM) was used to set up methodology for weaning food preparation. The microbial community responsible for fermentation was investigated using standard microbiological and Biochemical methods. Proximate composition analysis was done to ascertain the nutritional quality of the blended product. Sensory evaluation was conducted using the 9-point hedonic scale to determine the most acceptable sample product that met the requirements by World Health Organization (WHO) for weaning foods. Statistical analysis of the data was carried out using Minitab (Version 17.0). The final product after processing yielded dry flour of maize and cowpea blend. *Bacillus cereus*, *Bacillus subtilis*, *Corynebacterium* sp, *Micrococcus* sp, *Lactobacillus lactis* and *Streptococcus faecalis* were predominant in the samples. Results from proximate composition analysis showed variances in moisture, ash, crude protein, and crude fiber content of the different samples. Sample code WFC (cowpea substitution 35%, pH 5.5 and fermentation time 72 hours) was most acceptable among the weaning mothers/caregivers. However, Sample code WFA (cowpea substitution in maize 15%, pH 3.5, fermentation time 48 hours) was the only product that met the requirements of WHO standard for weaning food nutrient composition. The results of the statistical analysis showed the responses of the independent variables to the dependent variables in the ANOVA, the contour as well as surface plot values. The prospect of producing a traditional fortified weaning food of WHO standard can be achieved if the processing conditions of pH , blend ratio and fermentation time are carefully explored.

Keywords: Weaning food, protein deficiency, fortification, optimization, response surface methodology.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

The term "weaning" originates from the word "*wenian*" (Old English) which denotes "to accustom". The definition of this word differs from country to country but generally it involves a gradual process by which an infant shifts from total dependence on milk to adult diet (MacDonald, 2016). Aptly, weaning is the process of gradually introducing a mammal infant to what will be its adult diet and withdrawing the supply of its mother's milk (Nwakalor & Obi, 2014). Weaning is an important process in the life of an infant because is confers many advantages to the infants such as –

- Ensuring adequate energy intake.
- Bioavailability of nutrients such as iron, vitamin D, zinc, and copper.
- Better neuromuscular coordination involving mouth movements such as biting, chewing etc.
- Better speech developments resulting from better mouth and tongue movement.
- Easier acceptance for variety of tastes and flavours.

To ensure that infant's nutritional needs are met, a careful selection of solid foods in the proper amount is essential. Formulated weaning foods must be soft, acceptable and must contain the essential nutrients in the correct proportion to ensure proper growth and development of the mammalian progeny. Many studies have looked into the improvement of weaning food with the hope of achieving the desired goal of a balanced nutrition for infants (Kulkarni, Kulkarni, & Ingle, 1991; Nwakalor & Obi, 2014)). These above-mentioned studies were focused on different

properties and characteristics. However, in general the weaning food was evaluated for functional properties such as viscosity, dispersibility, water absorption, water holding capacity, particle size and color (Kulkarni et al., 1991). Additionally, nutritive value such as vitamin C, minerals and amino acids (Kikafunda, Abenayo, & Lukwago, 2006). Other studies considered additional parameters such as flavor, nutrient density, storage stability, moisture content and acceptability (Bonsi, Plahar, & Zabawa, 2014; Nwakalor & Obi., 2014).

In Nigeria, traditional weaning foods are cereal gruels that are high in carbohydrates. To achieve a nutritionally balanced infant food, the cereal is fortified with a legume paste. These cereals after undergoing fermentation by microorganisms supply mainly carbohydrates. High carbohydrate intake by infants leads to slow growth rate due to malnutrition. In fact, malnourished infants appear skinny or bloated unlike their counterparts who consume nutritionally balanced weaning foods. Child malnutrition due to poor quality of complementary foods is a major cause of mortality among children in many sub-Saharan Africa. These complementary foods are made from starchy staple foods, which due to their heavy viscosity must be diluted with water before being given to children. Socially, parents who desire to improve the physical looks of their infants through proper nutrition desperately try different combinations of the cereals with proteins. However, proteins are not easily digestible thereby dousing the aim of this fortification project.

Over the years, efforts have been made to improve the nutritive value of weaning food through fermentation processes of various cereal food (Adeyemo & Onilude, 2018; Asres Asres, Nana, & Nega, 2018). The fermentation processes have been shown to result in improved nutrient content, reduction in anti-nutritional factors such as phytate and tannin, enhanced digestibility and

improved diarrhea inhibition food (Adeyemo & Onilude, 2018; Asres et al., 2018). However, there is a huge challenge, which involves the optimization of dynamic microbial changes during the fermentation process to avoid negative effect of fermented food on the infant. This project intends to use heterogeneous mixture of microorganisms as initial microflora for the cereal. Therefore, the microbial succession throughout the entire fermentation period is of paramount importance and will be used as an optimization factor.

To be able to evaluate the different controllable factors (variables) and the response needed during the fermentation process, a simple mathematical model is needed. Response surface methodology (RSM) is the desired model in this case because of its simplicity and its ability to provide experimental variability estimate. Box and Wilson proposed this model, and it is a collection of mathematical and statistical techniques geared towards appropriate design and analysis of experiment. RSM has been employed in different fermentation processes to optimize the production of different substances using different bacteria (Zhang & Gao 2017).

1.2 Statement of the problem

- Economic challenges due to flooding, fuel subsidy removal, security unrest among other crises are peculiar issues to Nigerians causing food shortages and eventually malnutrition in children.
- Most available traditional weaning foods are mainly cereals, their protein supply is low and therefore the need for fortification.
- The microorganisms responsible for fermenting this weaning food needs to be studied and evaluated for better performance.

- The application of RSM will enhance optimization of the processing parameters to identify optimal conditions for the production process.

This research work will be applied in Food/Industrial microbiology to provide a traditional alternative of a fortified weaning food of finest quality for the teeming population of the poor and average populace in Nigeria.

1.3 **Justification of Study**

After breastfeeding for six months, it is crucial to continue and maintain the child's growth and development. Unfortunately, during this period, mothers/caregivers give food only to prevent the child from being hungry with little regard to the nutritional quality. To obtain a quality weaning food, fortification with a legume paste because of its high protein content is very important. The production process of the weaning food will be optimized with special attention to the microorganisms involved in the fermentation processes. The use of response surface model will enhance the process. It is therefore justified to carry out this research to provide Nigerian mothers/caregivers with a nutritionally balanced food of finest quality.

1.4 **Purpose of Study**

This study is to determine the microbial community and ideal processing conditions for the production of a Maize- Cowpea blend weaning formulae.

1.5 **AIM**

To evaluate the microbial community, optimize and generate model ideal processing conditions to get a fortified weaning formula using Response Surface Methodology (RSM).

1.6 OBJECTIVES

1. Production of a Maize -Cowpea blend weaning formulae.
2. Evaluation of microbial community involved during production.
3. Sensory evaluation of the weaning formulae
4. Proximate composition analysis (crude protein, crude fiber, ash, moisture, and fat) of the weaning formulae.
5. Optimization and modelling of processing parameters to generate ideal model designs using Response Surface Methodology and Minitab software (version 17.0).

1.7 SIGNIFICANCE OF STUDY

This study will go to a very great extent provide a comprehensive production design for a traditional weaning formula which ab intio was basically traditional. Generally, production will be optimized and weaning food formulae with the capacity to reduce protein deficiency among weaning children in developing nations is produced commercially. The results of this study will serve as a guide/design to manufacturers. In turn, mothers/caregivers who cannot afford conventional standard weaning food will be able to provide infants a smooth transition from breast milk to fortified traditional semi-solid/solid foods.

CHAPTER TWO

2.0 LITERATURE REVIEW

Weaning is the process of gradually introducing a mammal infant to what will be its adult diet and withdrawing the supply of its mother's milk (D'Auria et al., 2018; Nwakalor & Obi, 2014; Wharton, 1989). Additionally, weaning could be seen as a gradual process of introducing solid food with concomitant withdrawal of milk as the primary source of nutrition (Foote & Marriott, 2003). The source of energy and nutrients needed by an infant for the first few months of life is supplied by breast milk. Proper diet for a child is crucial for body development as soon as breast milk is stopped. By four to six months of age, most infants are able to handle most proteins. The kidney tubules become efficient by six to eight weeks, after which there is less concern over the use of a high-protein, high-sodium diet (Guthrie, 1975). A study done in Italy showed that infants introduced to solid foods using a weaning approach known as baby-led weaning were more likely to achieve crucial developmental milestones (Addessi et al., 2021; D'Auria et al., 2018). Furthermore, other studies have also shown that introduction of solid foods have lifelong consequences on infants in aspects such as cognitive, physical and socio-emotional wellbeing (Rose, Birch, & Savage, 2017; Seach, Dharmage, Lowe, & Dixon, 2010). Weaning is a complex process involving adjustment to a range of nutritional, immunological, biochemical, and psychological changes (Al-Gashanin & Ghazwani, 2022). Moreover, there is an in depth review of the effects of nutrition during highly sensitive infancy periods such as weaning on the gastrointestinal microflora of infants which has increased interest in dietary modulation (Mountzouris, McCartney, & Gibson, 2002). Poor nutrition during this critical period of life may increase the risk of growth faltering and may have adverse effects on health and mental development of the infant (Ijarotimi & Keshinro, 2013). Since infants have no storage for

nutrients, there is a need therefore to get a constant supply of required food nutrients from available food. Foods readily available in Nigeria like maize, sorghum, millet, wheat etc. are major sources of nutrient. In a study by (Antai & Nzeribe, 1992), sieving the maize mash led to heavy loss of protein, ash, and crude fiber. The availability of the above mentioned food sources makes them the only option for poor Nigerians who cannot afford the processed weaning foods.

To enrich an infant's diet, weaning foods act as supplements pending when solid foods will be fully introduced and there are some researches done on weaning food as supplements in different countries (Asma, El Fadil, & El Tinay, 2006; Bates et al., 1982; Mensa-Wilmot, Phillips, Lee, & Eitenmiller, 2003; Nti & Plahar, 1995; Sadana & Chabra, 2004; Sajilata, Singhal, & Kulkarni, 2002). In Africa generally, mothers who cannot afford enriched meals for their babies, resort to available foods which are mostly cereals and force feed their babies with it. This places the infant in high danger of malnutrition diseases like marasmus, growth retardation and kwashiorkor. The major criteria for a good-quality weaning food are high balanced-protein content, high caloric value per unit of food volume, soft texture with low fiber content, adequate vitamin and mineral contents, and absence of anti-nutritional factors. The high viscosity characteristic of cereal grain is obviously responsible for young children's inability to fulfill their energy requirement (Kikafunda et al., 2006). With these requirements kept in mind, weaning foods are usually formulated using a mixture of cereals and legumes which guarantee a proper balance of amino acids to provide a complete protein (Gan et al., 2019). Due to the prevailing unfavorable economic conditions in most developing countries of the world, Africa and Nigeria in particular, where over 40% of the population live below poverty line (Nzeagwu & Nwaejike, 2019), the incidence of protein-energy malnutrition among different age groups particularly children with an estimated 400 million children being reported to be malnourished worldwide is

highly prevalent and is on the increase on daily basis. In Nigeria, infants and children suffer from protein energy mal-nutrition (PEM) (Nkama, et al., 2001). It actually occurs as a result of ignorance and exorbitant cost of animal sources of protein thereby making it out of reach of the common man (Patrick, 1998). This point is one of the criticisms against the WHO 2001 global recommendation which did not factor in the disparity among nations (Foote & Marriott, 2003). Although breastmilk is adequate to meet the energy and nutrient requirements of an infant up to four to six months of age, thereafter it is insufficient to sustain normal growth and needs to be supplemented with other foods, such as weaning foods (Sajilata et al., 2002). Most weaning foods are usually cereal-based gruels fermented by lactic acid bacteria (LAB). The nutritional requirement of weaning infants could be supplied by supplementation with protein rich diets. So many infant formulae are available in the markets both commercialized ones and locally produced ones. Soybean is a cheap and available source of protein in Nigeria; but it also contains some complex sugars (raffinose) which is associated with abdominal discomfort in children of weaning age (Adeyemo & Onilude, 2018).

Cereal-legume blends have been found to be relatively high in protein (both quality and quantity) because the legumes supply the lysine cereals lack. While the cereals provide cysteine and methionine which are low in legumes. Soybeans have been used in making the roasted or fermented cereal into such a complimentary mix (Annan & Plahar., 1995). There are different blends of a cereal with legumes to produce a fortified weaning food in Nigeria. Local additives like ground groundnut, ground fish/Crayfish, banana, half cooked egg, peanut, Soyabean are usually added to cereal gruels for enrichment as well as fortification. Researchers also use one or more additives simultaneously to achieve the same purpose. Production methods of these infant

formulations are incorporation of indigenous methods with modern technology. 90% of naturally fermented foods and alcoholic beverages in different countries and regions of the world are still at home production under traditional conditions (Tamang, Shin, Jung, & Chae, 2016). Traditionally, the process involves pre-treatment, crushing to smaller pieces, homogenization, and fermentation among others. Pre-treatment often includes dehulling, milling, steeping, roasting, sorting, frying, and malting. Materials used are either the traditional equipment like buckets, kilns, mortar, pestles, grinding stones etc. Modifications to these traditional equipment are also used like; blenders, grinders, ovens, dehulling machines etc. It is worthy to note that some of these traditional methods and materials are still sacrosanct because of purported traditional flavors and texture.

Fermentation is an important stage in most weaning food production processes. Fermented foods confer health benefits when consumed due to the presence of microorganisms. Nutrient synthesis, prevention of cardiovascular disease, prevention of cancer, gastrointestinal disorders, diabetes are some of the diseases that consumption of fermented foods can prevent. The menstruum environment stimulates microbial activity through the interaction of the raw materials, utensils as well as processing methods employed. Thus, conferring the final product a certain/ specific taste, flavor, and aroma. The microorganisms associated with the fermentation process are not strictly controlled neither is the succession pattern and the contribution of each of the microbial genera to the fermentation process clearly understood (Anumudu, Omeje, & Obinwa, 2018). Microorganisms involved in fermentation usually show a succession pattern throughout the fermentation process. The fermenting microbial population in most cereal fermentations comprises mainly lactic acid bacteria (*Lactobacillus, Leuconostoc, Pediococcus* and *Weissella*

species) and yeasts (*Candida, Saccharomyces, Geotrichum, Kluyveromyces* and *Pichia* species (Obinna-Echem & Jane Beal, 2013). *Bacillus* spp is usually predominant in legume-based fermentation.

Response surface methodology is an important tool used in optimization of industrial processes. The benefits of RSM are listed as follows –

- To gain knowledge from experimental data from the area of interest.
- To accurately estimate variability associated with the experiments (pure error).
- To ensure that there is adequacy between obtained experimental data and proposed model. This makes it easier to spot the lack of fit.
- To be able to predict observed response as accurately as possible even in the absence of experimental points.
- To be able to utilize results obtained to float sequential approaches to carry out new experimentation.
- To sustain high efficiency when it comes to economical cost, time etc.
- To make it easy to spot outliers in the data.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Methodology for Weaning Food Preparation

The food materials used in this study include maize and cowpeas and were purchased at Ekeonunwa market located in Owerri municipal in Imo state.

A Box-Behnken Design method under Response Surface Methodology was used for the design and the design was done using Minitab version 17.0. It involved varying three factors (pH, fermentation time, blend ratio) across three levels. The levels for pH are (3.5, 4.5 and 5.5), the levels of fermentation time are (24, 48 and 72hrs), while the blend ratios are (M M85C15; M65C35 and M50C50) where M- Maize and C- Cowpea. (Table 3.1)

Fifteen samples were used, and each sample had a different fermentation time, pH, blend ratio (Table 3.1). Each sample weighed a total of 1 kg on the weighing scale. The water used for milling/steeping was strictly acidic by the use of water with low pH. A blender of 1500 watts was used to wet mill (using the buffer solution water) to get a slurry. The slurry was then poured into a covered plastic bucket and left to ferment depending on the fermentation time for the sample. After this, the slurry was poured into flat trays and put in the oven to dry. Thereafter, the samples were evaluated for proximate composition.

Modifications used in this study to optimize the processing of this weaning food includes;

1) Use of acidic pH water for steeping the Maize and Cowpea as well as during milling so as to limit the microorganisms to acidophiles.

2) The plastic buckets used were covered to prevent entry of unwanted microorganisms. Usually, indigenous technology requires open fermentation which allows other microorganisms into the fermenting vessel. This is in line with the findings of Anumudu, Omeje, Obinwa (2018) who observed that the microorganisms associated with the fermentation process are not strictly controlled neither is the succession pattern and the contribution of each of the microbial genera to the fermentation process clearly understood. Also, the water used during the milling was the steeping water to reduce unwanted microorganisms.

Table 3.1 **Independent variables and the level used for the design**.

Independent variables (Factors)	**Levels**		
Blend ratio	M85C15	M65C35	M50C50
pH	3.5	4.5	5.5
Fermentation time (hrs)	24	48	72

The variables which are the processing conditions (factors) includes: blend ratio, pH, and fermentation time. Each variable has three different values.

Table 3.2 Fifteen samples run showing different fermentation time, pH, and blend ratio.

Sample code	Blend ratio	pH	Fermentation time (hrs)
WFA	1	3.5	48
WFB	3	4.5	24
WFC	2	5.5	72
WFD	2	3.5	24
WFE	2	4.5	48
WFF	3	4.5	72
WFG	2	4.5	48
WFH	1	4.5	24
WFI	3	3.5	48
WFJ	2	4.5	48
WFK	1	4.5	72
WFL	2	5.5	24
WFM	3	5.5	48

WFN	1	5.5	48
WFO	2	3.5	72

Each sample had a unique set of processing conditions, each with its own value. There were a total number of 15 samples.

3.2.0 Methodology for Evaluation of Microbial Community

3.2.1: Preparation of media and diluents

Nutrient agar (NA) and MRS agar were prepared according to standard methods (Cheesebrough, 2006).

3.2.2 Preparation of samples and inoculation

Samples of the weaning food (10g) was dispersed in 90 ml of sterile distilled water. The samples were serially diluted decimally by transferring 1 ml from each tube until the required dilution was obtained. Aliquots of 0.1ml of appropriate dilutions was inoculated into the pre-sterilized and surface dried medium.

3.2.3 Determination of microbial population

Colony counts obtained on the media were expressed as colony forming units per gram (CFU/g) to obtain total population. (Harrigan & McCance,2000).

3.2.4 Microscopic Characterization

The Gram staining technique, spore staining test and motility test were carried out as described by Cheesbrough (2006). **See appendix 1A,1B,1C**

3.2.5 Biochemical characterization of bacterial isolates

Microorganisms that were not identified by the colonial and microscopic characteristics were further subjected to a few biochemical tests described by Cheesbrough (2006). **See Appendix 1D, 1E, 1F, 1G, 1H, 1I, 1J, 1L**

3.3 Methodology for Proximate Composition Analysis

The methods adopted for proximate composition were (AOAC, 2006) with slight modifications.

See appendix 2

3.4 Methodology for Sensory Evaluation of Weaning Food

Sensory evaluation was based on a 9-point hedonic scale. **See appendix 3**. The different sample formulations were given to 120 mothers. The formulation was prepared by mixing the dry powder with cold water to obtain a slightly thick consistency, then boiled hot water was added until it became a semi thick slurry. It was allowed to cool before serving to the babies.

3.5: Statistical Analysis

The analysis of the data was done using Minitab 17.0. The values obtained from the proximate analysis were entered into the data interface of Minitab 17.0 version and statistical analysis done on it. With the experimental data obtained, the regression coefficients and statistical significance of the model terms (ANOVA) were determined by fitting into the equation. Other parameters such as F-ratio were used to establish the significance of the model terms with the p-value < 0.05. The adequacy of the used model was ascertained using model analysis, coefficient of determination and lack of fit test.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Formulation and product characteristics

The final product of the weaning food formulation was dry and had a creamy colour. **See appendix 4**. The physical properties of the different sample slurries show that samples with high maize content were softer and smoother. Also, they had a good aroma, while samples with high cowpea content were thicker in consistency, grainier, and foamed during fermentation. They also had an awful smell. This could be attributed to the different blend ratios as well as the length of fermentation time.

4.2 Discussion on Table 4.1

Table 4.1 shows the average bacterial count on Nutrient agar/ MRS agar. Nutrient agar is a general purpose agar and will allow the growth of the desired acidophiles as well as any microorganism that may come in during contamination. de Man, Rogosa & Sharpe agar was used to favour the growth of *Lactobacillus*. Sample code WFG had the highest average colony counts on the two different media while sample WFA had the lowest colony count. Samples with high colony counts is an indication of the diversity of microorganisms present. Since sample code WFG has a high colony count, it may be attributed to contamination during the production process whereas sample code WFA had low colony counts because the modalities put in place as discussed above limited entry of unwanted microorganisms.

Table 4.1 Average bacterial count on Nutrient agar/ MRS agar

Sample codes	Colony Counts (CFU/g)
WFA	1.81×10^5
WFB	9.58×10^4
WFC	$3.1\ 3 \times 10^5$
WFD	2.47×10^5
WFE	8.65×10^4
WFF	3.71×10^5
WFG	9.66×10^4
WFH	5.57×10^5
WFI	9.16×10^4
WFJ	6.06×10^5
WFK	2.01×10^5
WFL	2.91×10^5

WFM	5.11 x 10^5
WFN	9.06 x 10^4
WFO	3.67 x 10^5

4.3: Discussion on Table 4.2

Table 4.2 below shows the colonial and microscopic characteristics of bacteria from the different blends during fermentation. The Table serves as a guide to identify to a reasonable extent the most probable identity of the microorganisms that exhibit similar characteristics. The different biochemical tests showed varied responses to the tests in respect to the microorganisms present. Total spore formers isolates were 33%, the motile isolates were 44 %. None was responsive to Oxidase and Coagulase. The isolates identified were *Corynebacterium sp, Streptococcus sp, Micrococcus sp, Lactobacillus sp, Bacillus sp, Enterococcus faecalis and Bacillus megaterium.*

.

Table 4.2 Microscopic and Biochemical Characteristics of Bacteria isolated from Different Blends

Spo	Mot	Gram rnx	Oxi	Cat	Coag	In	MR	VP	S	L	G	M	Identity of isolates
-	+	-R	-	+	-	+	-	+	+	+	+	-	*Corynebacterium* sp
-	-	-R	-	-	-	-	+	-	-	+	+	-	*Streptococcus* sp
-	-	+S	-	-	-	-	-	+	+	+	+	+	*Micrococcus luteus*
-	-	+R	-	-	-	-	+	-	+	+	+	+	*Lactobacillus* sp
+	+	+R	-	+	-	-	-	+	-	-	-	-	*Bacillus* sp
+	+	+R	-	+	-	-	-	+	-	-	-	+	*Bacillus* sp
-	-	+S	-	-	-	-	+	-	+	-	+	+	*Micrococcus roseus*
-	-	+S	-	-	-	-	+	-	+	-	_	-	*Enterococcus faecalis*
+	+	+R	-	+	-	-	+	-	+	+	+	-	*Bacillus megaterium*

Spo, Spore formation; Mot, Motility Test; Gram rnx, Gram reaction; Oxi, Oxidase; Cat, Catalase; Coag, Coagulase; In, Indole Test; MR, Methyl Red Test; VP, Voges Proskaeur Test; S, Sucrose; L, Lactose; G, Glucose; M, Maltose; +, Positive Test; -, Negative Test

4.3: Discussion on Table 4.3

Table 4.3 depicts the distribution of bacteria in different blend samples. Each of the sample had unique microbial communities present. This can be attributed to the variations in pH, blend ratios and fermentation time. *Bacillus* sp and *Corynebacterium* sp were consistently present in all blends. This is in line with Lei and Jakobsen (2003) who reported that microorganisms predominant in fermented cereals are *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus acidophilus.* Also, Obinna- Echem *et al*., (2013) reported that *Bacillus* sp is usually predominant in legume-based fermentation.

Table 4.3: Distribution of Bacteria in Different Blends Sample

Sample codes	Bacterial Isolates
WFA	*Bacillus cereus, Bacillus subtitis, Corynebacterium* sp; *Micrococcus luteus, Lactobacillus lactis, Streptococcus faecalis*
WFB	*Bacillus cereus, Bacillus subtitis, Corynebacterium* sp; *Micrococcus luteus, Lactobacillus lactis, Streptococcus faecalis*
WFC	*Bacillus cereus, Bacillus subtitis, Corynebacterium* sp; *Micrococcus luteus, Lactobacillus lactis, Streptococcus faecalis*
WFD	*Bacillus cereus, Bacillus subtitis, Corynebacterium* sp; *Micrococcus luteus, Lactobacillus lactis, Streptococcus faecalis*
WFE	*Bacillus cereus, Bacillus subtitis, Corynebacterium sp; Lactobacillus lactis*
WFF	*Bacillus cereus, Bacillus subtitis, Corynebacterium sp; Micrococcus luteus, Lactobacillus lactis*
WFI	*Bacillus cereus, Bacillus subtitis, Corynebacterium sp; Micrococcus luteus, Lactobacillus lactis, Streptococcus faecalis*
WFJ	*Bacillus cereus, Bacillus subtitis, Corynebacterium sp; Lactobacillus lactis; Micrococcus roseus*
WFK	*Bacillus cereus, Bacillus subtitis, Corynebacterium sp; Micrococcus luteus, Lactobacillus lactis*
WFL	*Bacillus cereus, Bacillus subtitis, Corynebacterium sp; Micrococcus luteus, Lactobacillus lactis, Streptococcus faecalis*
WFM	*Bacillus cereus, Bacillus subtitis, Corynebacterium sp; Lactobacillus lactis*
WFN	*Bacillus megaterium, Bacillus subtitis, Corynebacterium sp; Micrococcus luteus, Lactobacillus lactis, Micrococcus roseus*
WFO	*Bacillus cereus, Bacillus subtitis, Corynebacterium sp; Micrococcus luteus, Lactobacillus lactis, Streptococcus faecalis; Bacillus megaterium*

4.5: Discussion on Table 4.

Table 4.4 shows the predominant microorganisms were *Bacillus* sp, *Lactobacillus* sp *Micrococcus* sp, *Streptococuus faecalis* and *Corynebacterium* sp. *Bacillus cereus* had the highest percentage of occurrence followed by *Bacillus subtilis*. This can be attributed to the low pH environment. Streptococcus sp had the lowest occurrence. It grows symbiotically with *Lactobacillus* during fermentation and is a homofermenter.

The LAB group of bacteria is usually regarded as safe. Knowledge of the microorganisms involved may be utilized in improving the organoleptic qualities of the final product. Fermented foods exhibit beneficial effects on health by reducing blood cholesterol levels, increasing immunity, protecting against pathogens, fighting carcinogenesis, osteoporosis, diabetes, diabetes, obesity, allergies and atherosceloris and alleviating the symptoms of lactose intolerance (Tamang & Kaliasapathy., 2010). Maximizing these microorganisms by obtaining better strains with genetic manipulation will bring about optimization of the production of local weaning

Table 4.4: Percentage (%) Occurrence of Bacteria Isolated from Different sample blends.

Bacterial Isolates	Number of occurrence	% Occurrence
Bacillus cereus	23	18.4
Bacillus megaterium	8	6.4
***Streptococcus* sp**	4	3.2
Enterococcus faecalis	6	4.8
Bacillus subtilis	28	22.4
***Corynebacterium* sp**	18	14.4
Micrococcus roseus	10	8.0
Micrococcus luteus	14	11.2
***Lactobacillus* sp**	18	14.4
Total number of isolates	**129**	100%

4.5: SENSORY EVALUATION

A 9- point hedonic scale was used to compute the responses of participants to the evaluation. In agreement with other workers (Abubakar & Ziglar, 2021), It was observed from the results in Table 4.3 that the formulations were generally accepted by the mothers for their babies as the samples were presented to the mothers, who in turn fed their babies. The weaning food was reconstituted with cold water to get a light consistency and then hot water was added to get a thick slurry. Sample code WFC scored the highest with 95% acceptance. It was the most acceptable among the mothers. In other words, they were satisfied with the taste, colour, aroma and texture. The protein content of WFC was at 14% which falls short of WHO|FAO (1991) recommendation for protein which is 20%. Although taking into cognizance the specific processing parameters at play for this sample order WFC, it is safe to say that holding pH at 5.5, blend ratio of M65C35, fermentation time at 72 hours with a controlled fermentation culture, the final product will be of great commercial value since acceptability is very high. Conversely, sample order WFA fit into the standard recommendation of WHO|FAO (1991) as can be seen in Table 4.3. Further research will be on enhancing and harmonizing the processing parameters of the above sample codes to obtain not just an acceptable but a nutritious weaning food as well.

Table 4.5: Values for sensory evaluation (%)

Sample order	Colour	Taste	Texture	Flavour	Aroma	Acceptance
WFA	75	79	90	87	93	80
WFB	60	90	75	72	81	80
WFC	95	85	87	90	85	95
WFD	90	80	87	89	76	76
WFE	95	75	74	70	65	75
WFF	65	71	89	80	75	90
WFG	95	87	90	67	89	78
WFH	75	90	65	87	65	76
WFI	68	90	87	75	68	66
WFJ	95	76	76	80	87	76
WFK	75	76	65	87	87	68
WFL	95	77	74	78	96	88
WFM	63	78	77	68	65	88
WFN	75	65	78	86	71	83
WFO	95	65	72	88	90	88

4.6 PROXIMATE COMPOSITION ANALYSIS

The different processing conditions and their effects on the quality characteristics of cowpea and maize blend are shown in table 4.6. The independent or explanatory variables are pH, cowpea and maize blend and fermentation time. The dependent or response variable are crude protein, fat, ash, crude fiber, moisture and Nitrogen Free Extract (NFE).

The protein content ranged from 10.7 to 21.2 %. WFA had the highest protein content while WFL had the lowest protein content. The presence of protein can be attributed to the addition of cowpea to the maize (Adeyanju, Abioye, Ogunlakin, Oyelade, Adesina & Oloyede, 2022). Temple & Bassa (1991) reported that adding legumes to cereals improved protein intake. With the presence of protein, the objective of a fortified weaning food has been achieved. Protein is required for tissue replacement, the deposition of lean muscle mass, and growth. (Adeyanju et al., 2022. Further improvements will be on adjustments to be in line Recommended Daily Allowance for infants.

The fat content was between 3.5 and 5.7% which is within the range of WHO|FAO (1991) standard of 6%. High levels of fat may cause rancidity and with odour as reported by (Ihekoronye, 1985). Fat enhances taste and increases acceptance as well as adds to shelf life. It is therefore recommended that fat containing additives should be added to this weaning food to achieve a good weaning diet for infants.

Moisture content varied between 5.2- 17.1%. Moisture content measures the amount of water that a food contains thereby showing its storage capacity. Sample WFB had the highest moisture content, this may be as a result of its Maize-Cowpea blend ratio (M50 : C50) since the legume is of equal amount, the foaming from the legume increased the moisture of the formulation.

Compared to WFG which had the lowest moisture content closer to recommendation by PAG (1971) where recommended moisture value should be 5-10%. Variations in moisture may also be attributed to the amount of water squeezed out from the slurry, this is in line with Obinna-Echem *et al*., 2013).

The ash content of the samples was between 1.2 -3.7%. Ash content measures the amount of ash left over after burning. Low ash content is an indicator of higher quality of food. This is because processed foods have high ash content which is a pointer that a large portion of the food is not useful to the consumer. Since weaning should be a continuum of breastmilk, it is expected that a large portion if not all should be useful to the infant for proper growth

Crude fibre values ranged from 0.7 and 2.9%. Fibre is the indigestible part of food. Cereals and legumes have been reported to contain fibre. The benefits of fibre as revealed by Maćkowiak et al.,2016) includes: reducing the risk and lowering the incidence of numerous diseases. However, in weaning foods, NHS (National Health Service) suggests minimal amount of fibre in weaning foods because a lot of fibre can fill up small tummies leaving little room for other foods. So, the samples had the adequate amount of fibre for a weaning food.

Nitrogen free extract (NFE). This is calculated by adding other nutrient values and then subtracting the total from 100. It consists of carbohydrates, sugars, starches,and a major portion of materials classified as hemicellulose in feeds.(FAO, 1991) The Nitrogen free extract ranged from 57-72%.

Table 4.6: Mean values of proximate analysis (%) done in duplicates

	MEAN					
Sample						
	Crude protein	**Fat**	**Ash**	**Crude fibre**	**Moisture**	**NFE**
WFA	21.21	4.23	1.58	2.87	6.48	63.64
WFB	16.52	4.65	2.81	1.24	17.10	57.70
WFC	14.71	4.24	2.65	0.84	6.59	70.98
WFD	12.62	4.75	2.26	0.70	6.89	72.78
WFE	16.85	3.45	1.61	2.40	6.08	69.62
WFF	17.05	3.64	2.47	1.54	14.05	61.26
WFG	17.30	3.82	2.84	3.89	5.17	67.01
WFH	9.97	4.34	2.35	1.15	6.11	76.10
WFI	12.14	4.47	1.72	0.82	9.05	71.81
WFJ	14.27	4.12	2.19	1.00	8.64	69.79
WFK	11.37	5.63	1.24	1.27	8.76	71.75
WFL	11.12	5.13	2.59	0.96	8.12	72.10
WFM	15.06	4.43	2.98	3.35	9.69	64.50
WFN	10.75	4.84	3.24	0.93	9.52	70.73
WFO	15.37	5.71	2.70	2.94	11.24	62.05

Table 4.7: Standard deviation values of the proximate analysis

			SD			
Sample						
	Crude protein	**Fat**	**Ash**	**Crude fibre**	**Moisture**	**NFE**
WFA	0.24	0.04	0.03	0.03	0.10	0.31
WFB	0.12	0.03	0.02	0.03	0.43	0.33
WFC	0.18	0.03	0.02	0.02	0.02	0.17
WFD	0.05	0.05	0.01	0.01	0.01	0.01
WFE	0.15	0.07	0.03	0.10	0.03	0.18
WFF	0.06	0.01	0.03	0.05	0.39	0.44
WFG	0.04	0.04	0.03	0.06	0.04	0.08
WFH	0.07	0.03	0.03	0.01	0.02	0.10
WFI	0.20	0.03	0.02	0.02	0.05	0.28
WFJ	0.36	0.05	0.04	0.01	0.03	0.39
WFK	0.09	0.04	0.01	0.02	0.03	0.10
WFL	0.06	0.68	0.03	0.02	0.02	0.59
WFM	0.09	0.05	0.03	0.02	0.10	0.18
WFN	0.16	0.03	0.03	0.03	0.03	0.16
WFO	0.44	0.03	0.03	0.03	0.32	0.79

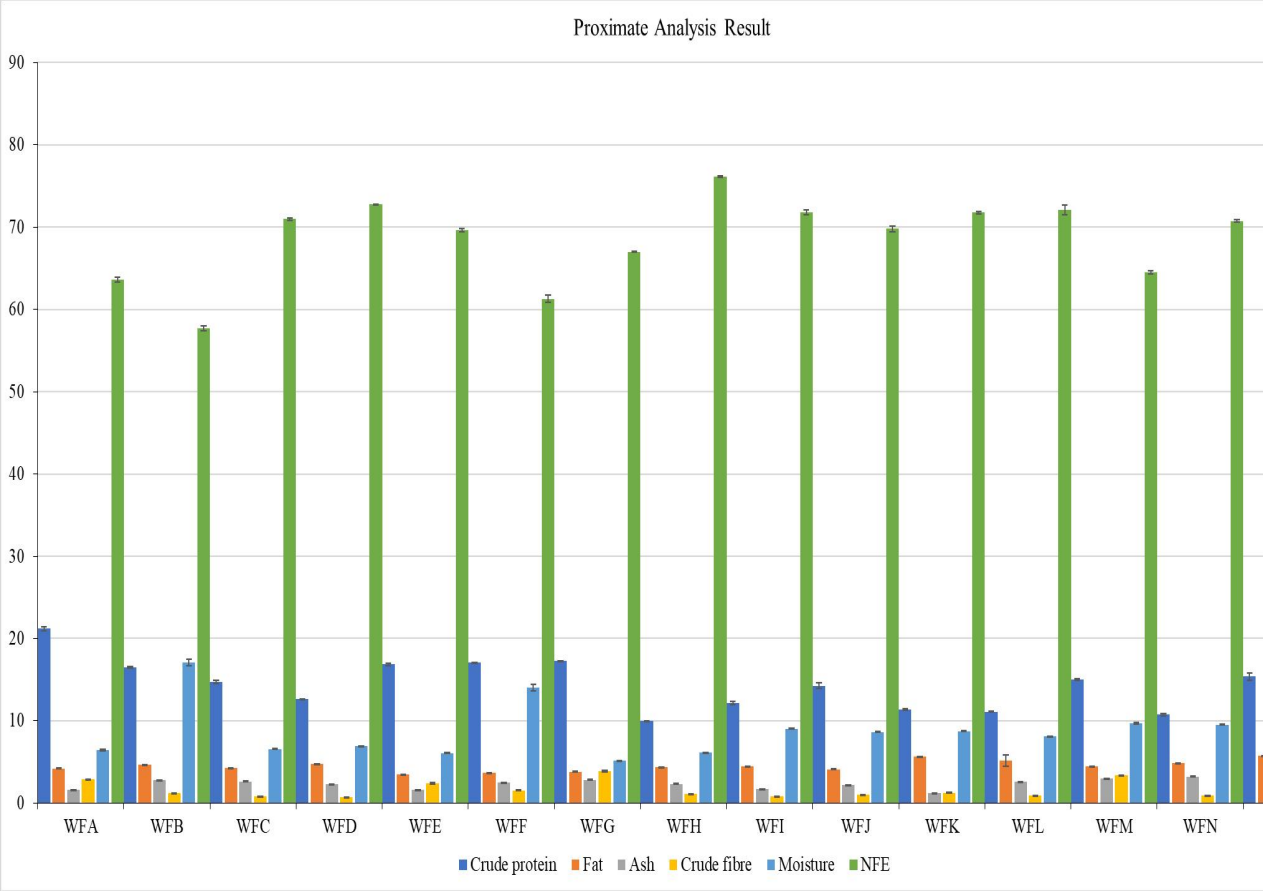

Fig 1: Values of proximate analysis on a bar graph with error bars

4.7 ANOVA, surface plots, contour plots of results of proximate composition analysis values

Three processing parameters were varied on three different levels (Table 3.1). This was done to maximize mainly protein as a fortifier for the maize cereal as well as other food components. The relationship between the independent variables (% cowpea substitution, pH and fermentation time) and the dependent variables (moisture, fat, protein content, ash content, fibre and NFE) was investigated.

4.8 FAT

The fat content of cowpea blended with maize ranged from 3.5 to 5.7% (Table 4.5). The p-value of the model is shown to be <0.05 which demonstrates the significance level for the model used. Model terms such as fermentation time, pH*pH, fermentation time*fermentation time, pH*fermentation time, blend ratio*fermentation time are all significant for the fat model because their p-value is <0.05 (Appendix 5A). Apart from p-value, other statistical parameters were used for example coefficient of determination (R^2), adjusted R^2 (R^2 adj) and standard deviation (S). The R^2 and R^2 adj of the fat model is shown to be 0.91 and 0.74 while the standard deviation is 0.36 (Appendix 5B). An R^2 close to unity (1) and a smaller standard deviation values signifies a better predicting response of the model used.

To determine the combined effect of the factors on fat content, 2D contour plots and 3D surface plots were used (**Fig 2A - Fig 2C).** These plots are graphical representation of regression equation. The 2D contour plots shows that there is an increase in fat content

(dark green) when pH is around 5.5 and blend ratio close to 15% with fermentation time held at 48 (**Fig 2A).** With blend ratio held at 2, there is increase in fat content when fermentation time is 72 hours and pH either at 3.5 or 5.5 (Fig 2B). Furthermore, when the pH is held at 4.5, the fat content increases as the fermentation time is approximately 70 hours and blend ratio around 15 **(Fig 2C).** Similar information is seen clearly with the 3D surface plots (**Fig 2D-2F).** Overall, with response optimization, it is shown that the optimum pH, blend ratio and fermentation time is 3.5, 15% and 72 hours respectively (**APPENDIX 6A & 6B)**

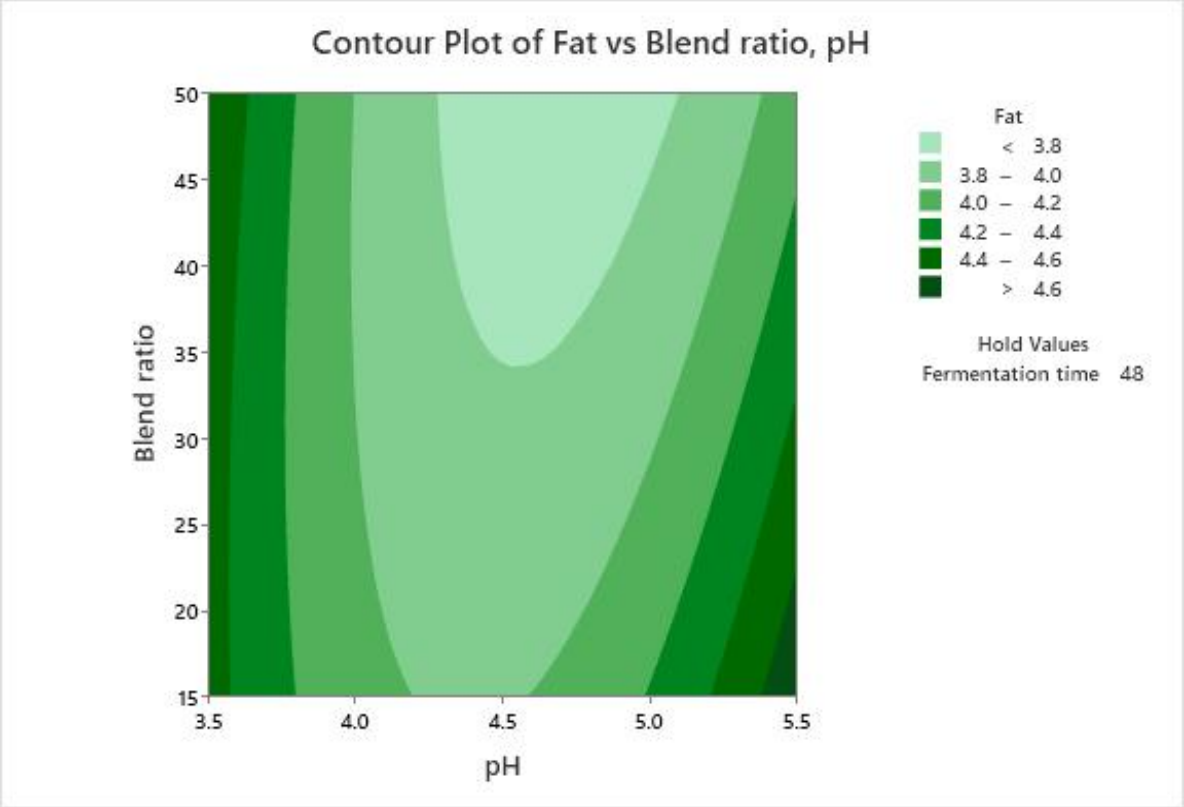

Figure 2A: Contour plot of fat vs blend ratio, pH

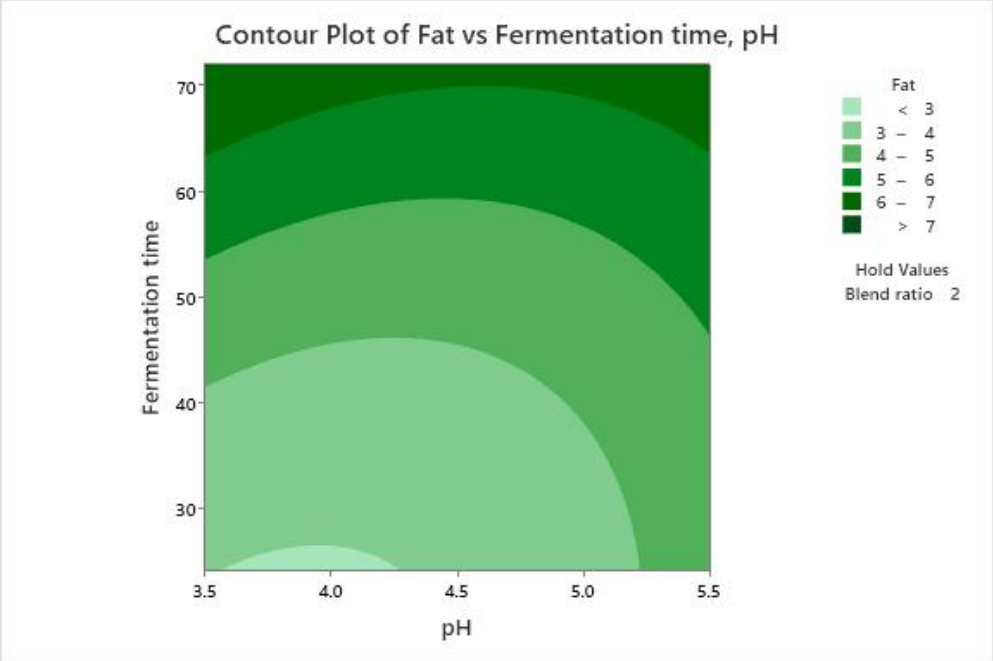

Figure 2B: Contour plot of fat vs fermentation time, pH

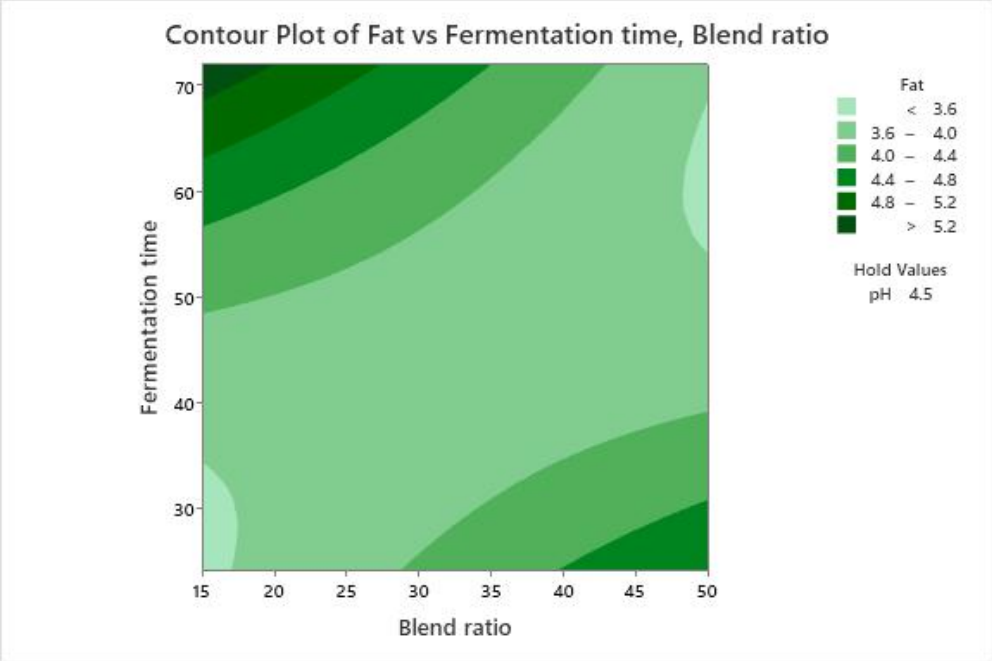

Figure 2C: Contour plot of fat vs fermentation time, pH

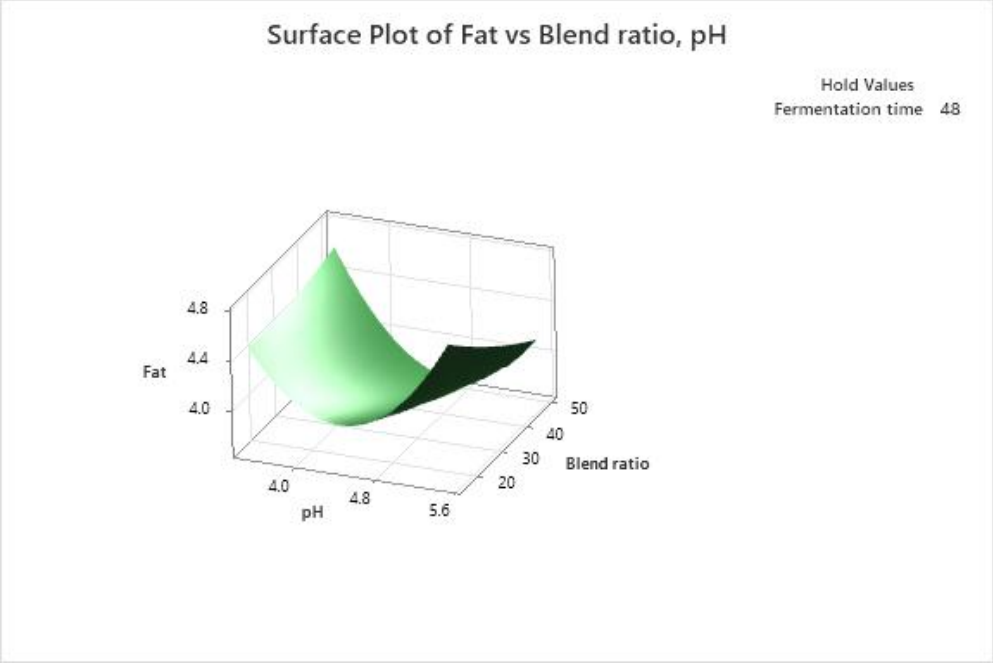

Figure 2D: Surface plot of fat vs blend ratio, pH

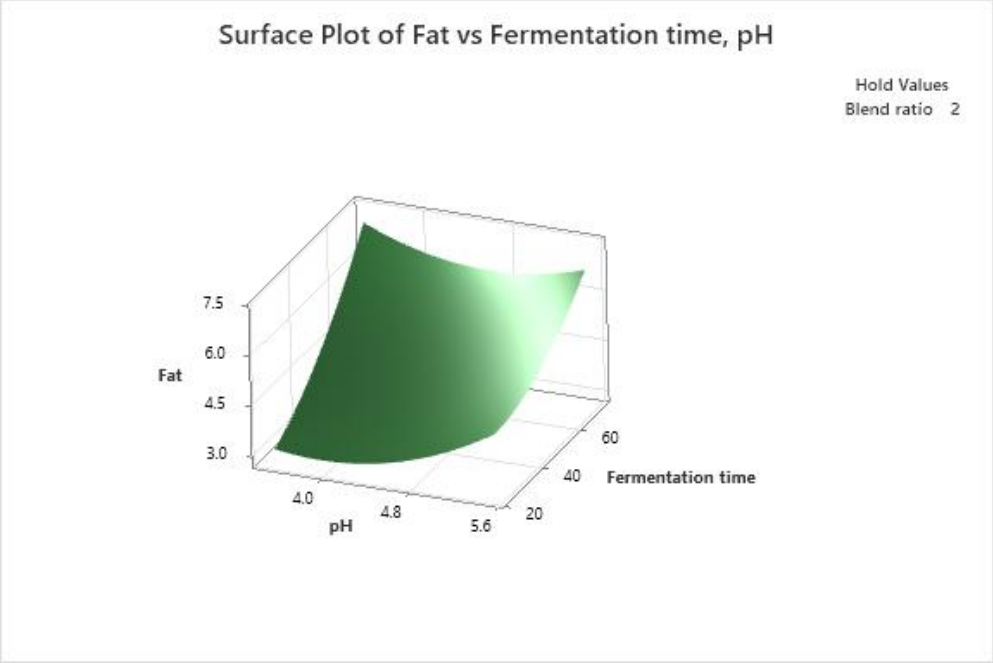

Figure 2E: Surface plot of fat vs fermentation time, pH

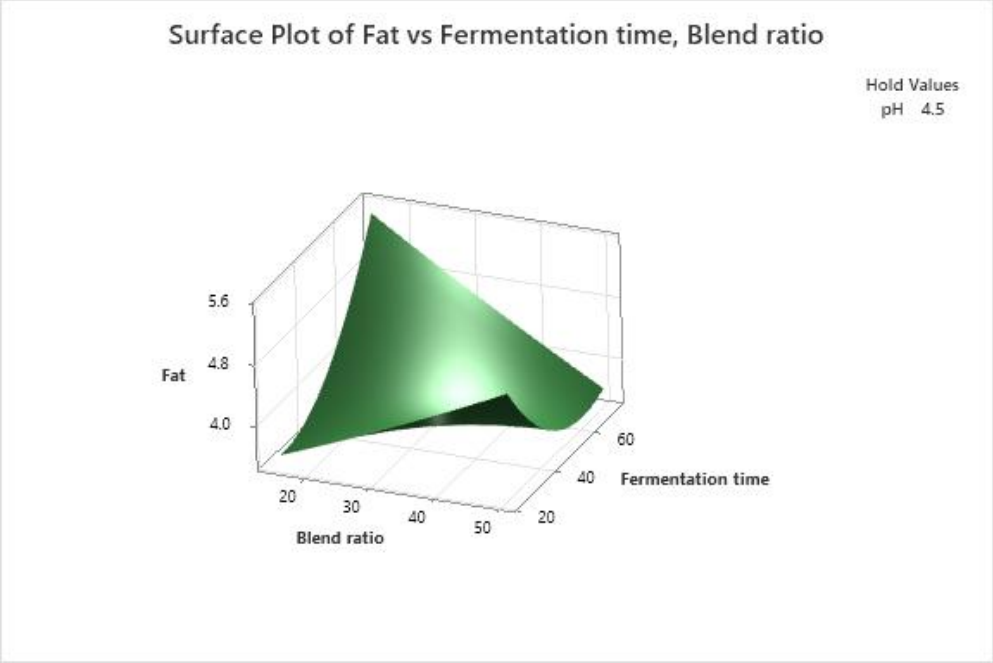

Figure 2F: Surface plot of Fat vs fermentation time, blend ratio

The optimized parameters for fat are as follows: pH(3.5), Blend ratio (M85: C15), Fermentation time (72 hours).

4.9 MOISTURE

The p-value of the model is shown to be <0.05 which demonstrates the significance level for the model used (Appendix 5B). The model terms such as blend ratio*blend ratio is significant for the moisture model because their p-value is <0.05. Apart from p-value, other statistical parameters were used for example coefficient of determination (R^2), adjusted R^2 (R^2 adj) and standard deviation (S). The R^2 and R^2 adj of the moisture content model is shown to be 0.77 and 0.38 while the standard deviation is 2.47 (Appendix 5C). An R^2 close to unity (1) and a smaller standard deviation values signifies a better predicting response of the model used.

To determine the combined effect of the factors on moisture, 2D contour plots and 3D surface plots were used **(Fig 3A- Fig 3C).** These plots are graphical representation of regression equation. The 2D contour plots shows that there is an increase in moisture content when pH is around 4.5 and blend ratio close to 50% with fermentation time held at 48 (**Fig 3A**). With blend ration held at 2, there is an increase in moisture content when fermentation time is 24 hours and pH of 5.5 **(Fig 3B).** Furthermore, when the pH is held at 4.5, the moisture content increases as the fermentation time is approximately 24 hours and blend ratio around 50% (**Fig 3C).** Similar information is seen clearly with the 3D surface plots (**Fig 3D-F).** Overall, with response optimization, it is shown that the optimum pH, blend ratio and fermentation time is 4.8, 50% and 24 hours respectively (Appendix 6C & 6D).

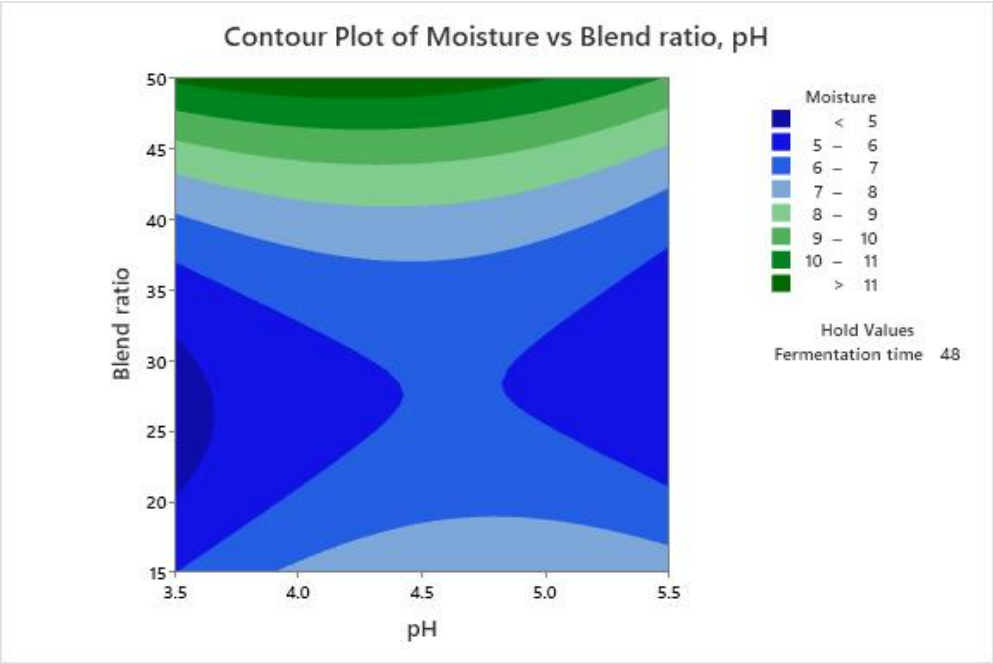

Figure 3A: Contour plot of moisture vs blend ratio, pH

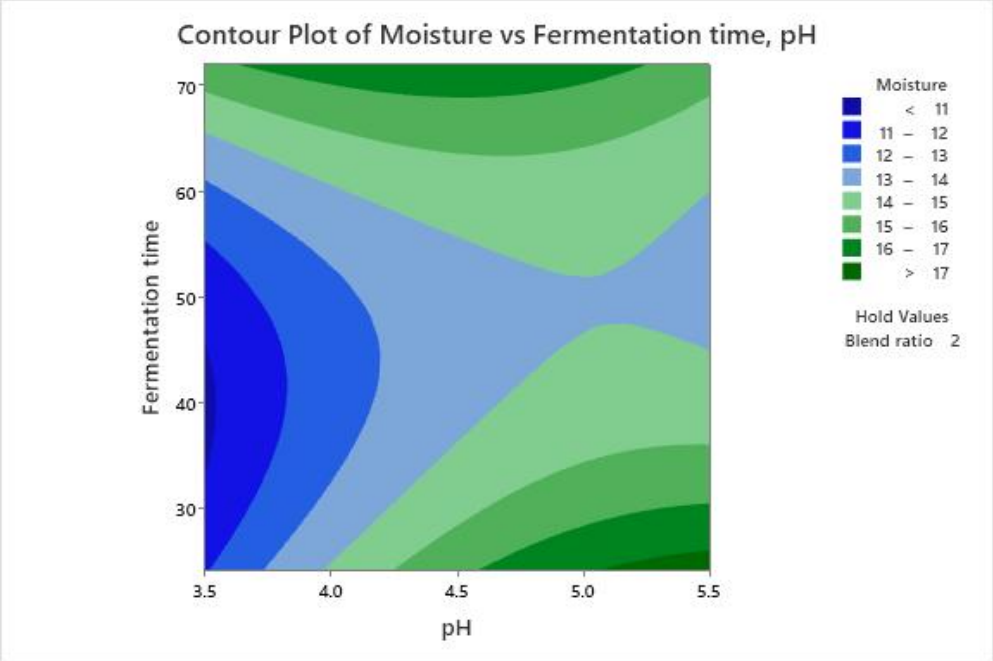

Figure 3B: Contour plot of moisture vs fermentation time, pH

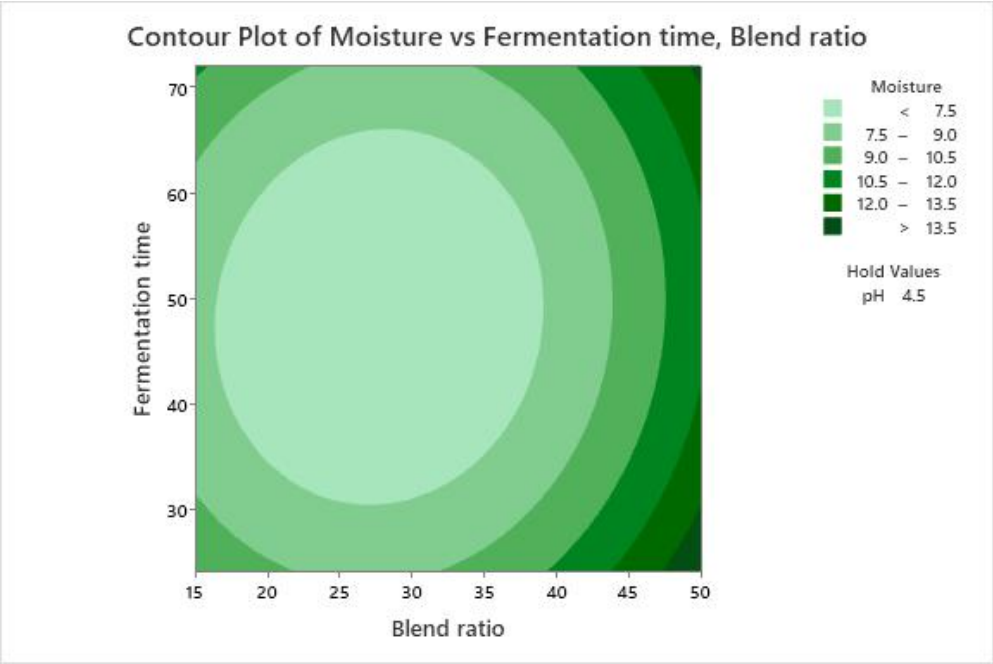

Figure 3C: Contour plot of moisture vs fermentation time, blend ratio

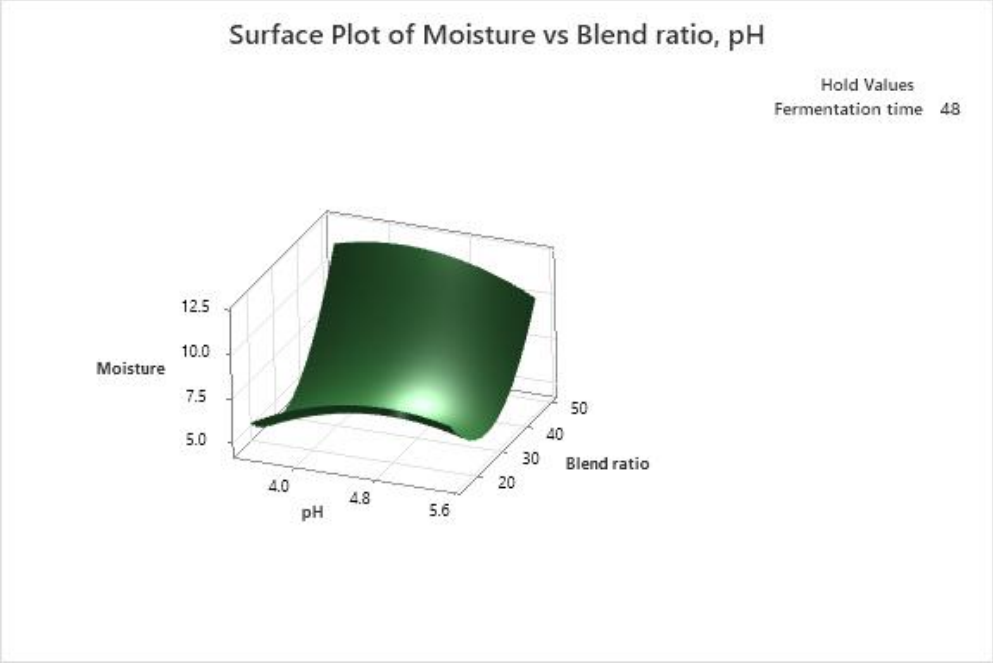

Figure 3D: Surface plot of moisture vs blend ratio, pH

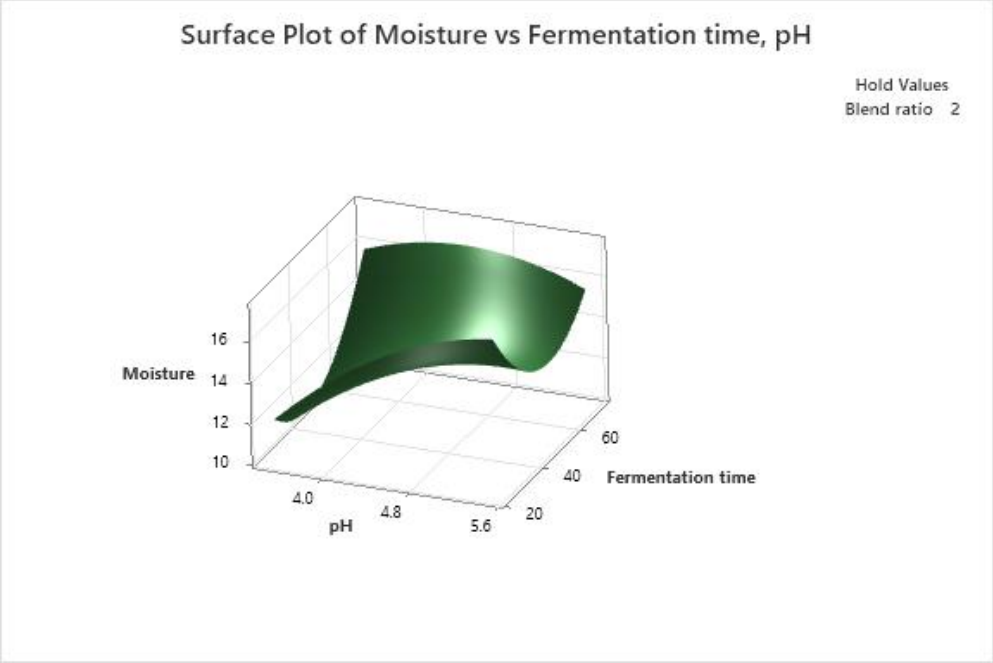

Figure 3E: Surface plot of moisture vs fermentation time, pH

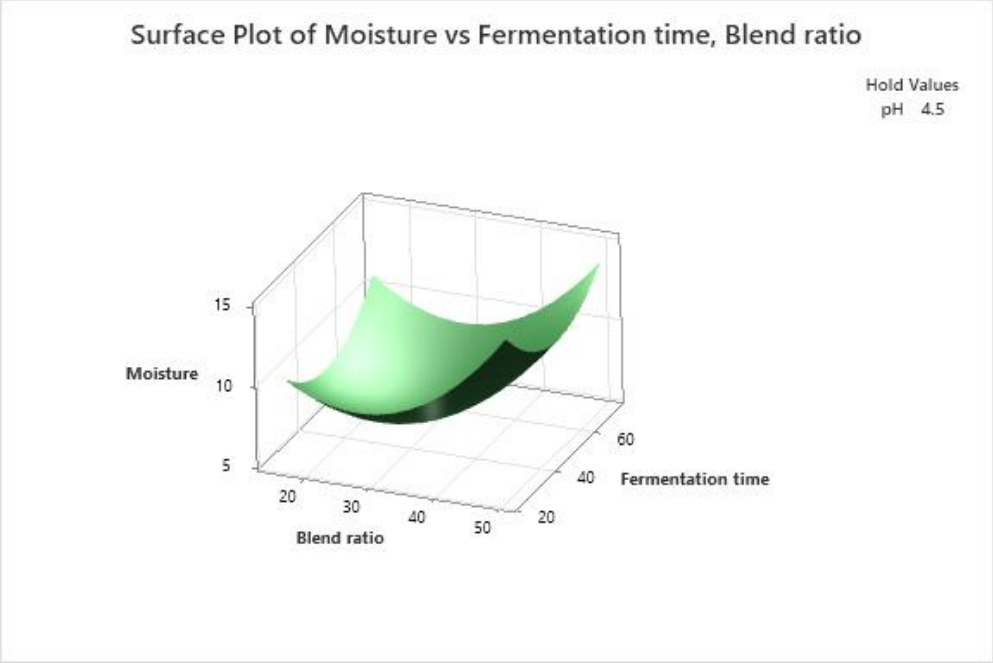

Figure 3F: Surface plot of moisture vs fermentation time, blend ratio

The optimized parameters for fat are as follows: pH (4.5), Blend ratio (M50: C50), Fermentation time (24 hours).

4.9.1 CRUDE PROTEIN

The p-value of the model is shown to be <0.05 which demonstrates the significance level for the model used (Appendix 5E).The model terms such as pH and pH*blend ratio are significant for the crude protein model because their p-value is <0.05. Apart from p-value, other statistical parameters were used for example coefficient of determination (R^2), adjusted R^2 (R^2 adj) and standard deviation (S). The R^2 and R^2 adj of the crude protein content model is shown to be 0.72 and 0.21 while the standard deviation is 2.5 (Appendix 5F). An R^2 close to unity (1) and a smaller standard deviation values signifies a better predicting response of the model used.

To determine the combined effect of the factors on crude protein, 2D contour plots and 3D surface plots were used **(Fig 4A- Fig 4C).** These plots are graphical representation of regression equation. The 2D contour plots shows that there is an increase in crude protein when pH is around 3.5 and blend ratio close to 15% with fermentation time held at 48 **(Fig 4A).** With blend ratio held at 2, there is an increase in crude protein content when fermentation time is 24 hours and pH of 3.5 **(Fig 4B).** Furthermore, when the pH is held at 4.5, the crude protein content increases as the fermentation time is approximately 24 hours and blend ratio around 15% **(Fig 4C**). Similar information is seen clearly with the 3D surface plots **(Fig 4D-F).** Overall, with response optimization, it is shown that the optimum pH, blend ratio and fermentation time is 3.5, 15% and 24 hours respectively **(Appendix 6E & 6F).**

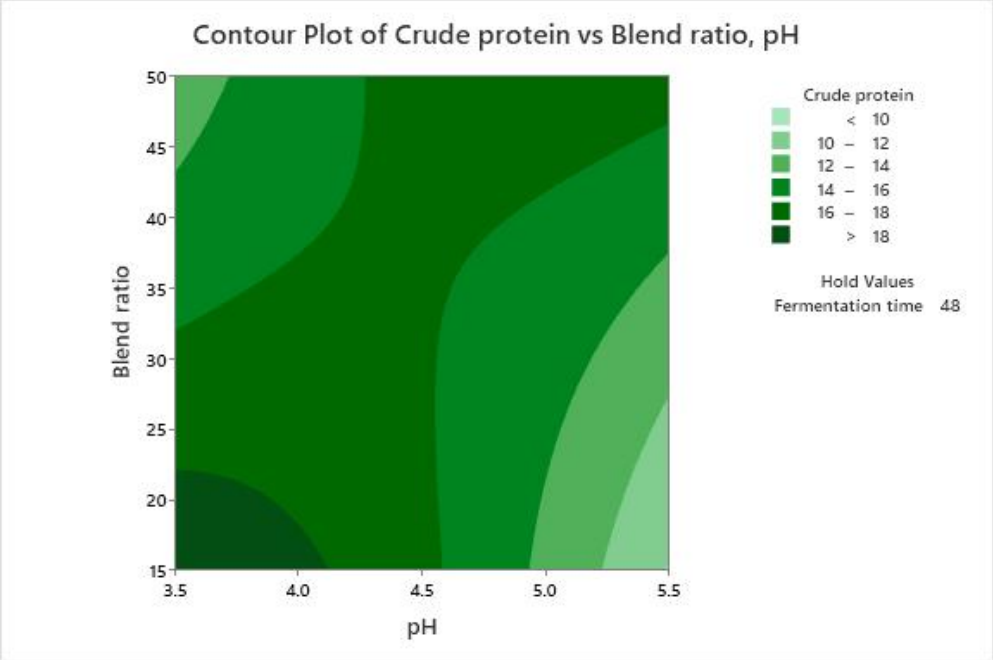

Figure 4A): Contour plot of crude protein vs blend ratio, pH

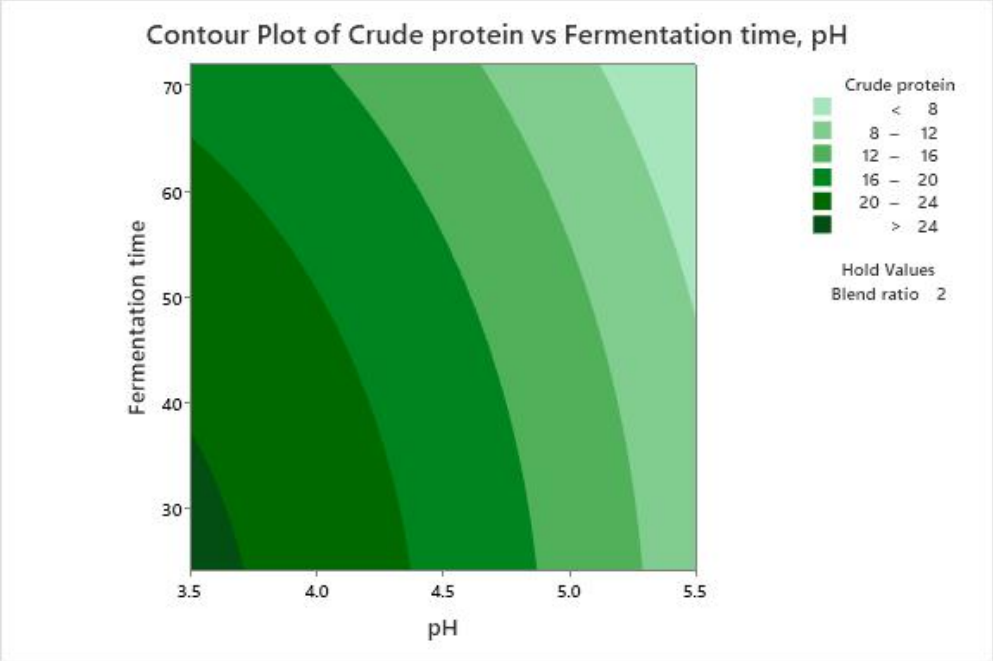

Figure 4B: Contour plot of crude protein vs fermentation time, pH

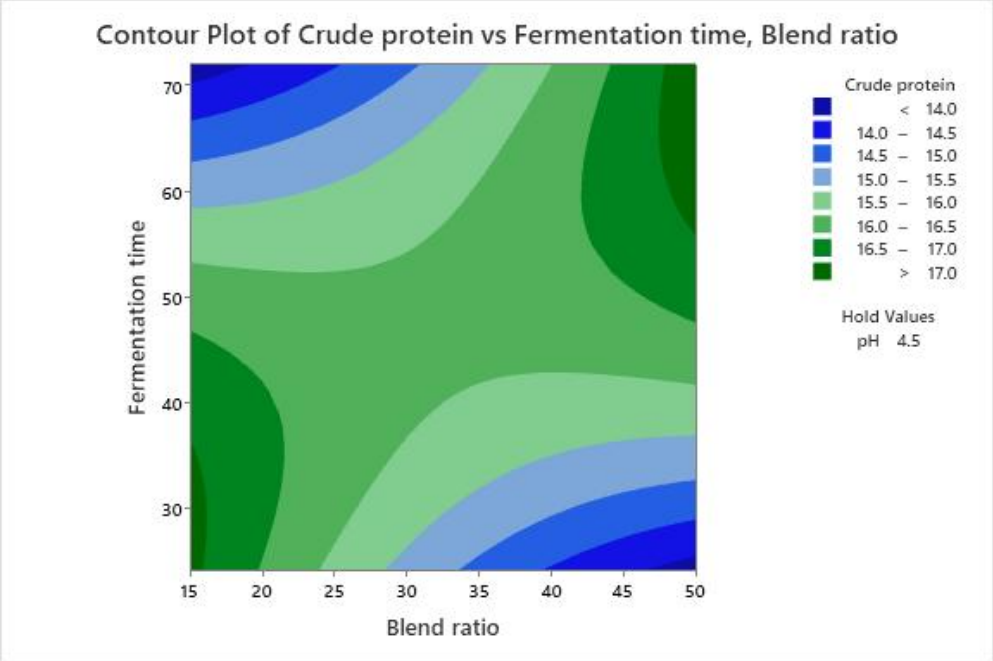

Figure 4C: Contour plot of crude protein vs fermentation time, blend ratio

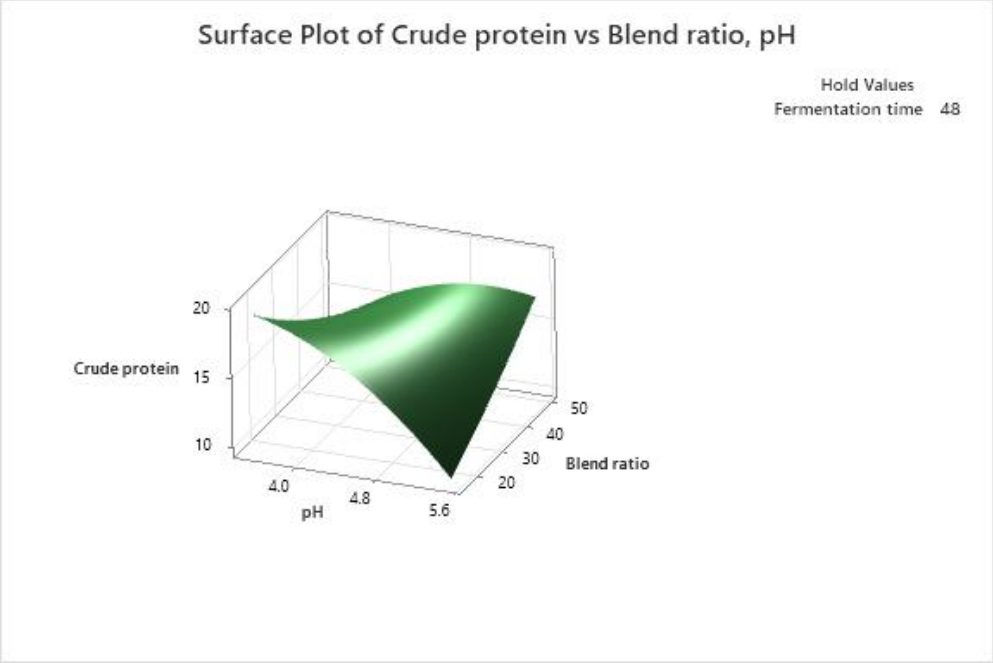

Figure 4D: Surface plot of crude protein vs blend ratio, pH

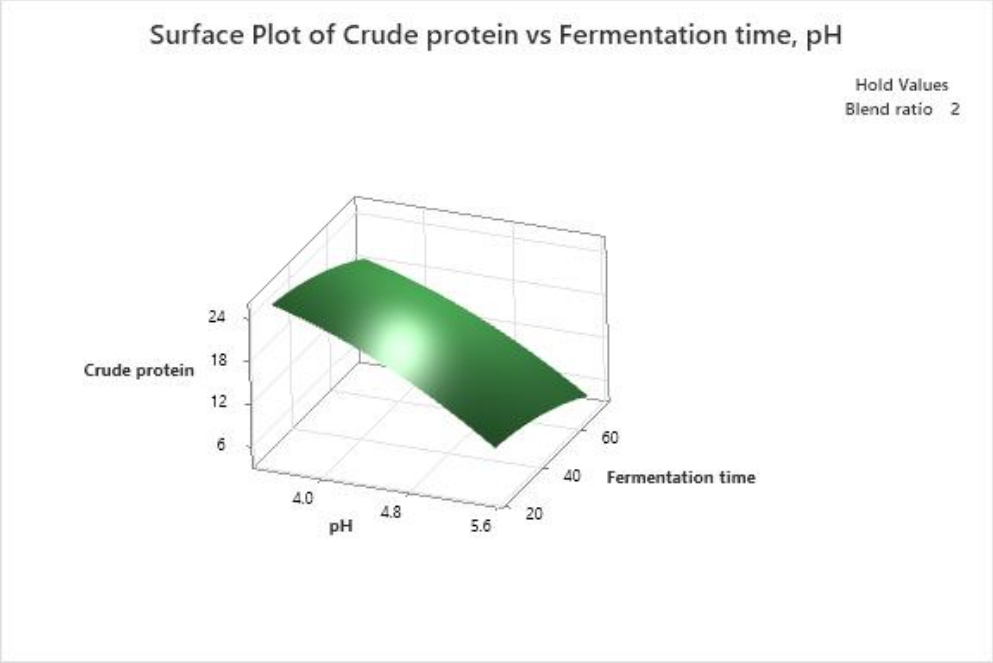

Figure 4E: Surface plot of crude protein vs fermentation time, pH

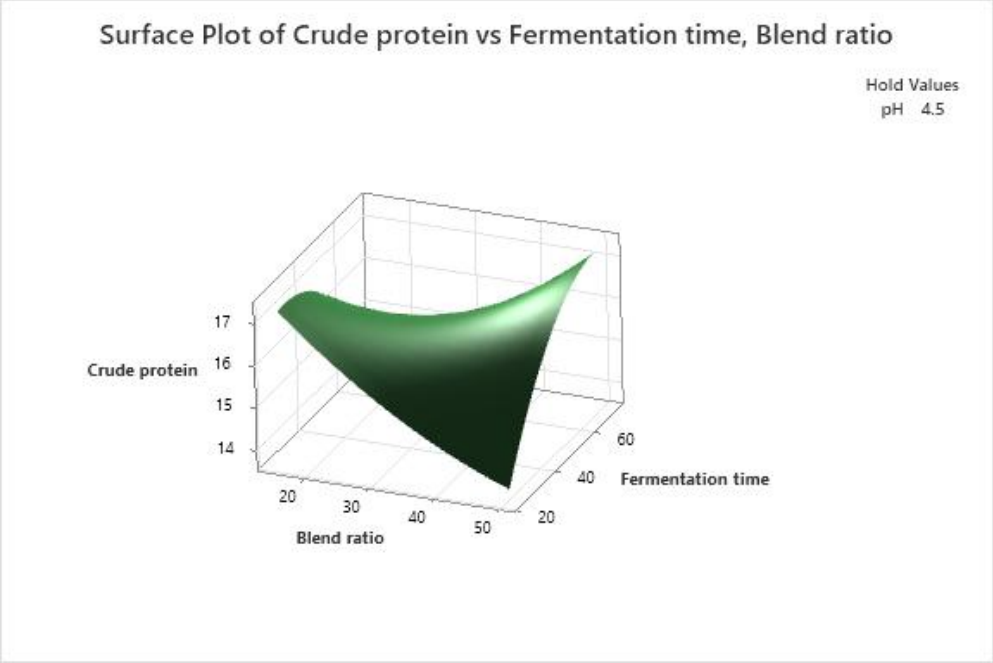

Figure 4F: Surface plot of crude protein vs fermentation time, pH

The optimized parameters for fat are as follows: pH (3.5), Blend ratio (M85: C15), Fermentation time (24 hours).

4.9.2 CRUDE FIBRE

The p-value of the model is shown to be <0.05, which demonstrates the significance level for the model used (Appendix 5D). The model terms are not significant for the crude fiber model because their p-value is <0.05. Apart from p-value, other statistical parameters were used for example coefficient of determination (R^2), adjusted R^2 (R^2 adj) and standard deviation (S). The R^2 and R^2 adj of the crude fiber content model is shown to be 0.60 and 0.00 while the standard deviation is 1.10 (Appendix 5E). An R^2 close to unity (1) and a smaller standard deviation values signifies a better predicting response of the model used.

To determine the combined effect of the factors on crude fiber, 2D contour plots and 3D surface plots were used (Fig 5A–Fig 5C). These plots are graphical representation of regression equation. The 2D contour plots shows that there is an increase in crude fiber when pH is around 3.5 and blend ratio close to 15% with fermentation time held at 48 (Fig 5A). With blend ration held at 2, there is an increase in crude fiber content when fermentation time is 55 hours and pH of 3.5 (Fig 5B). Furthermore, when the pH is held at 4.5, the crude fiber content increases as the fermentation time is approximately 55 hours and blend ratio around 35% (Fig 5C). Similar information is seen clearly with the 3D surface plots (Fig 5D-F). Overall, with response optimization, it is shown that the optimum pH, blend ratio and fermentation time is 3.5, 15% and 55 hours respectively (Appendix 6 G & H).

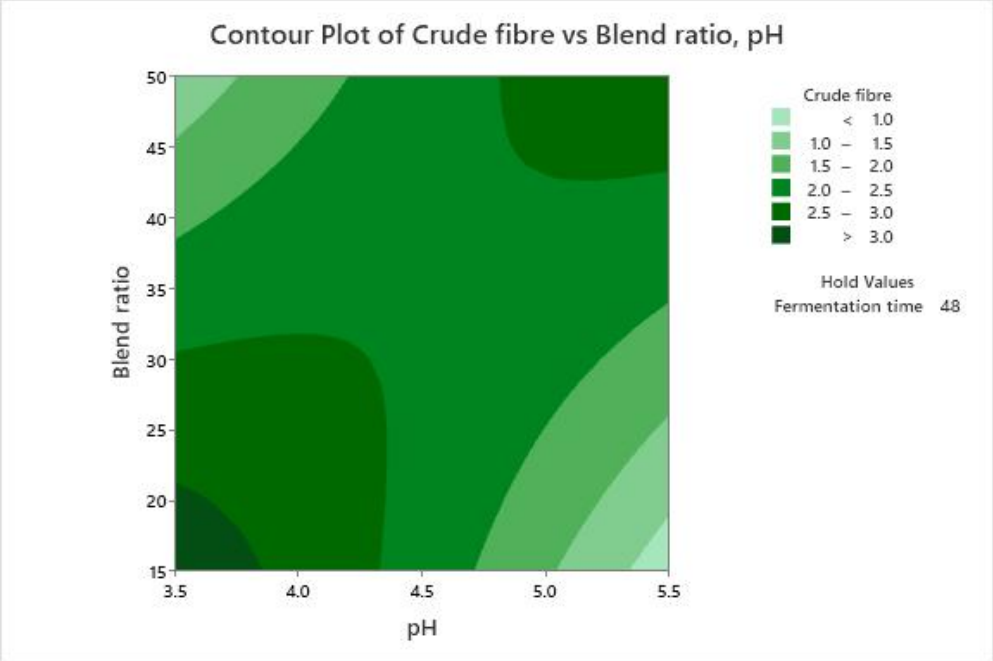

Figure 5A: Contour plot of crude fibre vs blend ratio, pH

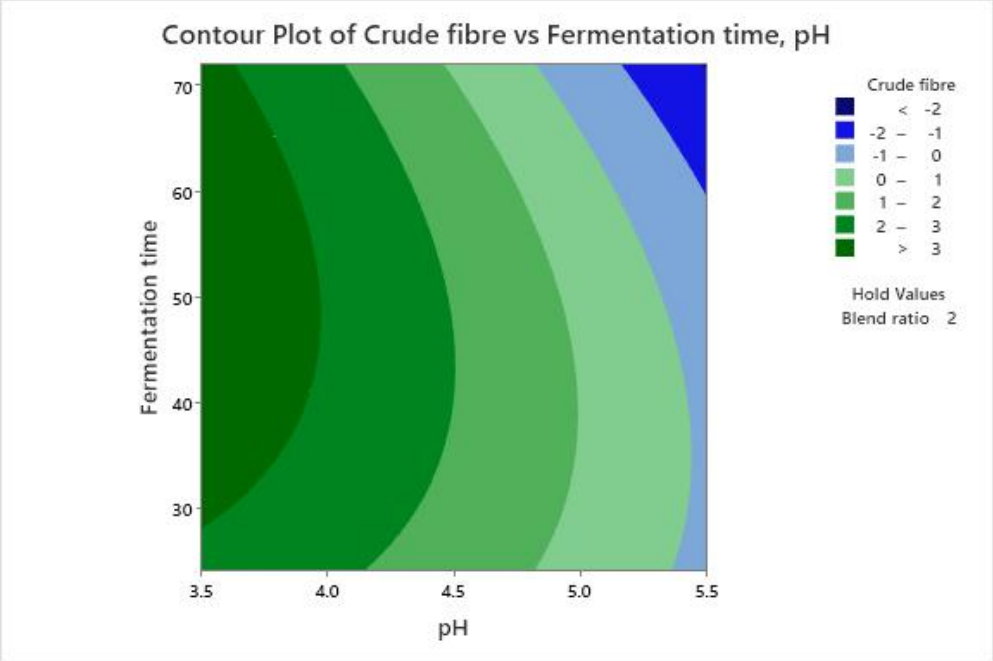

Figure 5B): Contour plot of crude fibre vs fermentation time, pH

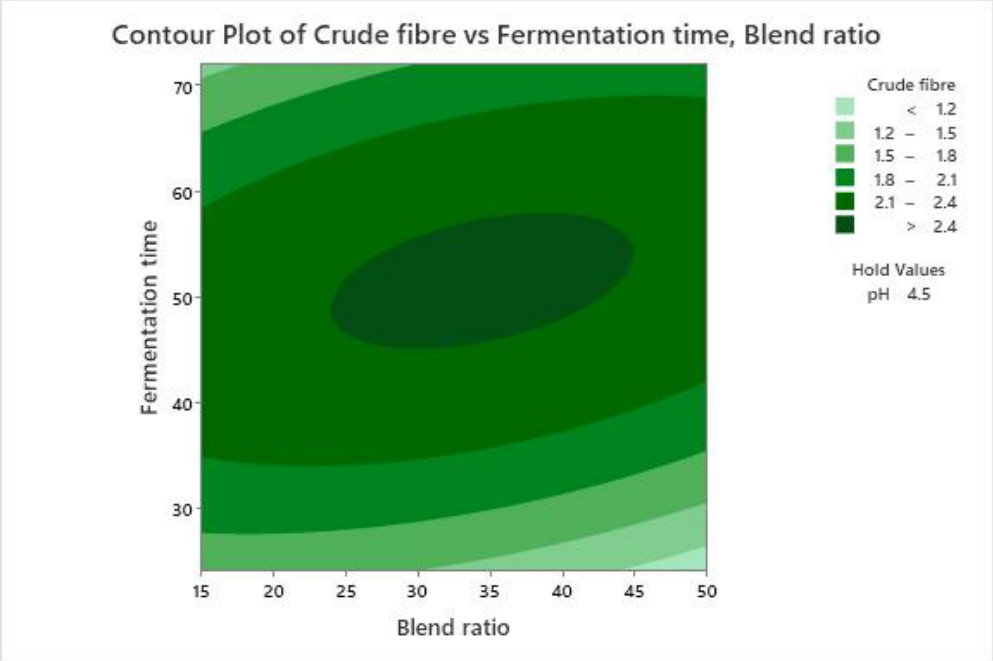

Figure 5C): Contour plot of crude fibre vs fermentation time, blend ratio

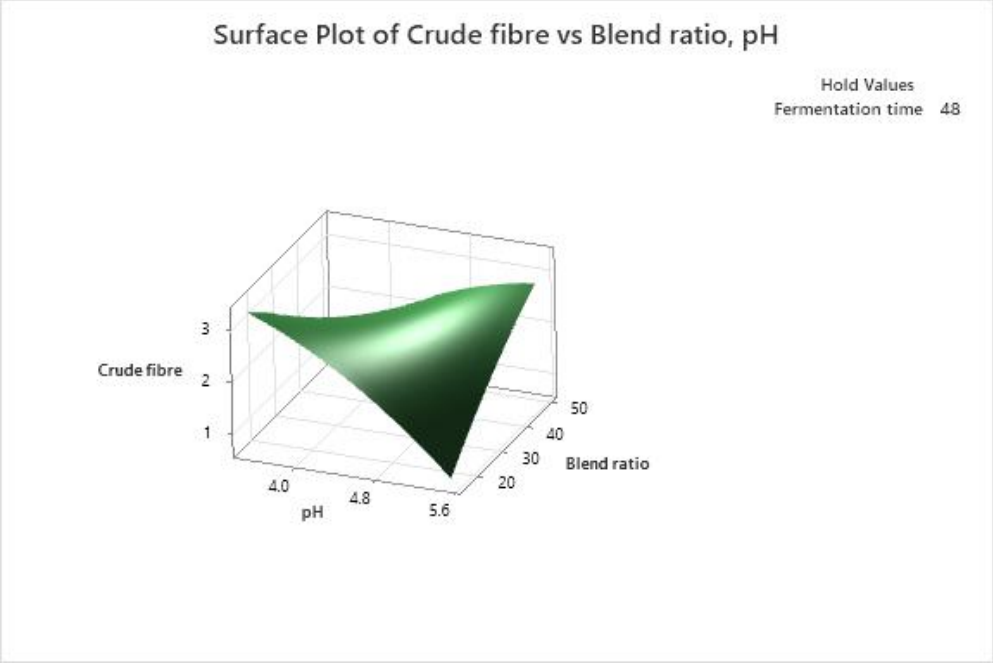

Figure 5D): Surface plot of crude fibre vs Blend ratio,pH

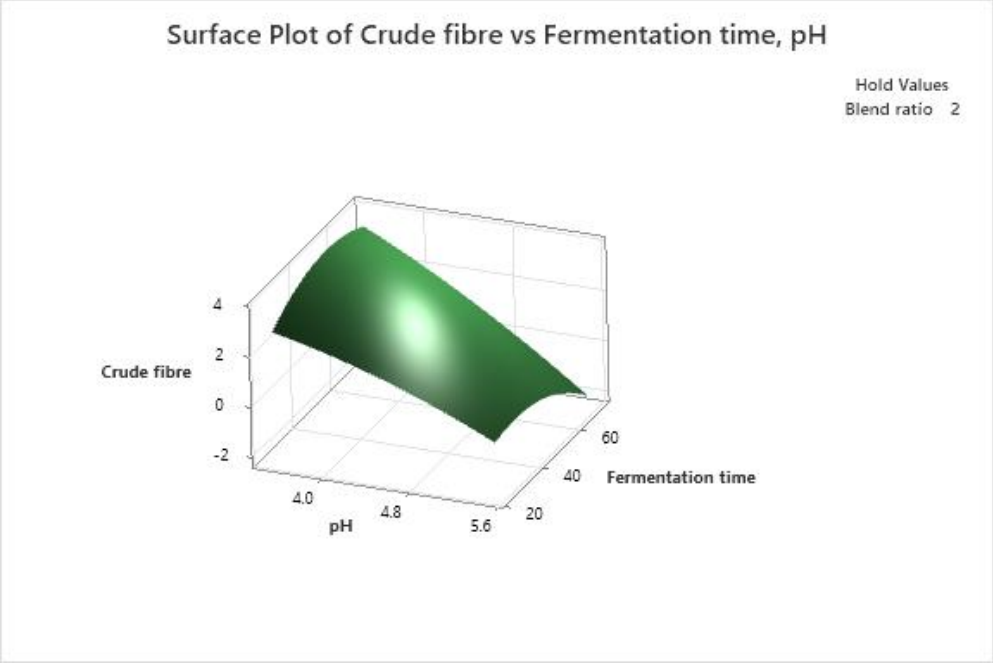

Figure 5E): Surface plot of crude Fibre vs Fermentation Time, pH

The optimized parameters for fat are as follows: pH (3.5), Blend ratio (M85: C15), Fermentation time (55 hours).

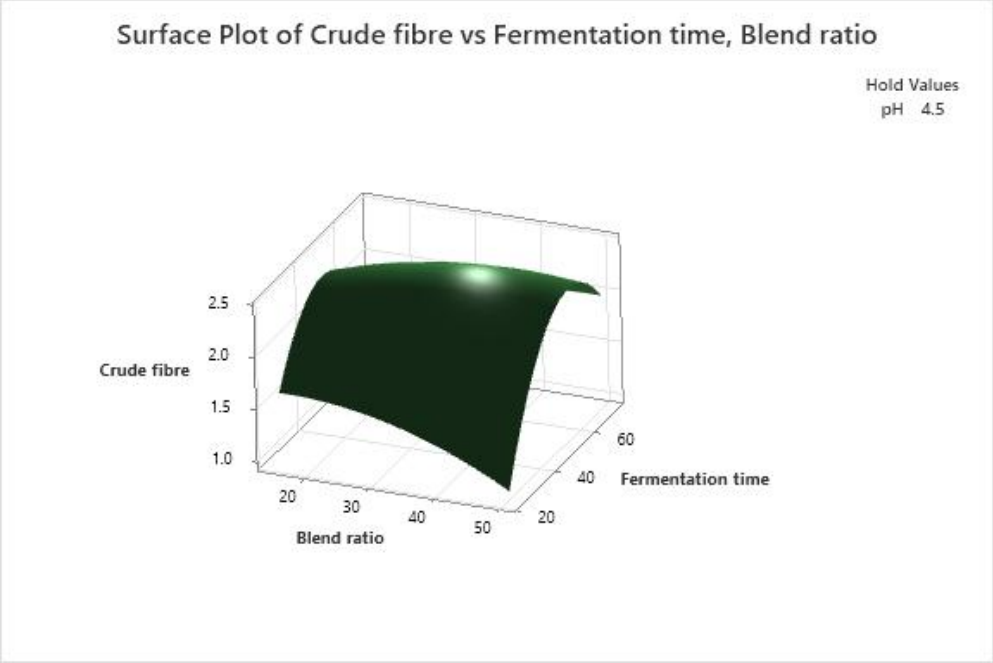

Figure 5F): Surface plot of Crude Fibre vs Fermentation Time, blend ratio

4.9.3 NITROGEN FREE EXTRACT

The p-value of the model is shown to be <0.05 which demonstrates the significance level for the model used (Appendix 5E). The model terms are not significant for the NFE model because their p-value is <0.05. Apart from p-value, other statistical parameters were used for example coefficient of determination (R^2), adjusted R^2 (R^2 adj) and standard deviation (S). The R^2 and R^2 adj of the NFE content model is shown to be 0.59 and 0.00 while the standard deviation is 5.0 (Appendix 5F). An R^2 close to unity (1) and a smaller standard deviation values signifies a better predicting response of the model used.

To determine the combined effect of the factors on NFE, 2D contour plots and 3D surface plots were used (Fig 6A-Fig 6C). These plots are graphical representation of regression equation. The 2D contour plots shows that there is an increase in NFE when pH is around 5.5 and blend ratio close to 16% with fermentation time held at 48 (Fig 6A). With blend ratio held at 2, there is an increase in NFE content when fermentation time is 70 hours and pH of 5.5 (Fig 6B). Furthermore, when the pH is held at 4.5, the NFE content increases as the fermentation time is approximately 70 hours and blend ratio approximately 20% (Fig 6C). Similar information is seen clearly with the 3D surface plots (Fig6D-F). Overall, with response optimization, it is shown that the optimum pH, blend ratio and fermentation time is 5.5, 16.7% and 72 hours respectively (Appendix 6I& J).

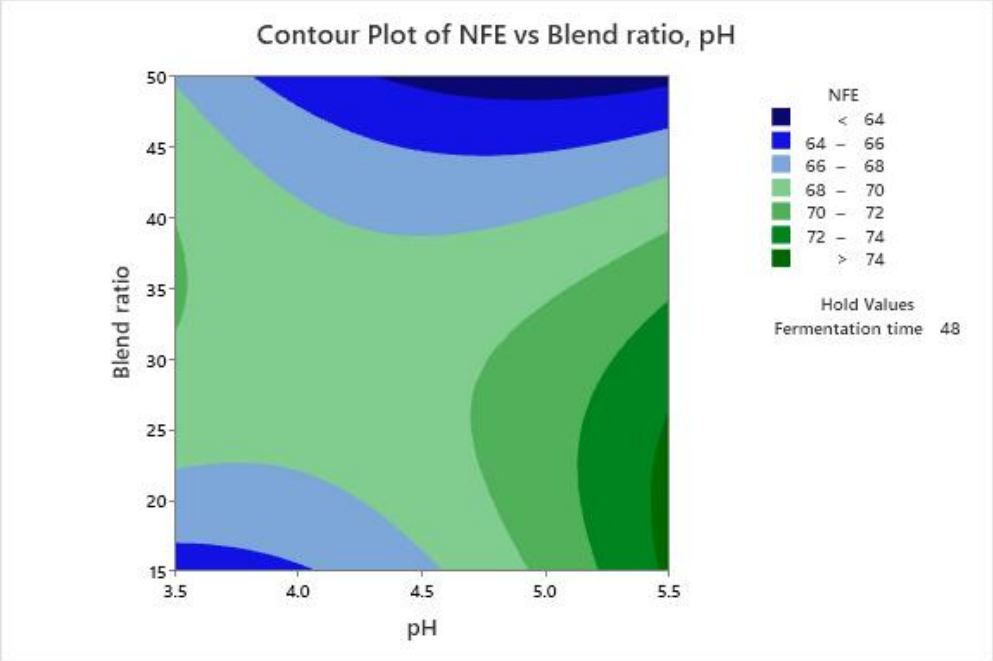

Figure 6A : Contour plot of NFE vs Blend ratio, pH

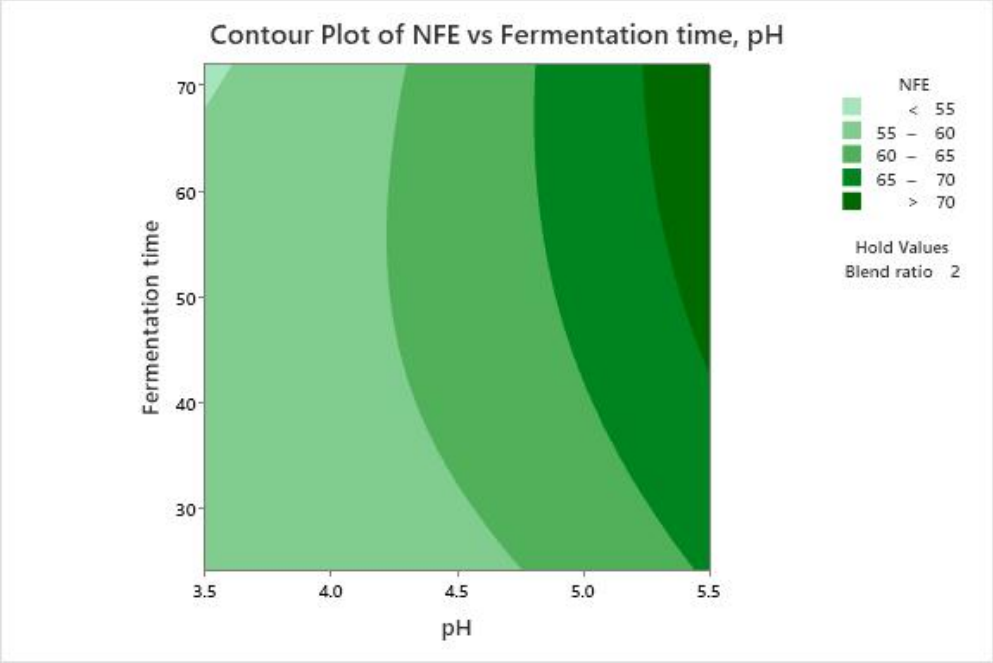

Figure 6B : Contour plot of NFE vs fermentation time, pH

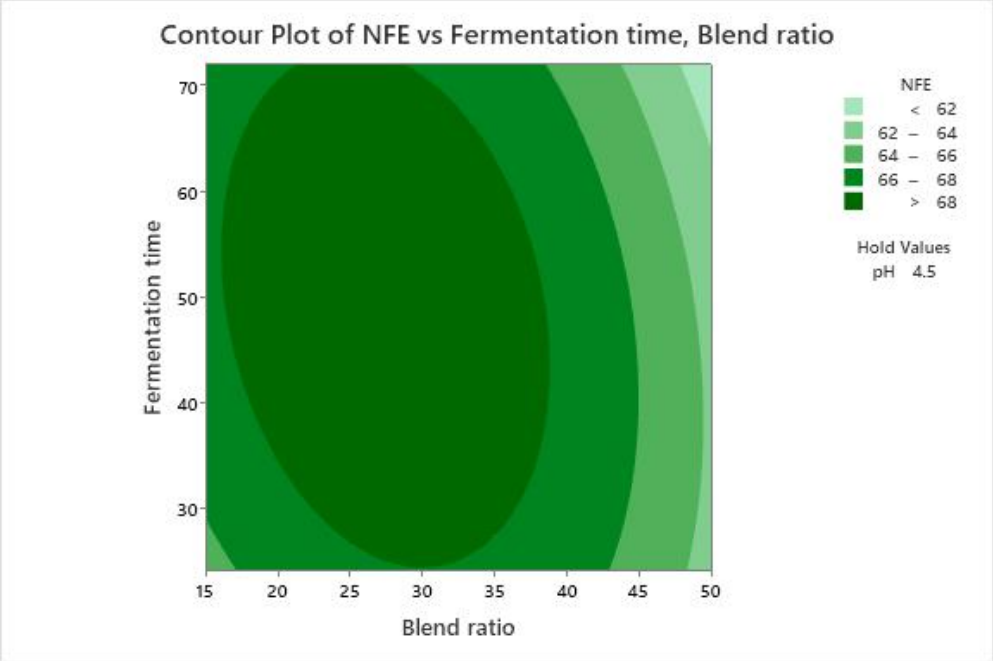

Figure 6C: Contour plot of NFE vs fermentation time, blend ratio

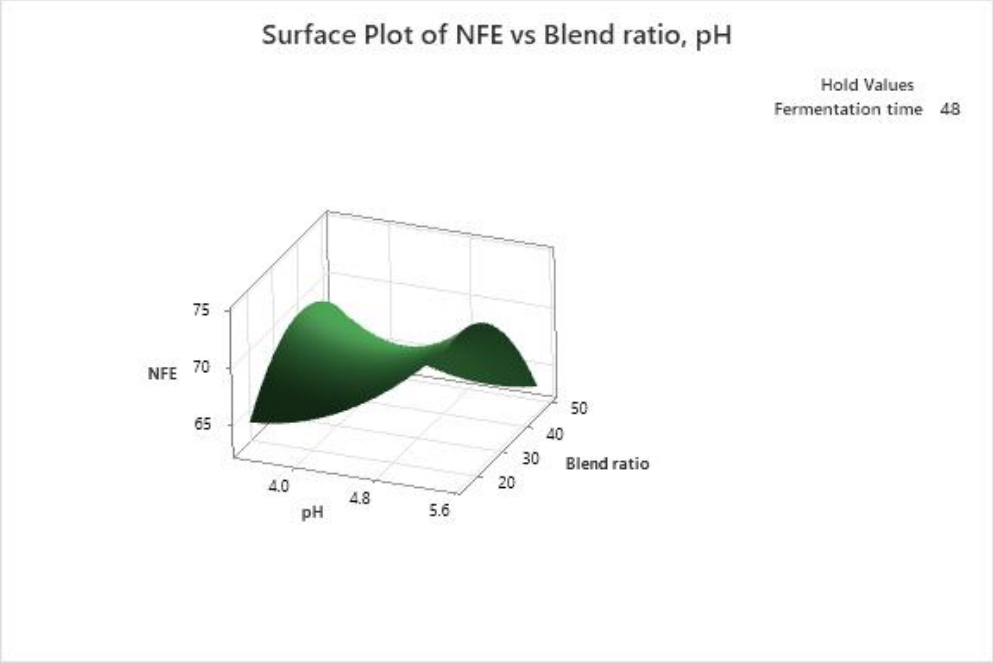

Figure 6D): Surface plot of NFE vs blend ratio, pH

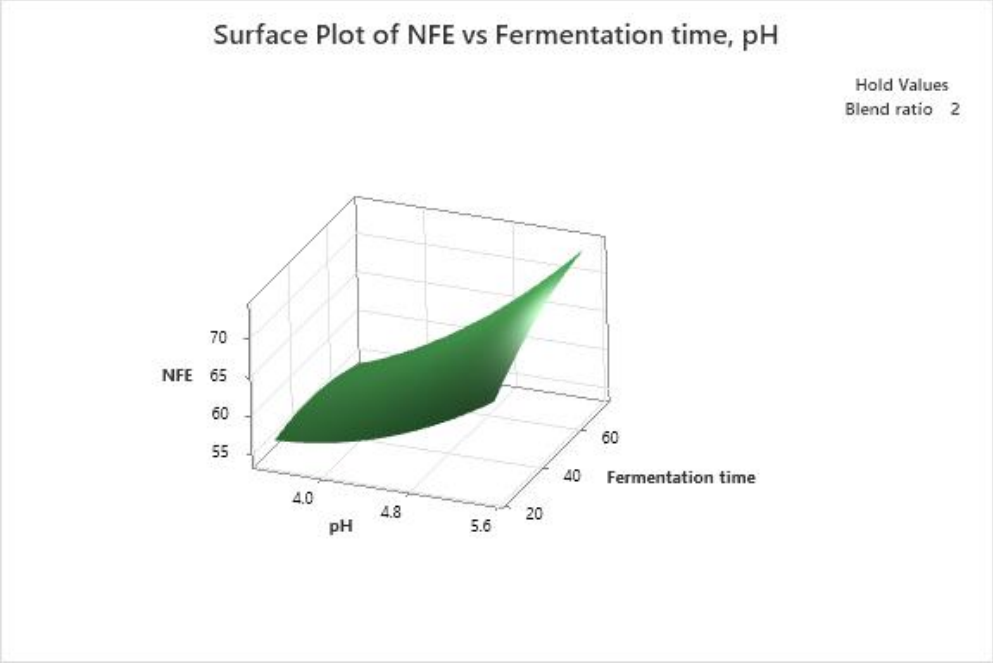

Figure 6E: Surface plot of NFE vs fermentation time, pH

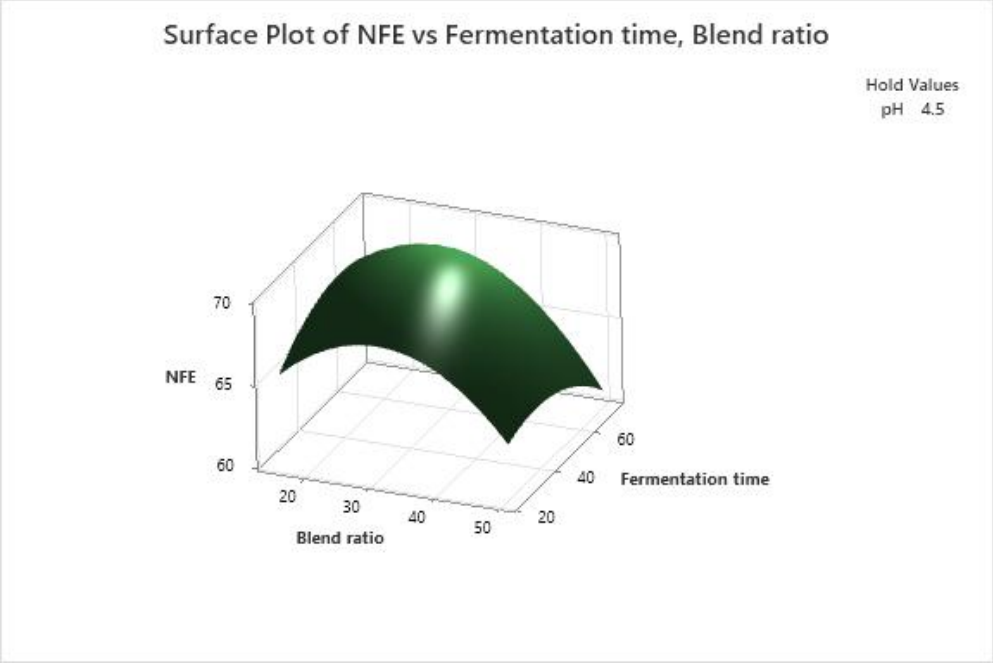

FIG 6F: Surface contour of NFE Vs fermentation time, blend ratio

The optimized parameters for fat are as follows: pH (5.5), Blend ratio (M85: C15), Fermentation time (72 hours).

4.9.4 ASH

The p-value of the model is shown to be <0.05 which demonstrates the significance level for the model used (Appendix 5I). The model terms are not significant for the Ash model because their p-value is <0.05. Apart from p-value, other statistical parameters were used for example coefficient of determination (R^2), adjusted R^2 (R^2 adj) and standard deviation (S). The R^2 and R^2 adj of the Ash content model is shown to be 0.49 and 0.00 while the standard deviation is 0.70 (Appendix 5J). An R^2 close to unity (1) and a smaller standard deviation values signifies a better predicting response of the model used.

To determine the combined effect of the factors on Ash, 2D contour plots and 3D surface plots were used (Fig 7A Fig 7C). These plots are graphical representation of regression equation. The 2D contour plots shows that there is an increase in Ash when pH is around 5.5 and blend ratio close approximately 30% with fermentation time held at 48 (Fig 7A). With blend ratio held at 2, there is an increase in Ash content when fermentation time is 24 hours and pH of 5.5 (Fig 7B). Furthermore, when the pH is held at 4.5, the Ash content increases as the fermentation time is approximately 70 hours and blend ratio approximately 50% (Fig 7C). Similar information is seen clearly with the 3D surface plots (Fig 7D-F). Overall, with response optimization, it is shown that the optimum pH, blend ratio and fermentation time was 5.5, 27.4% and 24 hours respectively (Appendix 6 I & J).

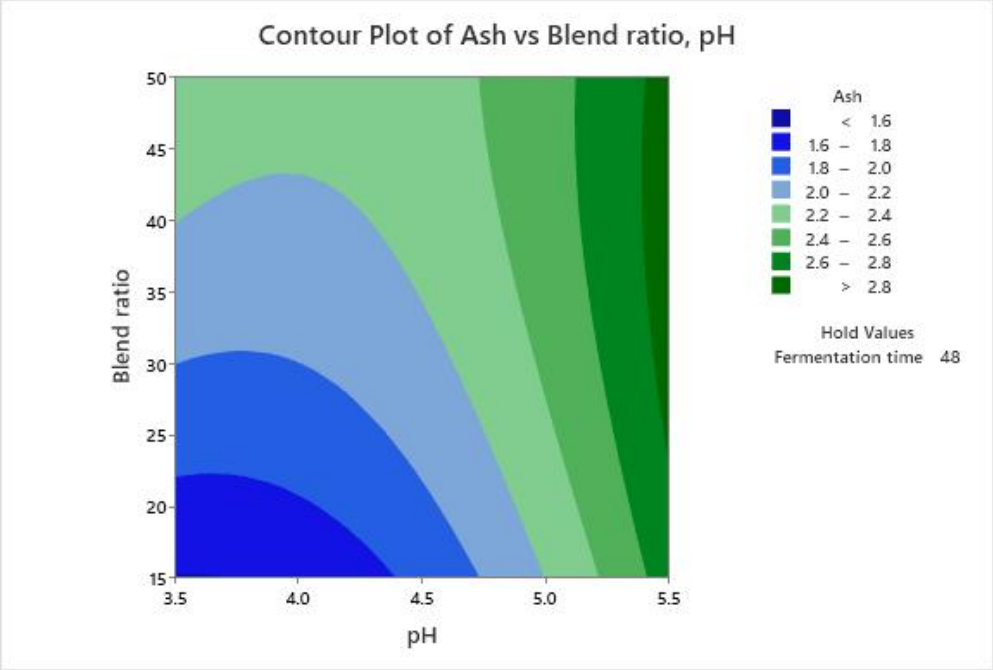

Figure 7A): Contour plot of ash vs blend ratio, pH

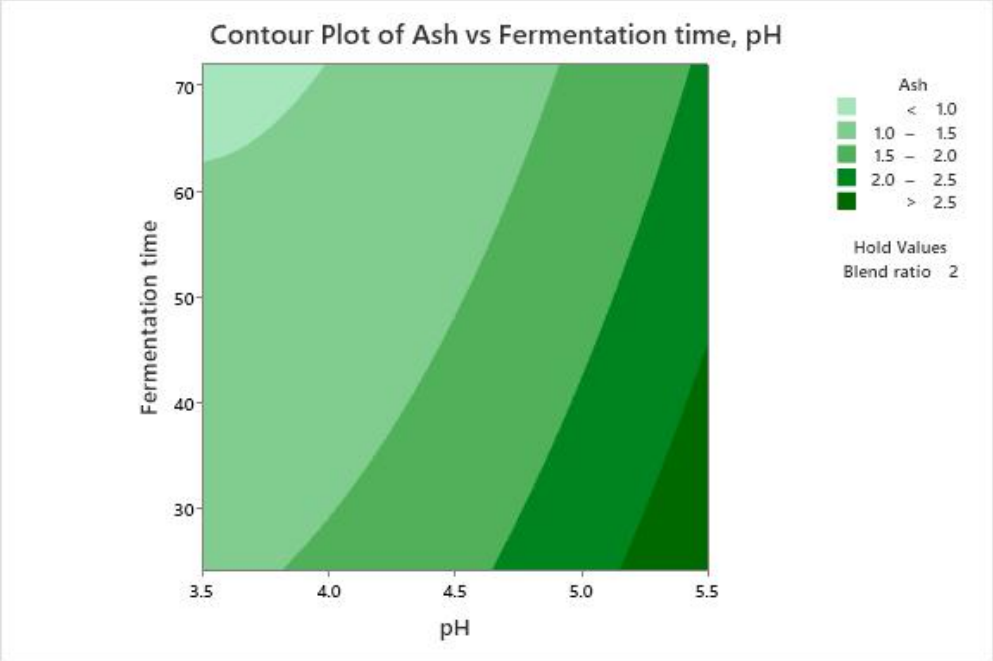

Figure 7B): Contour plot of ash vs fermentation time, pH

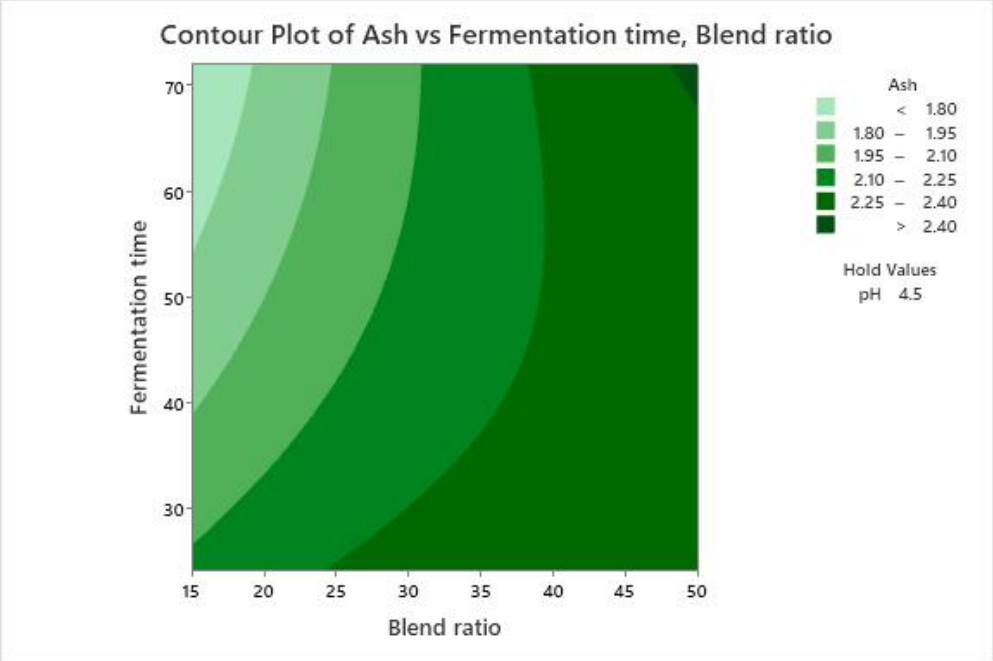

Figure 7C): Contour plot of ash vs fermentation time, blend ratio

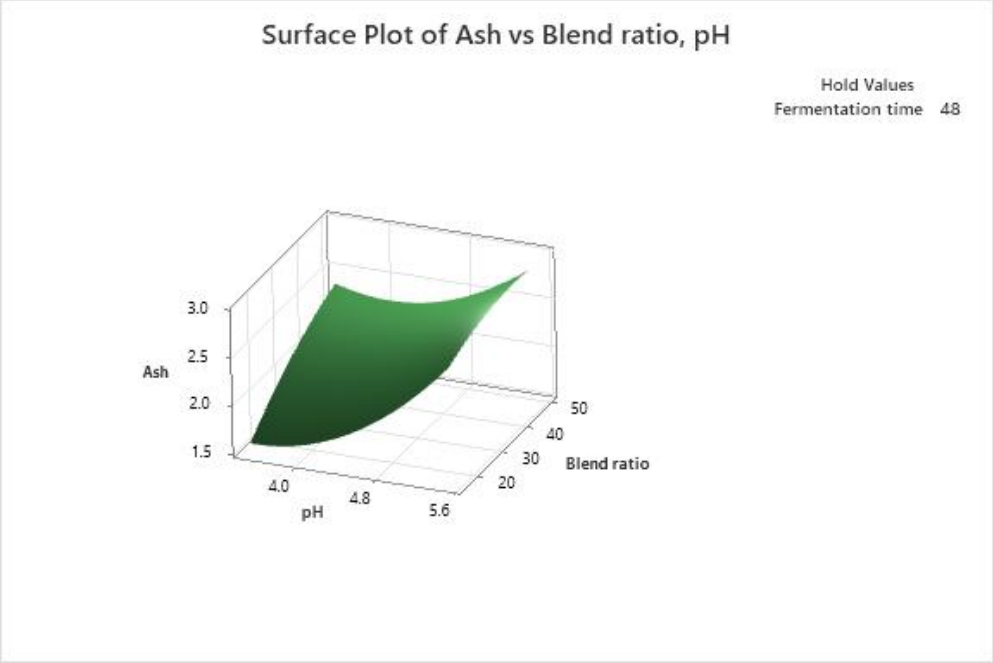

Figure 7D: Contour plot of ash vs blend ratio, pH

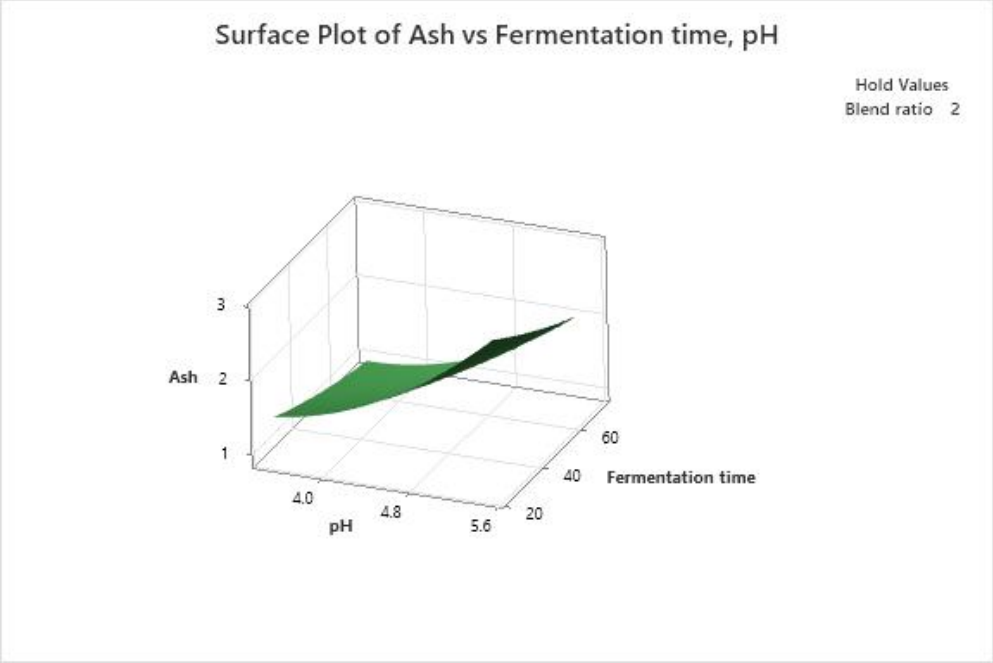

Figure 7E: Surface plot of ash vas fermentation time, pH

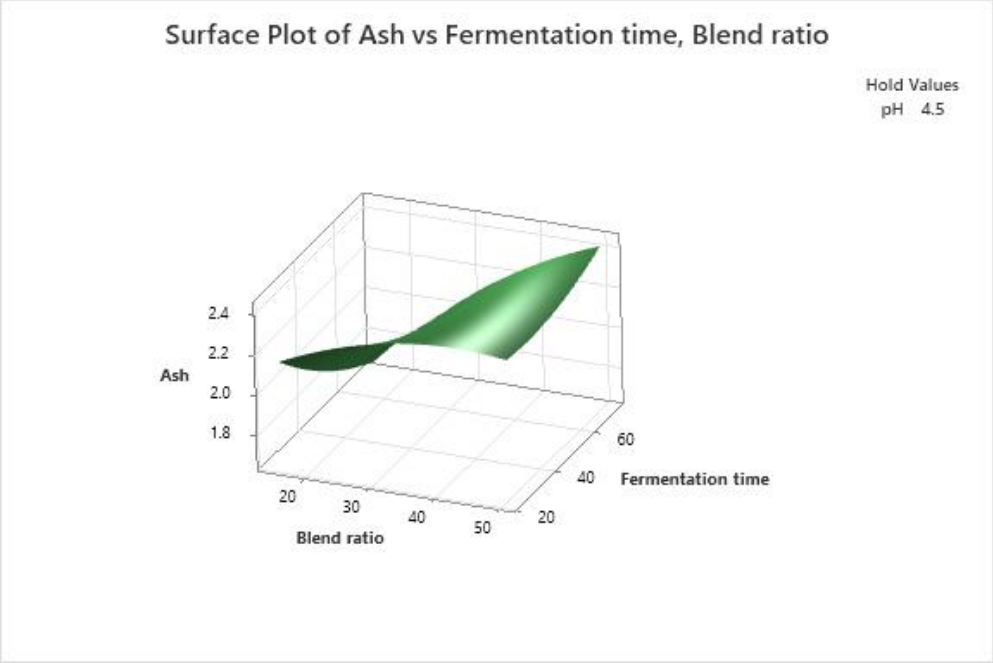

Figure 7F: Surface plot of Ash vs Fermentation Time, pH

The optimized parameters for fat are as follows: pH (5.5), Blend ratio (M73: C27), Fermentation time (24 hours).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The findings in this study shows that a maize and cowpea blend for a weaning formulation will yield a quality product. Evaluation of the microbial isolates would enhance the choice of starter culture to be used for production. WFA with processing parameters of pH 5.5, blend ratio 35% and fermentation time of 72 hours was the closest to the WHO|FAO (1991) standard of weaning food. Further work using these parameters would greatly optimize production as it would conserve time. This report is a reliable guide for industrial production of an affordable and fortified weaning food in Nigeria. This is to reduce malnutrition in weaned babies which is a global crisis.

6.2 RECOMMENDATION

Based on the findings of the present study, the following are recommended;

1. The use of cereal-legume blend as an alternative to expensive weaning foods.

2. Application of an articulated production design to ensure consistency in quality.

3. The use of genetically modified strains of the fermenting microorganisms to enhance better performance.

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APPENDIX 1: MICROSCOPIC AND BIOCHEMICAL CHARACTERIZATION

A) GRAM STAINING: A smear of the isolate was made on grease free glass slide with a drop of water and allowed to dry. The smear was fixed by mild heating, flooded with crystal violet and allowed to stand for 30 seconds. The crystal violet was rinsed off with water; Lugol's iodine was added and allowed to stand for 30 seconds. This was washed off with water and acid alcohol, till discoloration. It was counter stained with Safranin for 10 seconds and rinsed with water. The wet slide was allowed to air dry. A drop of oil immersion was added on the slide and viewed using X100 objective lens of the microscope.

B) SPORE STAINING TEST: The spore stain was used to confirm the presence of spores when indicated in the Gram stain. Isolates were heat fixed on a slide and flooded with 5% malachite green. It was steamed for 3 minutes (without allowing it to boil), dried and cooled. It was then rinsed off and stained with Safranin for 30 seconds. This was rinsed, dried with filter paper and viewed under the microscope using oil immersion tens. The positive spores showed green while the vegetative cells were stained pink.

C) MOTILITY TEST: This test was used to determine the motility of bacteria isolated. The test was carried out on a semi-solid agar medium in which motile bacteria swarm and gave a diffuse spreading growth. The medium was dispensed into test tubes, sterilized and allow to set in an upright position. It was then inoculated using an inoculation needle by stabbing it into the medium in the test tube. This was incubated at 37°C for 24 hours.

D) CATALASE TEST: The enzyme catalase is present in most cytochrome containing aerobic and facultative anaerobic bacteria. Catalase has one of the highest turnover numbers of all enzymes such that one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen in a second. Catalase activity can be detected by adding the substrate H_2O_2 to an appropriately incubated (18-24 hours) tryptic soy agar slant culture. Organisms which produce the enzyme breakdown the hydrogen and the resulting O_2 production produces bubbles in the reagent drop indicating a positive test. Organisms lacking the cytochrome system also lack the catalase enzyme and are unable to breakdown peroxide into O_2 and water and are catalase negative.

E) COAGULASE TEST: Coagulase is enzymes that clot blood plasma by a mechanism that is like normal clotting. The coagulase test identifies whether an organism produces this exoenzyme. This enzyme clots the plasma component of blood. The only significant disease-causing bacteria of humans that produce coagulase are *Staphylococcus aureus.* Thus, this enzyme is a good indicator of *S. aureus.* In the test, the sample is added to rabbit plasma and held at 37ºC for a specified period. Formation of clot within four hours is indicated as positive result and indicative of a virulent *Staphylococcus aureus* strain. The absence of coagulation after 24 hours of incubation is a negative result indicative of an avirulent strain.

F) OXIDASE TEST: Oxidase test is an important differential procedure that should b performed on all gram-negative bacteria for their rapid identification. The test depends on the ability of certain bacteria to produce indophenol blue from the oxidation of dimethyl-p-phenylenediamine and ∞-naphthol. This method uses N, N-dimethyl-p-phenylenediamine oxalate in which all Staphlococci are oxidase negative. In the presence of the enzyme cytochrome oxidase (gram negative bacteria) the N, N-dimethhyl-p-phenylenediamine oxalate and ∞-naphthol react to indophenol blue. *Pseudomonas aeruginosa* is an oxidase positive organism.

G) SUGAR FERMENTATION/OXIDATION: This test is used to differentiate between bacteria groups that oxidize carbohydrate such as members of Enterobacteriaceae. One milliliter (1ml) of 10% glucose, maltose, lactose, fructose, mannitol, and sucrose were separately under aseptic conditions transferred into duplicate tubes containing 9ml of sterile Hugh and Leifson's medium to obtain a final concentration of 1% of each of sugar. The tubes were stab-inoculated in duplicates while two uninoculated tubes serve as control. Vaseline was used to cover one set of the duplicate tubes, one control to discourage oxidative utilization of sugar. All tubes were incubated at 37^{o}c for 48h. After the incubation, they were observed for acid production in the culture. Yellow colouration indicates acid production in the open tubes only suggesting oxidative utilization of the sugar while acid production in the sealed tubes suggests a fermentative reaction.

H)HYDROGEN SULPHIDE PRODUCTION (H_2S) TEST: The test isolates were aseptically inoculated into a tube containing triple sugar iron agar started by stabbing the agar to the bottom and streaking the surface of the slant. The inoculated tube was incubated at 37^{o}c for 72h and was examined daily. Black precipitation and yellow colouration was checked for. Black precipitate indicates H_2S production and yellow colouration for sucrose, lactose and glucose fermentation.

I)UREASE TEST: Urease Agar slant in McCartney bottle was inoculated with the bacteria isolate at 30^{o}C for 4 hours and then overnight. A pink colour in the medium indicated a positive result.

J) IMVIC TEST: This test consists of four different test; they are Indole production, Methyl-Red test, Voges Proskaeur test and Citrate utilization test. This test is specifically designed to determine the physiological properties of microorganism. They are especially useful in the differentiation of Gram-negative intestinal bacilli, particularly *Escherichia coli* and the *Enterobacter-Klebsiella* group.

K) INDOLE TEST: This test demonstrates the ability of certain bacteria to decompose the amino acid-Tryptophan to Indole. The bacteria isolates were inoculated into the medium and incubated at 37°C for 48 hours. At the end of incubation period, 3 drops of kovac's reagents (see appendix) were added and then shaken. A red colour ring at the interface of the medium denotes a positive result.

Methyl red and Voges-Proskaeur test must be considered together since they are physiologically related. Opposite test is usually obtained from the MR and VP terst, that is, MR+, VP-, or MR-, VP+.

Methyl red test was performed to demonstrate the capacity of different organisms to produce acid from the fermentation of sugar (dextrose). Methyl-red positive organisms produce a red colouration when five drops of methyl-red indicator is added into 48h old MR-VP broth culture.

The Voges-Proskaeur test demonstrates the ability of organisms to produce acetoin from glucose metabolism. Some organisms metabolise glucose to produce pyruvic acid which is further broken down to yield Butane-diol and acetyl-methyl carbinol as an intermediate product.

Into one milliliter of the culture add one milliliter of six percent alcoholic solution of alpha-naphtol and one milliliter of 16% KOH and stand for 15-20 minutes. Development of red to pink colour is a positive test.

L)CITRATE UTILIZATION TEST: The slopes of the media were prepared in bijou bottles as recommended by the manufacturers. A sterile straight wire was used to the slope with a saline suspension of the test organisms before stabbing the butt. The bottles are incubated at 35^{o}C for 48 h. Bright blue colours in the medium means positive test while no change in colour of medium indicates negative citrate test (Cheesbrough, 2000).

Appendix 2

METHODOLOGY FOR PROXIMATE ANALYSIS

Moisture content (AOAC, 2006).

Mix the sample thoroughly. Determine the water content by weighing out 2g into glass petri dish, which has been previously dried and weighed. Place the dish including the sample inside it in hot

air oven for 5 hours at $130^0C \pm 3^0C$. Finally dry to constant weight, cool for ten minutes in a desiccator each time before weighing.

$$\% \text{ moisture} = \frac{W_t \text{ loss on drying, g} \times 100}{W_t \text{ test portion, g}}$$

W_t = Weight of sample

Nitrogen determination by micro Kjeldahl method (crude protein). According to (AOAC,2005)

The nitrogen of protein and other compounds are converted to ammonium sulphate by acid digestion with boiling sulphuric acid. A known weight of sample is placed in Kjeldahl flask and about 200 milligram of catalyst mixture (potassium sulphate, copper sulphate and selenium powder) is added.Add $10.0cm^2$ of concentrated sulphuric acid to the content of the flask. Heat gently for few minutes until frothing ceases and increase the heat to digest for 1 hour. Allow cooling and making to a known volume with distilled water ($100cm^3$). Distil $10.0cm^3$ aliquot of the dilute solution of the digest by pipetting the volume into distillation chamber of micro Kjeldhal distillation apparatus. Add $10.0cm^3$ of 40% sodium hydroxide solution and steam distil into $10.0cm^3$ of 4% boric acid containing mixed indicator (note colour from red-green) titrate with standard 0.01N or 0.02N hydrochloric acid to grey end point.

$$\% N = \frac{(a-b) \times 0.01 \times 14.0057 \times c \times 100}{dxe}$$

a = titre value for the sample

b = titre value for the blank

c = Volume to which digest is made up with distilled water

d = Aliquot taken for distillation

e = Weight of dried sample (mg)

To convert to % crude protein, multiply by necessary conversion factor (6.25).

Ash Determination (AOAC,2005) - method 942.05.

Weigh 2 g test portion into porcelain crucible and place in murfle furnance preheated to 600^0C. Hold at this temperature 2 hours. Transfer cucible directly to dessicator, cool, and weigh immediately, reporting percent ash to two decimal places.

Fat Determination (ether-extract) (AOAC, 2005) - method 945.16

This was slightly modified. Fit up a soxhlet extraction apparatus and 250ml quickfit flask which has been previously dried in the oven. Weigh 5 g of sample and transfer to a fat-free extraction thimble, plug lightly with cotton wool, place the thimble in the extractor and add about 150cm3 of petroleum ether (B.P. 40-60^0C) into the flask until it siphons over once. Adjust the source of heat (electrothermal heating mantle) so that the ether boils gently and leave it to siphon over for at least 6 hours. Detach the flask (which now contains all the oil). Filter extract(oil) through Whatman filter paper into weighed beaker, washing paper finally with small portion of hot fresh ether. Evaporate solvent at 100^oC and dry beaker containing residue in an air oven 1 hour at 100-105^{OC}C. Report as % oil to second decimal place.

Crude Fiber Determination (AOAC, 2005) - method 978.10

This was carried out with little modification. Transfer defatted ground sample from fat determination into 250 ml quick fit flask, add 150 ml of 1.25% sulphuric acid and fit it to reflux condenser. Reflux for 30 minutes, cool and filter using Buchner funnel fited with Whatman filter paper. Rinse three times hot distilled water, dry and carefully transfer the residue into quickfit flask. Add 150ml of 1.25% Sodium hydroxide and reflux for 30 minutes. Filter using Buchner funnel, rinse three times with hot distilled water, once with 1.25% sulphuric acid and finally with 95% ethanol. Remove the filter paper containing residue into porcelain crucible and dry in oven 2hours at 1300C. Cool in dessicator, ash at $550^0 \pm 10^0$C in murfle furnance, cool in dessicator and weigh.

APPENDIX 3: 9-POINT HEDONIC

SCALE

9-Point Hedonic Scale	
9	Like Extremely
8	Like Very Much
7	Like Moderately
6	Like Slightly
5	Neither Like nor Dislike
4	Dislike Slightly
3	Dislike Moderately
2	Dislike Very Much
1	Dislike Extremely

APPENDIX 4: PICTURES OF WORK ONGOING IN THE LABORATORY

Appendix 5

A: Analysis of variance on response surface for fat content

Source	DF	Adj SS	Adj MS	F-Value	P-Value

Model	9	6.11191	0.67910	5.34	0.040
Linear	3	2.64637	0.88212	6.93	0.031
Ph	1	0.08530	0.08530	0.67	0.450
Blend ratio	1	0.01947	0.01947	0.15	0.712
Fermentation time	1	2.54159	2.54159	19.97	0.007
Square	3	2.35457	0.78486	6.17	0.039
pH*Ph	1	1.52124	1.52124	11.95	0.018
Blend ratio*Blend ratio	1	0.00453	0.00453	0.04	0.858
Fermentation time*Fermentation time	1	1.00080	1.00080	7.86	0.038
2-Way Interaction	3	3.42834	1.14278	8.98	0.019
pH*Blend ratio	1	0.14823	0.14823	1.16	0.330
pH*Fermentation time	1	0.85562	0.85562	6.72	0.049
Blend ratio*Fermentation time	1	2.42449	2.42449	19.05	0.007
Error	5	0.63645	0.12729		
Lack-of-Fit	3	0.41140	0.13713	1.22	0.480
Pure Error	2	0.22505	0.11252		
Total	**14**	**6.74836**			

B: Fat content model summary

S	**R-sq**	**R-sq(adj)**
0.356777	90.57%	73.59%

C: Analysis of variance on response surface for moisture content

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**

Model	9	107.451	11.9390	1.95	0.239
Linear	3	32.630	10.8766	1.78	0.267
pH	1	1.909	1.9089	0.31	0.600
Blend ratio	1	30.503	30.5032	4.99	0.076
Fermentation time	1	0.218	0.2178	0.04	0.858
Square	3	73.141	24.3802	3.99	0.085
pH*pH	1	4.420	4.4204	0.72	0.434
Blend ratio*Blend ratio	1	42.814	42.8135	7.00	0.046
Fermentation time*Fermentation time	1	26.388	26.3879	4.32	0.092
2-Way Interaction	3	11.193	3.7309	0.61	0.637
pH*Blend ratio	1	2.098	2.0982	0.34	0.583
pH*Fermentation time	1	8.614	8.6142	1.41	0.289
Blend ratio*Fermentation time	1	0.480	0.4804	0.08	0.790
Error	5	30.574	6.1147		
Lack-of-Fit	3	24.085	8.0283	2.47	0.301
Pure Error	2	6.489	3.2444		
Total	14	138.025			

D: Moisture content model summary

S	**R-sq**	**R-sq(adj)**

2.47	77.85%	37.98%

E: Analysis of variance on response surface for crude protein content

Source	DF	Adj SS	Adj MS	F-Value	P-Value

Model	9	85.485	9.4983	1.44	0.360
Linear	3	68.013	22.6711	3.43	0.109
pH	1	58.193	58.1930	8.81	0.031
Blend ratio	1	0.397	0.3967	0.06	0.816
	1	9.424	9.4236	1.43	0.286
Fermentation time					
Square	3	14.384	4.7946	0.73	0.579
pH*pH	1	10.535	10.5352	1.60	0.262
Blend ratio*Blend ratio	1	0.436	0.4359	0.07	0.807
Fermentation time*Fermentation time	1	3.659	3.6585	0.55	0.490
2-Way Interaction	3	59.139	19.7130	2.99	0.135
pH*Blend ratio	1	46.915	46.9147	7.11	0.045
pH*Fermentation time	1	0.179	0.1785	0.03	0.876
Blend ratio*Fermentation time	1	12.046	12.0460	1.82	0.235
Error	5	33.013	6.6025		
Lack-of-Fit	3	27.677	9.2258	3.46	0.232
Pure Error	2	5.335	2.6675		
Total	14	118.497			

F: Crude protein content model summary

S	R-sq	R-sq(adj)
2.5	72.14%	21.99%

G: Analysis of variance on response surface for crude fiber content

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	9.3494	1.03882	0.85	0.608
Linear	3	4.8239	1.60796	1.32	0.366
pH	1	4.6436	4.64357	3.81	0.108
Blend ratio	1	0.0822	0.08217	0.07	0.806
Fermentation time	1	0.0982	0.09815	0.08	0.788
Square	3	2.4676	0.82252	0.67	0.604
pH*pH	1	0.3074	0.30741	0.25	0.637
Blend ratio*Blend ratio	1	0.0848	0.08484	0.07	0.802
Fermentation time*Fermentation time	1	2.2524	2.25240	1.85	0.232
2-Way Interaction	3	6.3004	2.10014	1.72	0.277
pH*Blend ratio	1	4.5788	4.57885	3.75	0.110
pH*Fermentation time	1	1.3865	1.38651	1.14	0.335
Blend ratio*Fermentation time	1	0.3351	0.33506	0.27	0.623
Error	5	6.0972	1.21945		
Lack-of-Fit	3	1.9344	0.64481	0.31	0.821
Pure Error	2	4.1628	2.08141		
Total	14	15.4466			

H: Crude fiber content model summary

S	R-sq	R-sq(adj)
1.10	60.53%	0.00%

I: Analysis of variance on response surface for NFE content

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	184.640	20.516	0.82	0.629
Linear	3	90.584	30.195	1.20	0.399
Ph	1	55.135	55.135	2.19	0.199
Blend ratio	1	32.668	32.668	1.30	0.306
Fermentation time	1	2.780	2.780	0.11	0.753
Square	3	75.011	25.004	0.99	0.467
pH*pH	1	16.896	16.896	0.67	0.450
Blend ratio*Blend ratio	1	46.454	46.454	1.85	0.232
Fermentation time*Fermentation time	1	7.934	7.934	0.32	0.599
2-Way Interaction	3	74.516	24.839	0.99	0.470
pH*Blend ratio	1	47.095	47.095	1.87	0.230
pH*Fermentation time	1	23.088	23.088	0.92	0.382
Blend ratio*Fermentation time	1	4.333	4.333	0.17	0.695
Error	5	125.817	25.163		
Lack-of-Fit	3	120.960	40.320	16.60	0.057
Pure Error	2	4.856	2.428		
Total	14	310.457			

J: NFE content model summary

S	R-sq	R-sq(adj)
5.0	59.47%	0.00%

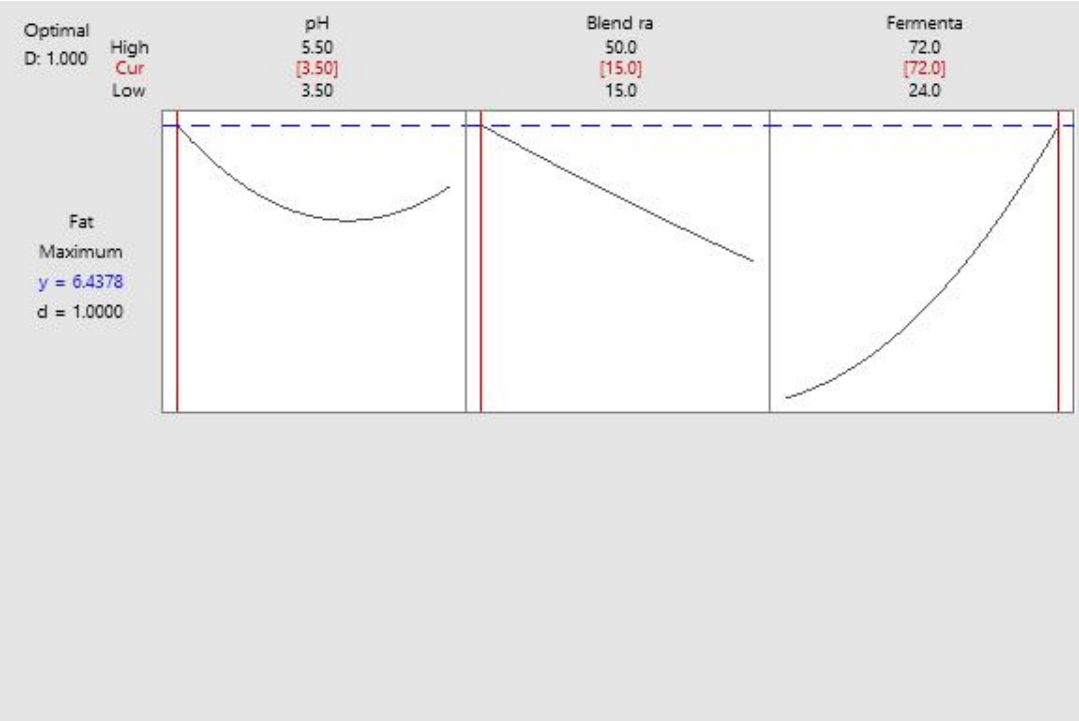
I: Analysis of variance on response surface for Ash content

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	2.35823	0.262025	0.53	0.806
Linear	3	0.67164	0.223880	0.46	0.725
pH	1	0.46830	0.468298	0.95	0.374
Blend ratio	1	0.08416	0.084159	0.17	0.696
Fermentation time	1	0.11918	0.119184	0.24	0.643
Square	3	0.35942	0.119805	0.24	0.863
pH*Ph	1	0.31231	0.312310	0.64	0.462
Blend ratio*Blend ratio	1	0.02680	0.026799	0.05	0.825
Fermentation time*Fermentation time	1	0.00863	0.008626	0.02	0.900
2-Way Interaction	3	0.20164	0.067213	0.14	0.934
pH*Blend ratio	1	0.08594	0.085938	0.17	0.693
pH*Fermentation time	1	0.03610	0.036100	0.07	0.797
Blend ratio*Fermentation time	1	0.07960	0.079601	0.16	0.704
Error	5	2.45817	0.491633		
Lack-of-Fit	3	1.70090	0.566966	1.50	0.424
Pure Error	2	0.75727	0.378633		
Total	14	4.81639			

J: Ash content model summary

S	R-sq	R-sq(adj)
0.70	48.96%	0.00%

APPENDIX 6: OPTIMIZATION RESPONSES

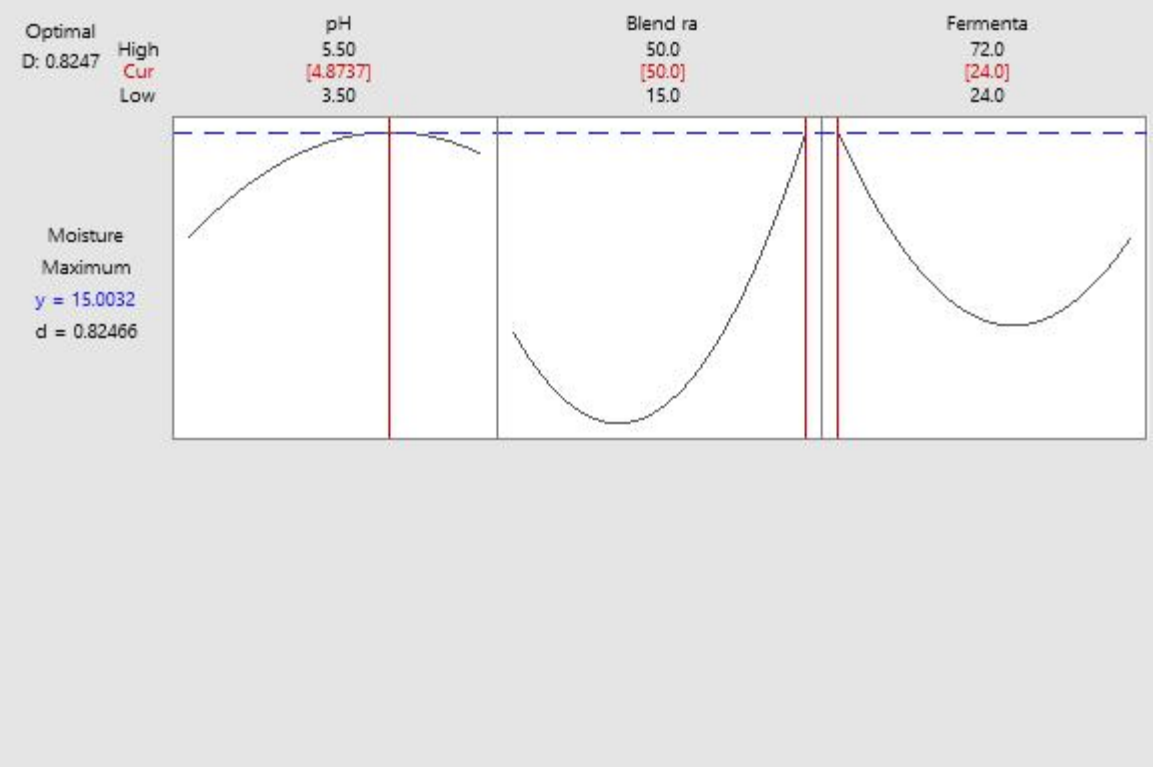


A: Response optimization: Fat content

B: Response optimization: Fat content

Variable	Setting
pH	3.5
Blend ratio	15
Fermentation time	72

Response	Fit	SE Fit	95% CI	95% PI
Fat	6.438	0.432	(5.328, 7.548)	(4.998, 7.877)

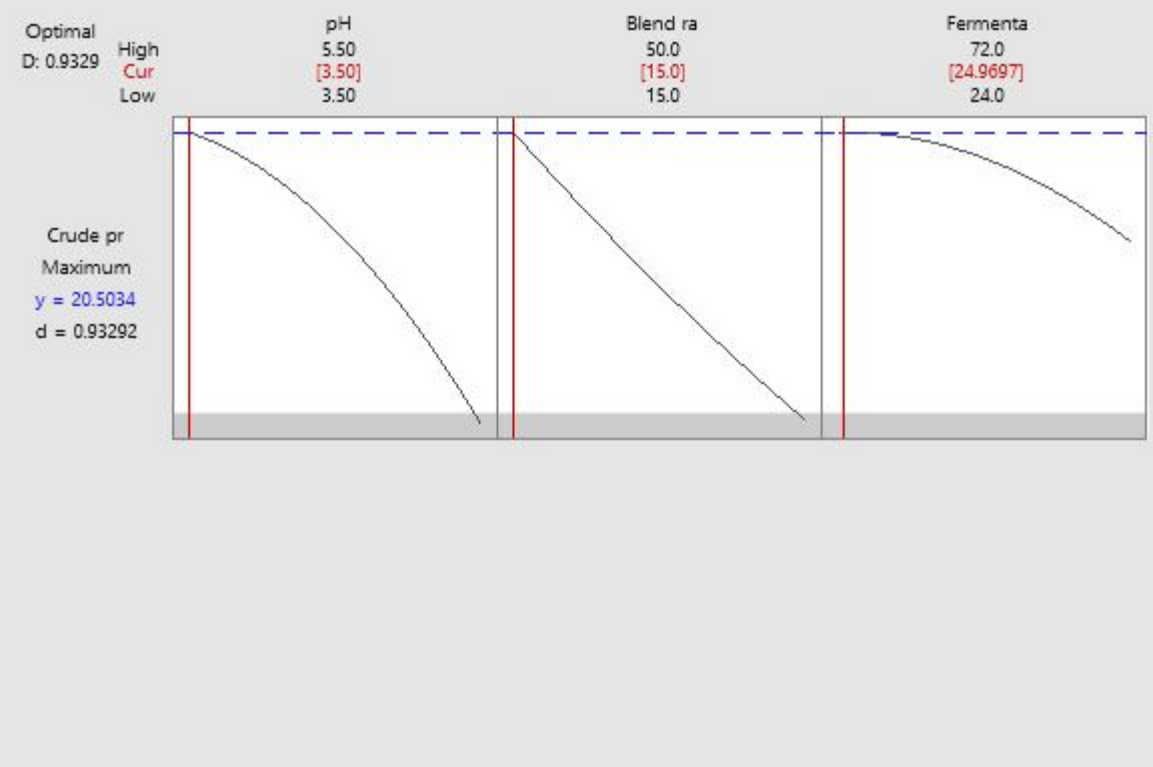


C: Response optimization: Moisture

D: Response optimization: Moisture content

Variable	Setting
pH	4.9
Blend ratio	50
Fermentation time	24

Response	Fit	SE Fit	95% CI	95% PI
Moisture	15.00	2.16	(9.44, 20.57)	(6.56, 23.45)

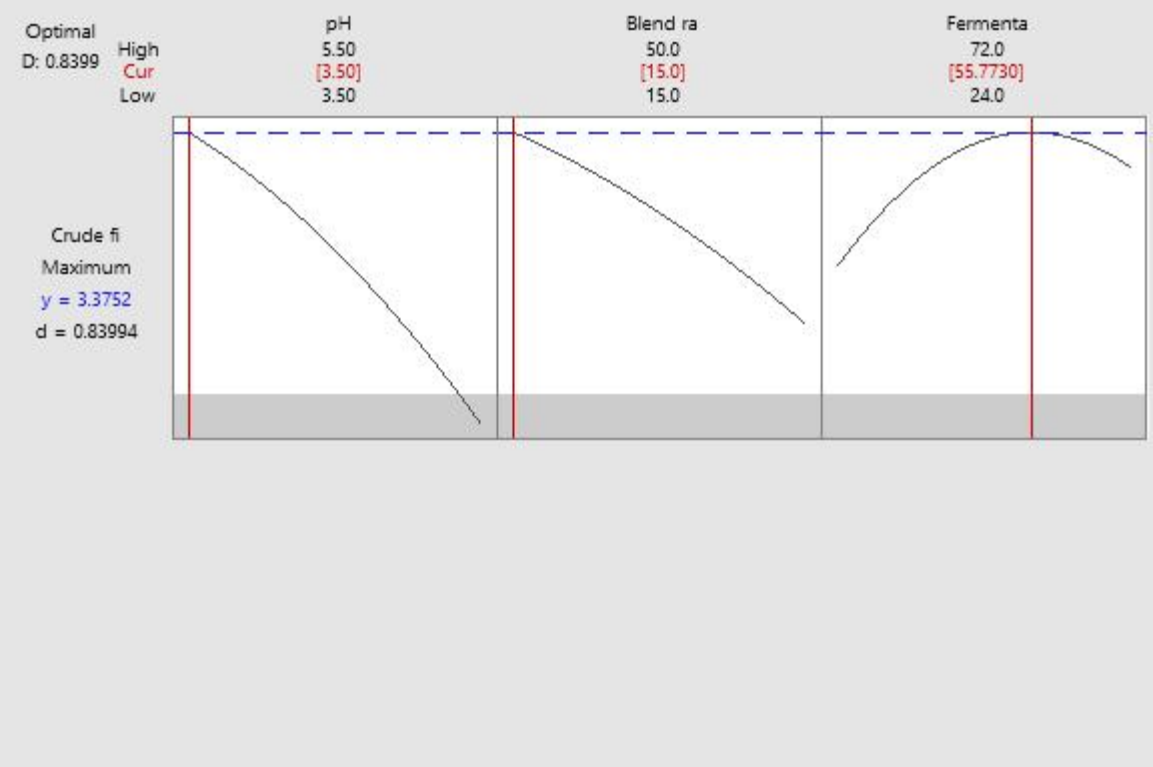


D: Response optimization: Crude Protein

E: Response optimization: Crude protein

Variable	Setting
pH	3.5
Blend ratio	15
Fermentation time	24.9

Response	Fit	SE Fit	95% CI	95% PI
Moisture	20.50	3.03	(12.71, 28.29)	(10.29, 30.72)

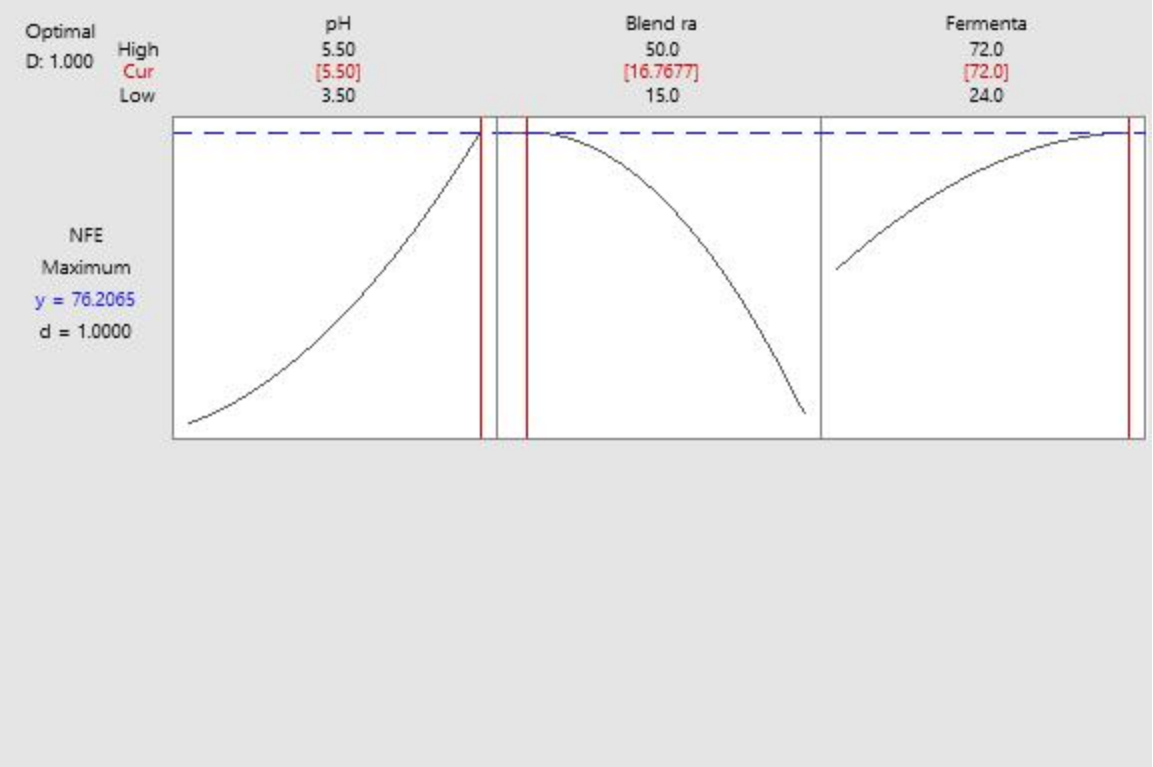


E: Response optimization: Crude fiber

F: Response optimization: Crude fiber

Variable	Setting
pH	3.5
Blend ratio	15
Fermentation time	55.7

Response	Fit	SE Fit	95% CI	95% PI
Moisture	3.38	1.01	(0.79, 5.96)	(-0.46, 7.22)

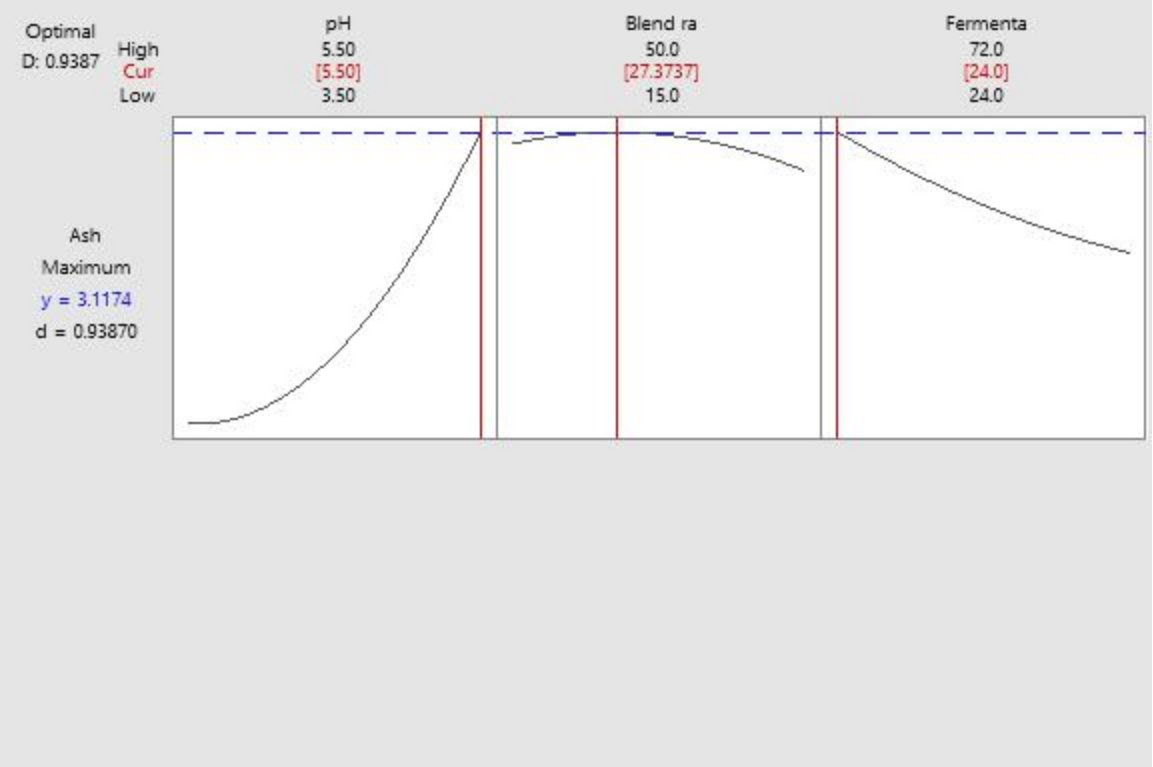


G: Response optimization: NFE

H: Response optimization: NFE

Variable	Setting
pH	5.5
Blend ratio	16.7
Fermentation time	72

Response	Fit	SE Fit	95% CI	95% PI
Moisture	76.21	5.71	(61.52, 90.90)	(56.66, 95.75)



I: Response optimization: Ash

J: Response optimization: Ash

Variable	Setting
Ph	5.5
Blend ratio	27.3737
Fermentation time	24

Response	Fit	SE Fit	95% CI	95% PI
Moisture	3.117	0.636	(1.482, 4.752)	(0.684, 5.551)