PHYSICOCHEMICAL AND NUTRITIONAL PROFILING OF FERMENTED TIGER NUT-CEREAL-BASED SYNBIOTIC DAIRY DRINK

THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF PHILOSOPHY FOOD SCIENCE DEGREE.

BY

STEPHEN YEBOAH

10636549

DECLARATION

I, **STEPHEN YEBOAH**, hereby declare that except for the references which have been duly cited, the work in this thesis, "**PHYSICOCHEMICAL AND NUTRITIONAL PROFILING OF FERMENTED TIGER NUT-CEREAL-BASED SYNBIOTIC DAIRY DRINK**" was done entirely by me in the Department of Nutrition and Food Science, College of Basic and Applied Sciences, University of Ghana, Legon. This work has never been presented either in whole or in part for any other degree in this University or elsewhere.

18th May, 2020

STEPHEN YEBOAH **DATE**

(STUDENT)

23rd May, 2020

DR ANGELA PARRY-HANSON KUNADU **DATE**

(PRINCIPAL SUPERVISOR)

24th May, 2020

DR. AGARTHA OHEMENG **DATE**

(CO - SUPERVISOR)

24th May, 2020

PROF. FIRIBU KWESI SAALIA **DATE**

(CO - SUPERVISOR)

ABSTRACT

Tiger nut and millet are indigenous crops in Ghana. These crops have been on the radar recently due to their versatile applications in food processing and their increase in consumption by low-income households. The nut is rich in fermentable carbohydrates and resistant starch, rich in fiber and micronutrients, and it is an ideal candidate for synbiotic foods. The objective of this study was to utilize whole tiger nut in dairy-cereal based beverage, make the product synbiotic, and assess the nutritional, physicochemical profile, microbial quality, and shelf life of the drink. The tiger nut-millet agglomerate was prepared by incorporating cellulase hydrolyzed tiger nut fibrous co-product (TNF), and non-hydrolyzed TNF (10% and 15%) into millet with a varying fermentation time of 12 and 24 hours. The agglomerate functional properties (i.e. Water Absorption Capacity (WAC), Bulk Density (BD), Swell Index) and textural characteristics (hardness, resilience, cohesiveness, adhesiveness gumminess and chewiness) were objectively measured in triplicates. The drink produced from composite tiger nut milk: dairy in a ratio of 40%:60% was inoculated with probiotics (*Lactobacillus casei*). The drink was analyzed in triplicate for physicochemical, proximate, and microbiological quality. Accelerated shelf-life study with the Arrhenius model was used to predict the shelf life of the drink. The obtained data were subjected to analysis of variance (ANOVA) in Minitab version 17. Agglomerate prepared from dough fermented at 12 hours had excellent textural and functional characteristics hence was selected for the synbiotic drink production. The moisture content of the product decreased with tiger nut incorporation, while sodium, potassium, magnesium, phosphorus, zinc, vitamin E, protein and total carbohydrate together with crude fibre increased with tiger nut incorporation. The products were microbiologically safe with no count for *E. coli*, *Staphylococcus aureus*, and yeast and moulds after five days of storage with increasing acidity. The shelf life observed for all the products at a

pH limit of 3.5 was five days, four days and three days at a storage temperature of 5℃, 25℃ and 35℃, respectively. *Lactobacillus casei* counts in the product increased by 1 log cycle each day for both refrigerated and room temperature samples. Incorporation of whole tiger nut and a known probiotic into dairy cereal-based beverage will increase the nutritional content of the product as a functional food. Consuming this product will provide consumers with the health benefits associated with the consumption of the whole tiger nuts based probiotic products.

DEDICATION

This thesis is dedicated to the Almighty God, to my uncle Lawyer John Owusu Agyeman and my supervisors. It is also dedicated to all who contributed in diverse ways to make this research come to fruition.

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AAS	Atomic Absorption Spectrophotometer
ANOVA	Analysis of variance
ATP	Adenosine triphosphosphate
BHI	Brain Heart Infusion
DNA	Deoxyribonucleic acid
FAO	Food and Agriculture Organization
HCl	Hydrochloric Acid
HPLC	High-performance liquid chromatography
HTST	High-temperature short time
LA	Lactic acid
LAB	Lactic acid bacteria
MC	Maconkey Agar
MRS	de Mann Rogosa Sharpe
NaOH	Sodium Hydroxide
PCA	Plate Count Agar
PET	Polyethylene Terephthalate
SEM	Scanning Electron Microscope

sigma	S degradation by ClpXP
TNF	Tiger nut fibrous co-product
TNM	Tiger Nut Milk
TPA	Texture Profile Analysis
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.0 Background

Tiger nut and millet are indigenous crops that have been on the radar recently due to their versatile applications in food processing and their growth and consumption by low-income households (Amadou et al., 2013). Consumers have become more aware of their health and wellbeing; they consider not only the taste but the health implications of the food they eat. This change has been the pivot compelling food industries and researchers to consider underutilized indigenous crops as alternative raw materials or food ingredients in food processing (Charalampopoulos et al., 2002).

Cyperus esculantus L (tiger nut) is a tropical crop. In Ghana, tiger nut remains classified as a minor crop and not captured in official records (Osei-Akoto et al., 2014). Enhancing the usability of indigenous plant products like tiger nut and millet in a developing country will help curb the problem of food and nutrition insecurity. It will also provide food ingredients that exhibit interesting functional and physical characteristics that have significant impact on the sensory properties of food (Sánchez-Zapata et al., 2012). There is conscious effort being made to standardize and enhance their usage as composite materials and nutritional supplements in food processing. Sánchez-Zapata et al. (2013) studied the tiger nut co-product (the fibrous remains after juicing tiger nut) as a fibrous component in sausage and also the liquid co-product (tiger nut milk) as a carbon source for development of probiotic products. Plant products and microorganisms have been used in food processing for many decades and they are still relevant because of their enormous benefits. Some of these benefits include source of enzymes e.g. papain from papaw for meat tenderizing; protease, lipase, pectinase, phytase, catalase, insulinase, and cellulase from *Aspergillus niger* for glucose and alcohol production; source of plant prebiotics such as inulin,

source of beneficial bacteria that promote gut health (probiotics) and source of a variety of preservatives. These have all helped functionally by improving on the texture, stability, nutritional and health benefits of foods(Kizzie-Hayford et al., 2018).

Tiger nut is of high nutritional value, the tubers contain pleasantly flavored oil much like olive and sweet almond oil(Kim et al., 2007). According to Arafat et al. (2009) tiger nut has long been recognized for its health-related benefits. They reported that digestible carbohydrate forms the major chunk (about 50% the entire mass), with 10% protein and 9% dietary fiber which consists mainly of cellulose and lignin. Tiger nuts have a good profile of essential minerals namely, zinc, magnesium, potassium, calcium, and vitamins C and E (Kim et al., 2007; Asante et al, 2014). The nut is consumed in Ghana mainly for its perceived aphrodisiac properties and also as an important source of nourishment serving as a ready-to-eat (RTE) snack. It is an ingredient in healthy and balanced diets due to its high nutritional quality (Asante et al., 2014; Ocloo, Okyere, & Asare, 2014). Tiger nut is a suitable composite material for the production of yogurt, and its yogurt quality is comparable to that of dairy milk (Sanful, 2009).

Millet in Ghana is one of the local grain staples for individuals living in mainly the Northern locales. The availability of the protein, vitamins, essential minerals, with high fiber content makes millet an essential ingredient to be considered in products like infant food, snacks, and dietary enhancements. Moreover, millet cuisines have progressed toward becoming a part of daily meals in homes and at restaurants (Subramanian & Viswanathan, 2007; Liu et al., 2012). Millet and tiger nuts are very fibrous; their fibres are readily“fermentable by colonic microscopic organisms, such as bifidobacteria and lactobacilli as a carbon source, making them a decent source of prebiotic (Elena Sánchez-Zapata et al., 2013).

The market for fermented products in Ghana keeps expanding(Tawiah, 2015). The production of fermented cereal foods is a common practice all over the world and it is done using a variety of cereals such as rice, millet, corn or sorghum (Blandino et al., 2003). In Accra, the capital city of Ghana, commonly consumed traditional fermented beverages include *asana, fura*, and fermented cereal-based dairy product locally referred to as *brukina* (Tawiah, 2015). *Brukina* is a millet-based purely fermented dairy drink ((Otwey, 2015;Baidoo & Kunadu, 2018). The nature and the composition of *brukina* generally make it a suitable candidate to be considered synbiotic product but limited studies have been done along that line. A synbiotic product is a food product that contains probiotic(s) and prebiotic(s) to sustain probiotics in the gut of the consumer. Otwey (2015) reported on the processing options, focusing on the development of the particle size of the millet agglomerate and sensory acceptability. Fermented composite milk (tiger nut and dairy milk) *brukina* has been reported to have good consumer acceptability and microbiological quality (Nyarko-Mensah, 2018). There is thus sufficient research that supports the development of a synbiotic *brukina* beverage to enhance the nutrition and wellness of many Ghanaians that consume this traditional beverage.

1.1 Problem statement

Tiger nut is a nutritious crop; the tubers have a lot of benefits (highly fibrous, source of prebiotic) as an ideal composite material in synbiotic food processing. However, the level of patronage indicates that tiger nut is an underutilized crop. It is typically consumed raw as snack. Nyarko-Mensah (2018) developed *brukina* from millet and tiger composite milk maximizing the usability of the nut in Ghana. Also, Asante et al. (2014) developed a tiger nut cocoa beverage and studied the shelf life of the product. Prior to Asante's work, Sanful et al. (2009) developed yogurt from composite milk of dairy and tiger nut. All of these works prove the feasibility of the tiger in

developing nutritious affordable food products. However, none of these products focused on developing the pre-probiotic (synbiotic) product that has incorporated tiger nut fibre and a suitable probiotic into the final product. The work of Sánchez-Zapata et al. (2013) which incorporated tiger nut fibre into sausage did not make the product synbiotic, no suitable probiotic was added. Also, pasteurization of tiger nut juice is known to help reduce microbial load present in tiger nut-based beverages, but heating tiger nut juice a little above 60°C causes gelling, which affects the functional and physicochemical properties of the food. For that reason, use of tiger nut milk in heated foods is limited.

1.2 Rationale

Tiger nut is a nutritious tuber. The utilization of the whole tuber can be improved by producing a synbiotic drink with the nut as a major component. This drink will be affordable, nutritious and microbiologically safe beverage. The utilization of enzyme in the extraction of the tiger nut juice has been proven to reduce the problem of gellification but the enzyme used did not address the issue of breaking the cellulose component (Tapsoba, 2016). Cellulase hydrolysis is a safe method for tiger nut starch and fiber hydrolysis. It is thus an avenue that can be exploited to enhance the quality of tiger nut milk. Cellulase hydrolysis will help reduce gellification that will result from pasteurization and increase the reducing sugar content of the tiger milk. Improving the microbial quality of this product by fermentation, heat application and also the unique incorporation of the tiger nut fibrous co-product into the millet agglomerate of symbiotic *brukina* has the potential of increasing shelf life and improve local consumption.

1.3 Main objective

To utilize whole tiger nut in the production of dairy-cereal based beverage, making it synbiotic and assess the nutritional profile, physicochemical profile, and microbial quality of the drink.

1.4 Specific objectives

- To develop composite agglomerate of millet and tiger nut co-product
- To determine physicochemical, nutritional profile and microbial quality of the fermented tiger nut-cereal based dairy synbiotic drink
- To determine the shelf life of the fermented tiger nut-cereal based dairy synbiotic drink

CHAPTER TWO

LITERATURE REVIEW

2.1 Tiger nut

Tiger nut (*Cyperus esculentus L.)*, is a member of the family Cyperaceae. It is a perennial spherical grass-like plant. *chufa* is the recognized name of the nut in most locations across the globe. It has different commonplace names, which include; earthnut, yellow nutsedge, and *Zulu* nut. In Ghana, the *Akans* call it *atadwe*, *atangme* via the *Gas, fie* via the *Ewes* and *nansaxa* by the Dagombas (Dokosi, 1998). The commonly known varieties of tiger nuts are, *Esculentus, Hermannii*, *Leptostachyus*, *Macrostachyus*, and *Sativa*. In Ghana, the varieties of interest are *esculantus*, and the *sativa*, they referred to as the black and brown nut and their categorization is based on form, colour, and length of the nuts (Tetteh & Ofori, 2011). Tiger nut is 1.0 to 2.0 cm tuber obtuse in shape and depending on the state, weather soaked in water or dry will come off as a round or irregular tuber. The brown and black nut are the outward appearances as the nut has whitish and juicy flesh on the inside (Tetteh & Ofori, 2011).

2.1.1 Handling and use of the nut in Ghana

Tiger nuts are heat sensitive. Storage is always carried out in jute sacks and baskets in airy locations (Tetteh & Ofori, 2011). The tubers are in particular cleaned of soil particles by means of rubbing the nuts collectively in a basket. In some locations, the tubers are washed in water bodies to eliminate the adhering soil earlier before storage (Tetteh & Ofori, 2011). The popular locations in Ghana to locate tiger nuts include areas with heavy vehicular traffic and heavily populated locations inclusive of lorry stations, areas round motor tollbooths and street intersections. Vendors patronize the nut in head pans tied in plain polythene bags. Presently, the majority of harvested

tubers grown in Ghana are eaten raw as a snack or used in the preparation of other beverages as a composite material. Normally, beverages are prepared from tiger nut are offered at lorry stations, marketplace locations, and creation sites. It is eaten by all including babies and adults. Drivers who journey for long hours chew it to save you them from dosing off whiles driving.

Records suggest that before the early 70's a few quantities of tubers had been exported to Spain, Japan, USA and England (Ofori, 1994;Pascual et al, 2000)). In 2004 for instance, approximately 3 million kilograms were exported to Spain from Ghana, Nigeria, and Togo (Arranz et al., 2006).

2.1.2 Nutritional characteristics of tiger nut

Tiger nut has high carbohydrate content which is unique and defines the nature of any product from the nut. The carbohydrate is the major component of the nut with the corresponding energy value of 400-413.8 kcal/100g and has been reported on by several authors. (Table 2). The carbohydrate content has been reported to be around 43.3 g/100g. Comparatively, the starch content in the wet matter of tiger nut has been reported to be around 25- 29.9%, which is similar to that of cassava and more than twice the starch content of the potato (Sánchez-Zapata et al., 2012; Asante, & Saalia, 2014). Dietary fibre content, of 8.81 g/100g, is much higher than in other tubers, which contain 0.66–2.55 g/100g. The insoluble dietary fibre, is mainly cellulose and constitutes 99.8%. Sucrose content has also been reported to be 13.03 g/100g.

Table 2.1Comparative proximate analysis of tiger nut

Parameter	**Black type**	**Unspecified**	**Brown type**
Crude protein (%)	5.04	9.15	5.66
Fat (%)	24.49	33.33	17.6
Carbohydrate (%)	43.3	46.99	64.16
Starch (%)	-	-	-
Crude fibre (%)	8.91	11.11	11.62
Moisture (%)	-	8.66	-
Ash (%)	1.7	22.33	1.23
Energy (kJ/100g)	413.8	1754.98	1867.07
References	Sanchez-Zapata, (2012)	Salau et al. (2012)	Asante et al. (2014a)

Source (Tapsoba, 2016)

Skimmed milk has been reported to have an energy value of 33 kcal/100 mL, while tiger nut milk and milk, soy milk have been reported to have energy values 33 to 58 kcal/100 mL(Roselló-Soto et al., 2018). The overall plant-based milk alternatives present a more extensive range (12–92 kcal/100 mL), which is impressive and can be used as a composite material as a skimmed milk alternative. Tiger nut beverages processed using various methods have been reported to have protein content ranging 0.47–2.51% (Linssen et al., 1989). Plant-based milk with the highest overall protein content has been found to be in soy-based beverages, ranging from 2.50 - 3.16% whiles the rice and almond-based milk alternatives exhibit the lowest protein content with 0.28% and 0.31–0.59%, respectively (Arafat et al., 2009). The tiger nut of essential amino acids is 14.27

g/100 g, which represents 28% of the total amino acid content (Yeboah et al., 2012; Belewu and Belewu, 2009)

2.1.3 Functionality of the tiger fibrous co-product

The tiger nut co-product which is obtained after milk extraction contains a high proportion of total dietary fibre (59.71 g/100 g) (Verdú et al., 2017). This is mainly of insoluble dietary fibre (99.8%). Sánchez-Zapata et al., (2010) reported that the fibrous co-product of tiger nut has a high water-holding capacity (8.01 g/g) and oil-holding capacity (6.92 g/g) and a low water absorption (1.79 g/g) and water adsorption (0.23 g/g) capacities, in comparison with other dietary fibre sources . The emulsifying ability was 70.33 mL/100 mL, and the fibrous co-product showed high emulsion stability (100 mL/100 mL). The co-product high content of fibre allows application in the development of dietary fibre rich foods . E. Sánchez-Zapata et al., (2010) evaluated the increasing utilisation of TNF coproduct at increasing levels (0%-control, 5%, 10%, and 15%) development of burger. The pork burgers that had TNF recorded a higher nutritional value (higher fibre content) with better cooking characteristics (higher cooking yield, fat retention and moisture retention) than control burgers. Textural parameters, particularly chewiness and springiness increased. Burgers with TNF were perceived as less greasy, less juicy, and more grainy and with less meaty flavour than controls. No significant differences were detected with the scores of control, 5% and 10% TNF burgers. The TNF addition to burgers is a promising and convenient application as a source of dietary fibre to produce fibre rich foods. The fibre in the diet consists mostly of the plant polysaccharides that cannot be digested by human digestive enzymes and they include cellulose, hemicellulose lignin and some materials that make up the cell wall (Soudham et al., 2013).

Cellulose and hemicellulose typically represent about 40 to 50% and 20 to 30% of plants biomass respectively, these polysaccharides that can be broken down into sugars through hydrolysis by enzymes or acids, and these sugars can be fermented as a carbon source for probiotic microorganisms (Amarasekara & Owereh, 2009; Soudham et al., 2013).

2.2 Synbiotic foods

Synbiotics are food products that contain probiotics and prebiotics. They exert positive synergy between the prebiotics and probiotics within the product. Probiotics are distinct living microorganisms that have beneficial health significance in adequate amounts when used in either food processing or the pharmaceutical industry (Pineiro et al., 2008; Hill et al., 2014). Probiotics have been observed to aid in the growth beneficial bacteria over most pathogenic and spoilage microorganisms. The none digestible nature of prebiotics as food components are beneficial to the host, it aid in selectively stimulating the growth and/or activity as a preferred substrate for a selected group of microorganisms living in the colon (Hill et al., 2014).

An important rationale for using a synbiotic is that a true probiotic, without its prebiotic food, does not survive well in the digestive system. Without the necessary food source for the probiotic, it will have a greater intolerance for oxygen, low pH, and temperature. By harnessing both the advantages of the prebiotics and probiotics into synergy, the number of good bacteria increases several-fold in the digestive system (Rastall et al., 2005; Ewaschuk & Dieleman, 2006; Petschow et al., 2013). Although the most commonly used probiotics include species of *Lactobacillus* and *Bifidobacterium*, the yeast, *Saccharomyces cerevisiae*, and some *Escherichia coli*, *Bacillus* species are also used as probiotics. Lactic acid bacteria (LAB) including species of

Lactobacillus serve as agents of food fermentation and potentially impart health benefits. (Ziemer & Gibson, 1998).

Generally recognized prebiotics consist of fructooligosaccharides (FOS), galacto-oligosaccharides (GOS), lactulose, pyrodextrins, and soybean-oligosaccharides (SOS) (Anadón et al., 2016). Fermentation of FOS in the colon increases the numbers of Bifidobacteria as well as the absorption of calcium and fecal weight in the colon. In addition, it leads to a decrease in gastrointestinal transit time (Sekhon & Jairath, 2010; Saikh, 2014). The increase in colonic Bifidobacteria produces compounds that inhibit potential pathogens by lowering blood ammonia levels and by producing vitamins and digestive enzymes . There is also a significant increase of short-chain fatty acids (SCFAs), ketones, carbon disulfide, and methyl acetate following the intake of synbiotics (Vitali et al., 2010). Synbiotics lead to a modulation of the gut metabolic activities with the maintenance of the gut biostructure and act by delivering specific health benefits. (Kolida & Gibson, 2011). Compared to probiotics and prebiotics, synbiotics products are the least investigated substances in regard to health effects, safety, and toxicity.

2.3 Plant-based milk processing

Plant-based milk is a milk-like product that represents another possible way of meeting the demands for proteins, vitamins, minerals, carbohydrates, and beneficial health ingredients from plant sources (Aidoo et al., 2010). However, the overall quality of the plant-based beverage is dependent on the processing methods. Tiger nut milk production involves soaking, blending, milling, pasteurization, fermentation, and the addition of preservatives (Tunde-Akintunde & Souley, 2009). The extraction for the uses different production methods, but the choice is mostly

dependent on the nature and type of the plant product with much emphasis on the sensorial quality of the beverage after the processing.

The primary process of juice extraction from plant source is mostly by blending the wet (fresh or soaked) plant material with water; or by milling the dried plant material to get a powder which can be dissolved in water and sieved (Aidoo et al., 2010). Djomdi et al., (2007)reported that water uptake during soaking has direct impacts on the texture and the grinding properties of the plant material as well as the milk solids. Water used in soaking as it has been taken up by plant cells causes the cells to swell, and softening the plant tissues, with a corresponding increase in cells cytoplasmic content which results in higher milk solids.

Milling if used is a processing method that helps in the qualitative and quantifiable variation of the properties of plant-based milk (Kizzie-Hayford et al., 2015). To standardize the tiger nut milk extraction method, Kizzie-Hayford et al. (2015), observed tiger nut milk obtained from different milling intensities improved during 16 hrs storage in milk solids and also showed positive figures in the nutritional parameters (Table 2.2), This is because higher processing force (milling intensity) brought about the conglomeration of organic polymers which brought about colloidal destabilization. It improved the lightness, stability and decreased the browning rate of the tiger nut milk during storage .

Table 2.2: Effect of milling intensity on the transfer of nutrient compounds into tiger nut milk (g /100 g**)**

Nutrient compound	**TNM -1**	**TNM-2**	**TNM-3**
PROTEIN	80.34	80.43	81.71
FAT	73.49	77.89	79.14
ASH	76.46	77.32	78.18
INSOLUBLE FIBRE	16.63	14.54	13.13
SOLUBLE FIBRE	80.71	68.85	86.97
CARBOHYDRATE	38.57	43.31	40.68

Source (Kizzie-Hayford et al, 2015)

Tiger nut milk after 1 min milling (TNM.1), after 2 min milling (TNM.2) or after 3 min milling (TNM.3)

Pasteurization is the processing that has been proven over time to have a significant impact on microbial load reduction. Pasteurization of the plant-based beverage affects the flavour and colour (darker) of the final product as well as the milk solids (Asante et al., 2014).

Plant-based milk as compared to cow or any other animal milk is cholesterol-free and lactose-free. According to Yadav et al., (2003) soymilk is one plant-based milk that has crude protein content (3.12%) comparable cow milk crude protein content (3.18%).

The calcium, riboflavin and vitamin B12 content in cow milk are relatively higher than plant-based milk. The most common early signs of vitamin B12 deficiency is fatigue or lack of energy.

Riboflavin plays a crucial role in certain metabolic reactions, mostly the conversion of carbohydrates into sugar, which is "burned" to produce energy. These facts have led to the fortification of plant-based milk by manufacturers and the development of composite plant-based milk. One popular composite plant-based milk is cowpea-peanut milk in which cowpea with its low energy content is added to peanut to balance the energy deficiency (Aidoo et al., 2010).

Generally, plant-based beverages have the characteristic flavour and colour of the particular plant material. Plant-based milk extracted from unroasted peanuts or soybeans is characterized by their intense flavour, suspension instability, and chalky mouthfeel (Buzzell et al., 2013).

2.4.1 Resistant starches

Dietary carbohydrates according to Englyst et al. (2007) has the following classifications :

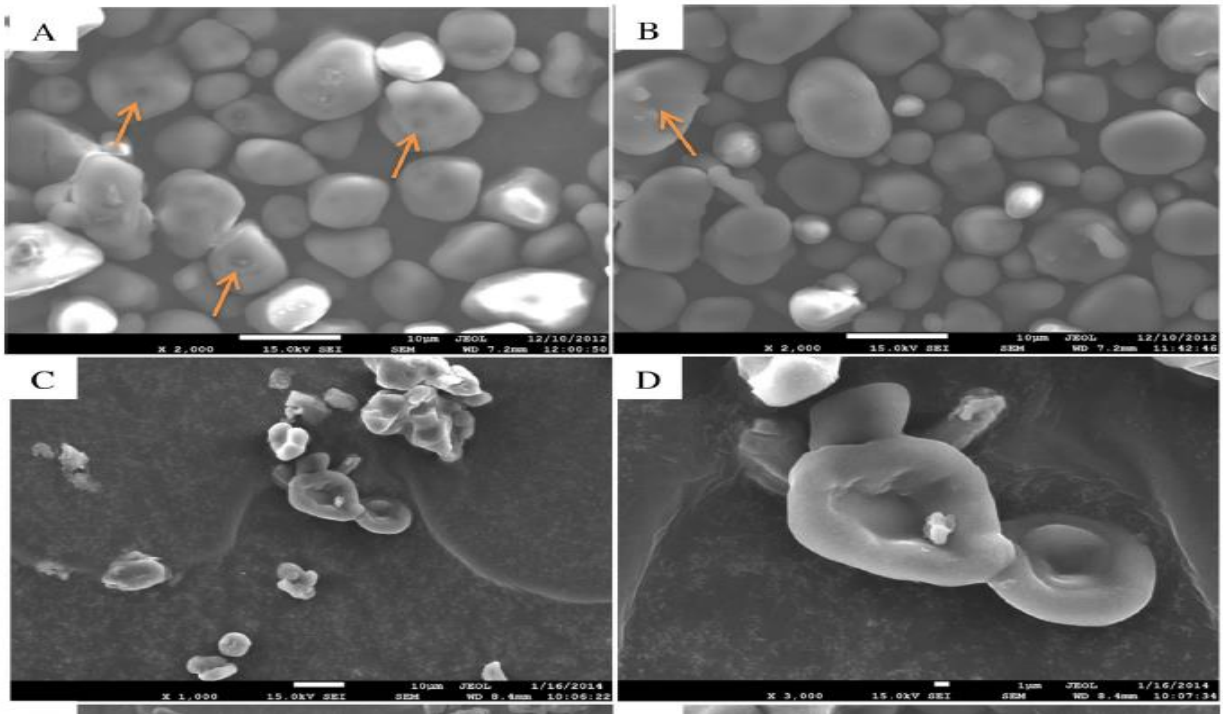
(i) Accessible starches: these types of starch are processed are adequately metabolized and assimilated in or before reaching the small intestine.

(ii) Resistant starches: these starches also oppose assimilation in the intestinal tract or are inadequately metabolized.

Nutritionally, dietary fibre remains the most prominent resistant carbohydrate. Be that as it may, various different resistant starches occur in limited quantities in food naturally or have been created during food processing for specific purposes. These resistant starches include gums, oligosaccharides, fructans, polydextrose, safe maltodextrins, and resistant starches (Englyst et al., 2007). Resistant Starch (RS) is a starch group that resists intestinal breakdown into D-glucose

within 120 mins of being consumed, however, it serves as a fermentable substrate in the large intestine for gut microbiota (Lei Friis, & Michaelsen, 2006).

A small number of features may make the starch be resistant to assimilation, and they include the size of the starch-containing parts been coarsely ground grains, the structure and adaptation and the development of retrograded crystallites because of formulating and chemical transformation (Figure 2.1). RS is a primary carbohydrate in many food components including grains, oats, vegetables, seeds, tubers, and a few nuts (Yang et al., 2016).



Source: (Yang et al., 2016)

Figure 2.1: Morphology of high-amylose starch granules by scanning electron microscopy (A) and B are native starch granules respectively (C) and (D) were obtained after hydrothermal treatment for 0.5 h, respectively The arrows in (A) and (B) indicate the small depressions on the granule surfaces of native starches which appear pronounce in (C) and (D) .

2.4.2 Subtypes of resistant starches

Resistant starches have been grouped into five general subtypes named RS1–RS5, which are portrayed beneath:

Type 1 resistant starches are physically out of reach starches bolted inside cell walls and food medium, in a manner that forestalls amylolysis. Processing and biting by consumers can make these starches progressively available and less resistant. It is stable to heat processing in most ordinary cooking operations, which allows its utilization in food processing (Abdel-Aal, 2009).

Resistant Starch type 2 is made out of starch granules from individual plants containing uncooked starch or starch that was gelatinized ineffectively and hydrolyzed poorly by R-amylases (e.g. high-AM corn starches) (Abdel-Aal, 2009; Yao, Paez, & White, 2009).

Type 2 again portrays starch granules that are shielded from processing by the adaptation or natural structure of the starch granule. This structure has low bioaccessibility to various amylases in the stomach, and is responsible for the resistant nature of RS2, for example, ungelatinized starch consumed in foods like a banana (Sajilata et al., 2006). RS2 is able to hold its structure notwithstanding the subjected handling and processing in food preparation (Charalampopoulos et al., 2002). This enables it to be steady in most ordinary cooking tasks and allows its utilization in food preparation whiles maintaining its structure (Sanz et al, 2010).

During food preparation, much of the time in which steam is included (boiling and steam cooking), RS type 1 and 2 can be transformed into RS type3 (Sanz et al., 2010). According to Story et al. (2011), polysaccharide called 'retrograded resistant maltodextrins' is a type 3 RS. They are gotten from starch that is handled to improve or hydrolyse starch granules intentionally, and

consequent retrogradation to render them impervious to assimilation. This procedure brings about the development of inedible crystallites that have sub-atomic comparability to type 3.

Type 4 RS includes chemically modified or re-polymerized starches (e.g. chain linkage altered dextrins, ethers, or esters) used by food manufacturers to alter the functional characteristics of the starch and include starches which have been esterified, or cross bonded with chemicals in such a manner as to decrease their digestibility (Nugent, 2005). RS4 can be produced by chemical modifications, such as conversion, substitution, or cross-linking, which can prevent its digestion by blocking enzyme access and forming atypical linkages (Aravind et al., 2013). Type 5 RS is an Amylose(AM)-lipid complexed starch, which is formed from high amylose(AM) starches that require higher temperatures for gelatinization and are more susceptible to retrograde (Cummings & Stephen, 2007). Also, Frohberg and Quanz (2008) defined RS5 as a polysaccharide that consists of water-insoluble linear poly alpha-1,4-glucan that is not susceptible to degradation by alpha-amylases. They also found that the poly-alpha- 1,4-D-glucans promote the formation of short-chain fatty acids (SCFA), particularly butyrate, in the colon and are thus suitable for use as nutritional supplements for the prevention of colorectal diseases.

2.5.1 Plant-based cellulose hemicellulose and lignin.

Cellulose is a linear homopolymer fundamentally thought of to be uncomplicated because of the homopolymeric nature. The chemical structure of cellulose, which is a linear polymer of β-(1→4)-linked to D-glucopyranose monomer units, is very straightforward. Normally, cellulose chains in essential plant cell dividers have a level of polymerization in the range from 5,000-7,500 glucose

monomer units. Most of the polysaccharides found in plant cell walls are classed either as cellulose, hemicellulose, or pectin (Mandels & Reese, 1999).

The plant cell cellulose as the dominant structural polysaccharide. Cellulose is the rigid, load-bearing component of the cell wall. The rigid cellulose is embedded within a structured matrix of hemicellulose that prides itself on its load-bearing ability (Mandels & Reese, 1999). The hemicellulose is also called the cross-linking glycans, and it is involved in the elongation and modulation of the cell wall. (Guan et al., 2008) The chemical composition of hemicellulose varies widely. Hemicelluloses are hetero-polysaccharides defined as a non-cellulose, non-pectin component of the cell wall. It consists of several polymers, varying in the composition of monosaccharides, glycosidic linkages, substitution patterns and degree of polymerization. Their chemical conformation and structural features vary extensively across species, subcellular location, and developmental stages (Guan et al., 2008).

Chemically, their association or cross-linking to other polysaccharides, proteins or lignin have been reported by several authors. Studies of bacterial cellulose produced by *Acetobacter xylinum* in the presence of various hemicellulose fibres have shown that these hemicelluloses readily become complexed into the interior and along the surface of the cellulose microfibril (Rahman et al., 2007).

Also, it is worthy of stating that the hydrolysis of lignocellulosic biomass produces reducing sugars and resistant starches that are fermentable by colonic microbiota (Rahman et al., 2007). Pretreatment of lignocellulosic biomass by steam followed by enzymatic hydrolysis and fermentation using the enzyme cellulase and xylanases on both hemicellulose and cellulose yielded reducing sugar near-theoretical glucose yield (96-104%) (Amarasekara, 2013).

2.5.2 Hydrolysis of cellulose

Enzymes cellulases catalyze the reaction of water with the glucose molecules in these chains to release single glucose molecules monomers by the following reaction :

$(C_6 H_{10}O_5)n + nH_2O \rightarrow nC_6 H_{12}O_6$ RXN 1

In which H_2O is water, $(C_6 H_{10}O_5)n$ is a chain made up of n glucose molecules that are often termed glucan, and $C_6H_{12}O_6$ is a glucose monomer (Amarasekara, 2013).

Thus, each glucose unit in the long-chain combines with a water molecule and 180 mass units of glucose are released from 162 mass units of glucan and 18 mass units of water, an 11.1% mass gain. Oligomers made up of several glucose molecules may also be released as intermediates in cellulose hydrolysis and often contain only 2 to perhaps three glucose units. Cellulase enzymes are particular in only catalyzing the addition of water to glucan chains, and the optimum temperature needed for reaction (RXN 1) is only about 50℃, virtually eliminating degradation reactions. Thus, only glucose is formed via enzymatically driven hydrolysis of cellulose, and yields can approach 100%. (Taherzadeh & Karimi, 2007; Amarasekara, 2013). On the other hand, the use of dilute acids (e.g 1.0% sulfuric acid) to drive reaction (RXN 1) requires much higher temperatures of about 220℃. The acid also triggers the breakdown of glucose to degradation products such as hydroxymethylfurfural, limiting yields. Concentrated acids (e.g., 75% sulfuric acid) can be used at moderate temperatures to achieve high yields similar to those for cellulase enzymes(Amarasekara, 2013).

Hemicellulose can also be hydrolyzed by the addition of water to release individual sugar chains contained in the longer hemicellulose molecule. The stoichiometry for the reaction of the hexose sugars galactose, glucose, and mannose that are in hemicellulose is the same as shown in reaction

(RXN 1), and an 11.1% mass gain results for these molecules. On the other hand, the addition of water to the five-carbon sugar molecules arabinan and xylan in the hemicellulose molecule follows the following stoichiometry(Amarasekara, 2013).

$(C5H8O4)n + nH2O \rightarrow nC6H12O^{-}6$ RXN 2

With $(C_5H_8O_4)n$ is a chain made up of n arabinose or xylose (pentose) molecules that can be termed arabinan or xylan, respectively, and $C_5H_{10}O_5$ being one of the corresponding pentose sugars formed by hemicellulose hydrolysis. Based on this reaction, the molecular weight of the sugar molecule released increases from 132 mass units before hydrolysis to 150 mass units of pentose sugars formed, a gain of over 13.6%. Hemicellulose is open to attack at intermediate positions along its long backbone with the release of oligomers made up of many sugar molecules that can be successively broken down to shorter chained oligomers before single sugar molecules are formed(Taherzadeh & Karimi, 2007).

A suitable mixture of enzymes known collectively as hemicellulase can catalyse the addition of water to hemicellulose with high specificity at modest temperatures, thus avoiding sugar degradation and resulting in high sugar yields(Öhgren et al., 2007).

Dilute acids (e.g., sulfuric) can also catalyse hemicellulose hydrolysis to sugars at temperatures of about 100 to 200^{o}C, but furfural and other degradation products are formed from the sugars at these temperatures if one targets good yields of hemicellulose sugars in solution. Nonetheless, the degradation of the sugars released can be modest enough to recover about 80 to 90% of the maximum possible sugars (Amarasekara, 2013). On the other hand, operation without adding acid limits recovery of hemicellulose sugars to about 65% or less, with most in the oligomeric form (Amarasekara, 2013).

2.5.3 Cellulase structure and function 2.5.3.1 Cellulase activity Gilligan and Reese (2009) first showed that the amount of reducing sugar released from cellulose by the combined fractions of fungal culture filtrate was higher than the sum of the amounts released by the individual fractions Since that preliminary report, many investigators "have used a range of fungal preparations to validate a synergistic interaction between homologous exo- and endo-acting cellulase components. Cross-synergism between endo- and exo-acting enzymes from filtrates of diverse aerobic fungi has also been demonstrated many times (Sadana & Patil, 1985).

These enzymes usually find available "ends" at the reducing and non-reducing termini of cellulose microstructure. random internal cleavage of surface cellulose chains by endoglucanases provides numerous additional sites for attack by cellobiohydrolases. Therefore, each hydrolytic event by endoglucanase yields both a new reducing and a new non-reducing site. Thus, logical consideration of catalyst efficiency dictates the presence of exoglucanases specific for reducing termini and non-reducing termini (Taherzadeh & Karimi, 2007).

2.5.3.2 Classification of cellulase enzymes

2.5.3.2.1 Endocellulase

The endo-1,4-β-glucanases or 1,4-β-D-glucan 4-glucanohydrolases (EC 3.2.1.4), which act randomly on soluble and insoluble 1,4-β-glucan substrates, are commonly measured by detecting the decrease in viscosity or reducing groups released from carboxymethylcellulose (CMC) (Sun & Cheng, 2002).

2.5.3.2.2 Exocellulase

The exo-1,4-β-D-glucanases, include both the 1,4-β-D-glucan glucohydrolases (EC 3.2.1.74), which liberate D-glucose from 1,4-β-D-glucans and hydrolyse D-cellobiose slowly, and 1,4-β-D-glucan cellobiohydrolase (EC 3.2.1.91), which liberates D-cellobiose from 1,4-β-glucans (Sun & Cheng, 2002)These enzymes can be further distinguished by their ability to aid the liberation of free sugars from either the reducing or non-reducing end of the cellulose chain. Determination of which preference a given enzyme has is usually carried out through synergy studies with enzymes of known orientation (Sun & Cheng, 2002).

2.5.3.2.3.Beta-D-Glucosidase

The β-D-glucosidases or β-D-glucoside glucohydrolases (EC 3.2.1.21) action discharges D-glucose units from cellobiose and soluble cellodextrins, and range of glycosides (Sun & Cheng, 2002).

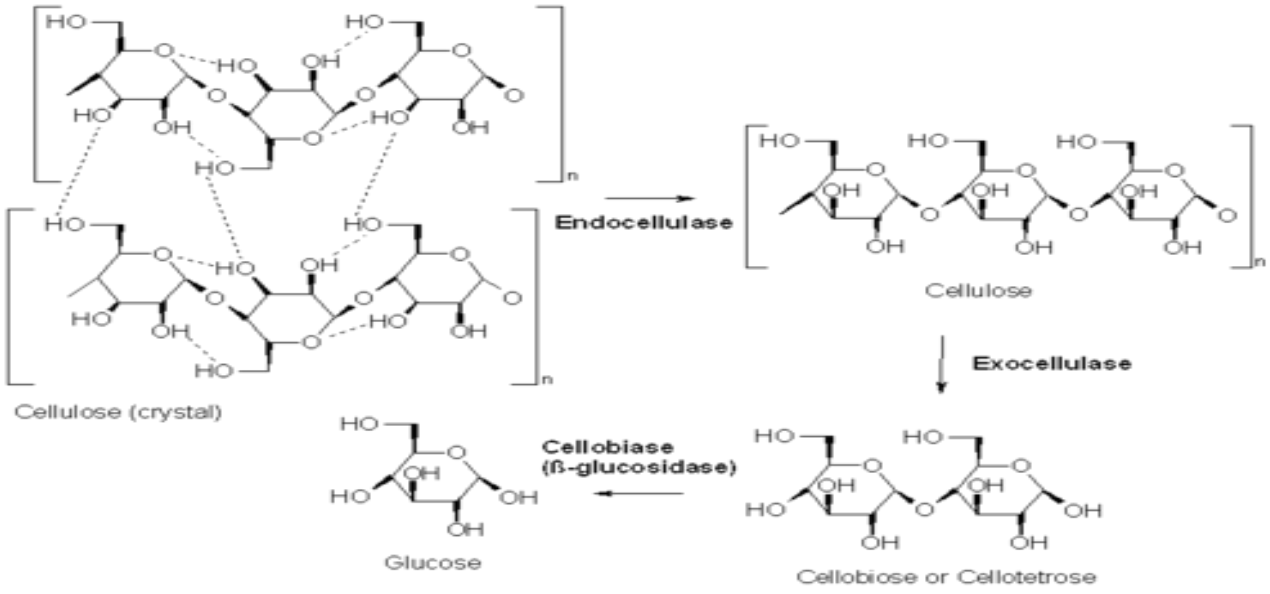


Figure 2.2: Cellulase synergistic mechanism of action (i)Breakage of the non-covalent interactions in the crystalline structure of cellulose by endo-cellulase (ii)Hydrolysis of the individual cellulose fibers to break it into smaller sugars by exo-cellulase (iii). Hydrolysis of disaccharides and tetrasaccharides into glucose by beta-glucosidase

(Source: McHale & Coughlan, 2003)

2.6 Microbiological quality of tiger nut milk and related composite beverages

Although tiger nut milk and its related products have several benefits, the primary factor limiting its production is partly losses due to spoilage at ambient temperatures. There have been some studies of the microbiological quality of tiger nut. However, information on the assessment of the microbiological quality of the composite tiger nut milk and its related product is limited. Spoilage of tiger nut is by microorganisms naturally present in the raw tiger nut, in the surrounding atmosphere partly due to handling since tiger nut is an underground tuber and will have much dirt which is home for most microorganisms.

Microorganisms that occur in processed tiger milk are usually due to unhygienic tiger nut handling and processing (Ayeh-Kumi et al., 2014). In their study, Ayeh-Kumi et al. (2014) surveyed pathogens associated with tiger nuts tubers sold in Accra and observed that consumers need to pay critical attention to proper washing and surface sterilization of the tiger nuts before consumption. In their study, they isolated several pathogens including some parasitic microorganisms and bacteria. The parasites identified were; *Cryptosporidium parvum* oocysts, *Ancylostoma duodenale* ova, *Strongyloides stercoralis* larvae, and *Cyclospora cayetanensis* oocysts and the bacteria isolates were all not pathogenic. They included *Proteus* which is a spoilage organism, the *Enterobacters and Klebsiella oxytoca* also coliforms, with *K. oxytoca* not typically associated with foodborne illness and the only *Staphylococcus* spp of foodborne disease relevance is *S. aureus.* In another study conducted on the microbiological quality of Spanish commercial tiger-nut beverages as well as home-made samples collected from supermarkets, street vendors, juice bars and ice-cream parlours located in Valencia, the authors observed total viable count, Enterobacteriaceae, *Escherichia coli, Staphylococcus aureus, Salmonella, Bacillus cereus,* yeasts, moulds, *Yersinia enterocolitis, Clostridium perfringens* and *Vibrio* spp. The obtained result indicated a high contamination level in home-made tiger-nut beverages (Sebastià et al., 2012.

The local production of tiger nut beverage deserves much attention than its currently receiving in terms of the microbial quality. According to Baidu et al. (2018), the microbial quality of nutritious (Kunun Aya) non-alcoholic tiger nut drinks that are often processed and consumed within the University of Maiduguri campus, Nigeria encountered microorganisms of significant public health concerns. The most important pathogenic ones isolated were *Salmonella, Shigella, Staphylococcus aureus, E. coli,* and, *Saccharomyces cerevisiae* and *Rhizopus oryzae* are also isolated but have no history of health implication.

2.7 Fermentation and its significance in plant-based beverages

Spontaneous fermentation is one of the oldest forms of food preservation in the world (Caplice & Fitzgerald, 1999). Natural fermentations are carried out by yeast and lactic acid bacteria, forming a complex microbiota that acts in cooperation. Yeast has a prominent role in the production of beverages, due to the ability to accumulate high levels of ethanol and to produce highly desirable aroma compounds, but lactic acid bacteria are particularly crucial in fermentation because they produce desirable acids, flavour compounds, and peptides that inhibit the growth of undesirable organisms .

2.8 Bacterial in food processing

Food is a complex system that provides varying conditions for living organisms residing in it. Bacteria used in food processing are affected by their surrounding environment, and they react with dynamism to variations that occur both externally and internally within the food matrix. They have specialized systems for distinguishing and adapting to these external stimuli, which can include exposure to extreme temperatures or stresses generated by cell growth, such as the production of acidic metabolites and nutrient depletion (Ehrnsperger et al., 1998). The versatility of bacteria in food processing has been used in Ghana for the production of most food products. Their metabolite serves as a flavor and taste-enhancing agent in beverages like *pito*, *brukina*, *asana*, texture improving agent in the likes of *gakomi*, and *banku*. The metabolite of these bacteria most importantly lactic acid bacteria(LAB) also have additional preservatives effect due to the production of lactic acid, acetic acid, ethanol, and carbon dioxide which aids to improve shelf life of product when used as fermentation culture (Tano & Celligoi, 2000; Parkouda et al., 2010).

2.8.1 *Lactobacillus* species

Lactic acid bacteria (LAB) are a group of organism that is very important to the food processor, the genera Lactobacillus is the largest and one of the most important within the LAB group of organisms per the classified outline of prokaryotes (Garrity et al., 2004)

Taxonomically, genus *Lactobacillus* belongs to the phylum Firmicutes, class Bacilli, Order Lactobacillales, family Lactobacillaceae. The genus has not less than 140 designated species with about 80- 90% of them were of industrial significance (Singh et al., 2009). The natural habitation of lactobacillus species both in raw milk and the GIT of mammals have been reported widely (Gorbach, 1990; De Roos & Katan, 2000; Brizuela et al., 2001).

Health benefits of *Lactobacillus* sp. are enormous, and it spans from the species been able to control the level of the pathogenic organism in food matrix to the intestinal, urogenital tracts (Sgouras et al, 2005;(Maragkoudakis et al., 2006). Fuller & Fuller, (1992) reported on the presence of LAB in the gut and its beneficial effect on health. Several other researchers have as reported on the lab *sp*. preventing intestinal disorders, hypercholesteremic effects, binding of mutagenic compounds, lowering the environmental pH and immune enhancement (Gorbach, 1990; De Roos and Katan, 2000; Brizuela et al., 2001)

Leal-Sánchez et al., (2002) and Cotter et al., (2005) in separate studies both reported on the ability of some lactobacilli strains producing bacteriocins or hydrogen peroxide, that exert antimicrobial activity on other species, resulting in inhibited growth and cell death.

2.8.2 *Lactobacillus casei* as a probiotic

Lactobacillus casei has tolerance for the high acid environment. They are rod-shaped LAB. Their habitats and applications have made them the predominant species of the Lactobacillus genus. According to Scheer (2006), the isolation the *Lactobacillus casei* is mainly from a variety of environments including raw and fermented milk and meat or plant products, as well as the oral, intestinal, and reproductive tracts of humans and animals.

Lactobacillus casei is a facultative heterofermentative species which produce lactic acid from hexose sugars through (Figure 2.1) the glycolytic pathway and from pentoses by the 6-phosphogluconate/ phosphoketolase pathway (Axelsson et al., 1998) 1 mol of ATP per mole of hexose and equimolar concentrations of lactic acid, acetic acid, ethanol, and carbon dioxide are generated. The quality of the fermented product along this pathway under the availability of substrate and suitable incubation temperature is significant in food processing, it helps to improve texture, flavour and eliminate other microorganisms that are pathogenic or may cause spoilage (Parry-Hanson kunadu et al., 2009)

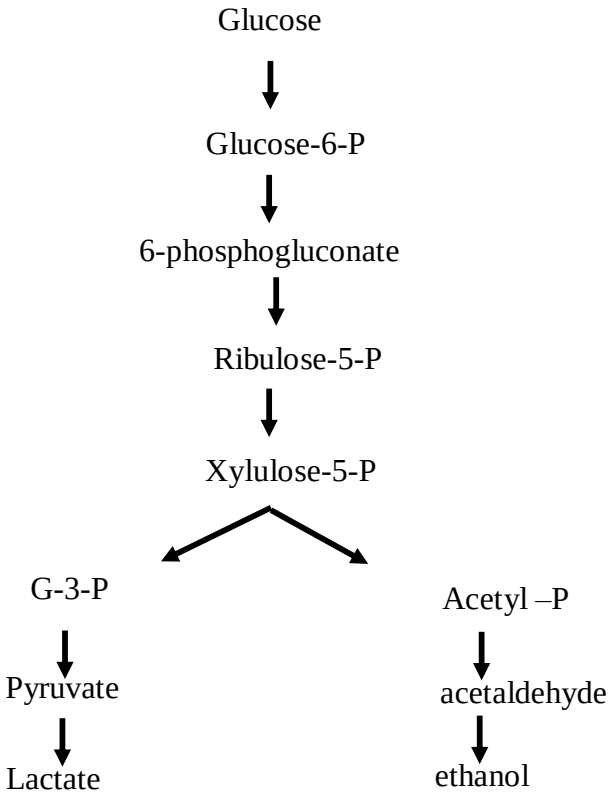


Figure 2.3: Fermentation of hexoses in heterofermentative lactic acid bacteria bacteria

(Caplice and Fitzgerald, 1999)

2.8.3 Industrial application *Lactobacillus casei*

Lactobacillus casei belongs to the distinguished class of thermophilic LAB; it has been widely used in the food industry for the processing of dairy products due to its biotechnological significance of heat resistance and ability to survive adverse processing conditions in food preparation (Kosin & Rakshit, 2006). According to Crow et al. (2001), *Lactobacillus casei/paracasei* are deliberately added to most food as starters during manufacture, and they have been found to survive pasteurisation in low numbers and grow in the food matrix. *Lactobacillus casei* are of utmost importance during the ripening of cheese due to their proven flavour producing and distinctive aroma enhancement (McSweeney, 2011).

Martínez-Cuesta et al. (2001) also has reported on the usage of *Lactobacillus casei* species isolated from high-quality cheese varieties as a potential to be used as adjunct cultures, which are added to the cheese milk for acceleration or intensification of flavour development and also to help control clostridia and gas-forming lactobacilli.

Lactobacillus casei in food matrix has low numbers post-manufacture due to manufacturing processes: however, as the food ages and their number increases, it becomes the dominant species representing up 96% of the non-starter LAB population at the end of ripening in cheese. Law and Law et al. (2000) found that dominant strains of *Lactobacillus casei* in 6-month-old cheeses appeared to be affected more by adjunct treatment and not cheese variety, and this property of *Lactobacillus casei* as an adjunct and ability to control competing pathogens can be utilised in the manufacturing of the synbiotic fermented based millet *brukina* with probiotic potential.

2.9 Millet

Millet is among the nutritionally significant cereals and are close to wheat, rice, and maize in terms of how it is consumed in Africa (Macauley, 2015). Millets are primary foods and are considered a major staple for inhabitants of hot and dry geographic locations. According to (Van Rijn et al., 2012), millet are cereals that grow in conditions of limited rainfall where other significant cereals fail to give substantial yields.

2.9.1 Nutritional composition of millet grains

Millets are rich in calcium, dietary fibre, and protein compared to other cereals (Devi et al., 2014). They are generally endowed with essential amino acids particularly the Sulfur-containing amino acids (methionine and cysteine); with higher fat content compared to maize, rice and sorghum (Belton et al., 2002). In general, cereal proteins, including millets, are limited in lysine and tryptophan content and vary with cultivar. However, most cereals contain essential amino acids as well as vitamins and minerals (FAO, 2009).

Table 2. 3: Nutritional composition of millet varieties

Component g/100g	**Proso millet**	**Pearl millet**	**Finger millet**
Protein	11.58	14.8	8.2
Ash	NA	1.6	2.7
Fat	4.9	4.86	1.8
Total CHO	80.1	59.8	83.3
Crude fibre	0.7	12.19	3.5
Reference	Bagdi et al. (2011)	Taylor et al. (2010)	Devi et al. (2011)

Source: (Tawiah, 2015)

Modification of a protein is usually realized by physical, chemical, biological actions such as fermentation or enzymatic treatment, which changes its structure and consequently its physicochemical and functional properties (Ieistienne et al., 2007). Millet foods are characterised to be potential prebiotic that can enhance the viability or functionality of probiotics with significant health benefits due to its high fibre content .

2.10 Texture

According to Phadungath, (2005), texture is quite difficult to define as it means different things to different people. Texture was formerly used to refer to the structure, feel and appearance of fabrics (Rosenthal, 1999). Food technologist attempted to define texture in terms of food because the meaning of texture did not cover the food aspect (Phadungath, 2005). International Organization for Standardization (ISO 4287, 1997) defines texture as a sensory characteristic perceived largely by way of the senses of touch and movement. Texture is the primary quality characteristic of solid food products. Texture analysis refers to the mechanical testing of food. Texture of products plays a very important aspect in the preference and acceptance of food products. Texture Profile Analysis (TPA) is a type of texture analysis that can be done on soft solid products. Instrumental measurement of the textural properties of food (TPA) is frequently used to understand consumers' perception of both food quality and the influence of the processing technology on quality (Liliana and Tudoreanu, 2009).

2.10.1 Relation between instrumental and sensory measures of food texture

The responsiveness of food texture depends highly on how the structure deforms and breaks when handled and eaten (Rosenthal, 1999). Therefore, any factor (ingredient interactions, processing conditions, storage, and packaging) that influences the structural properties of the food will affect its texture. Textural properties of food can be categorized into three groups depending on the sensation mechanisms : the visual, the acoustic and the tactile. For visual texture, properties such as smoothness, glossiness, thin and viscous are the most common examples. The appearance and light reflection of the food determine visual texture (Cook et al., 2005).

Texture features are closely associated with other sensory stimuli detected by mechanoreceptors. Internal scull vibration has also been shown to be important to the sensation of these texture features(Brenner & Nishinari, 2014). Of all texture features, the tactile texture is probably the most common and often the core focus of texture study. Tactile texture features are sensed by the direct contact between the food and human hands or oral surface which involves move movement and application of force and this is what TPA is objectively depicting using mechanical force and distance approach (de Cassia Silva et al., 2012).

CHAPTER THREE

MATERIAL AND METHODOLOGY

3.1. Sources of materials

The brown variety of tiger nut tubers (*Cyperus Esculentus L.*) was obtained from Twifo Praso in the Central region. The millet (Pearl millet) and Dairy milk powder were obtained from the Madina market.

Probiotic bacteria (*Lactobacillus casei*) strain isolated from human saliva was obtained from the microbiology research laboratory of the Department of Nutrition and Food Science, University of Ghana. The cultures were stored at −30 °C in de Man Rogosa Sharpe(MRS) broth (Sigma-Aldrich Co. Ltd., Dorset, UK) containing 10% (v/v) of glycerol (Rathore et al., 2012).

3.2 Tiger nut milk preparation

The tiger nuts were sorted to remove contaminated, defective tubers and whole tubers were washed with clean sterile water afterward. The nuts(200g) were then soaked in sterile clean water for 12 hours, then blended with water in a ratio of 1:7 (w/v) in a waring commercial blender(CB14). The obtained slurry was divided into two and labeled sample A and sample B. Sample A were enzymatically treated with Cellulases (NS 22186) 5% (v/w) (obtained from CSIR- Institute of Industrial Research Ghana Novozymes (A/S, Denmark) and sample B was raw blended tiger nut without enzymatic treatment. The slurry obtained from samples A and B were filtered through a 200 µm mesh nut milk bag (30.48 cm x 30.48 cm) to obtain the tiger nut milk separately. The milk was passed through the colloid mill to aid homogenization (Sanful, 2009; Asante et al., 2014).

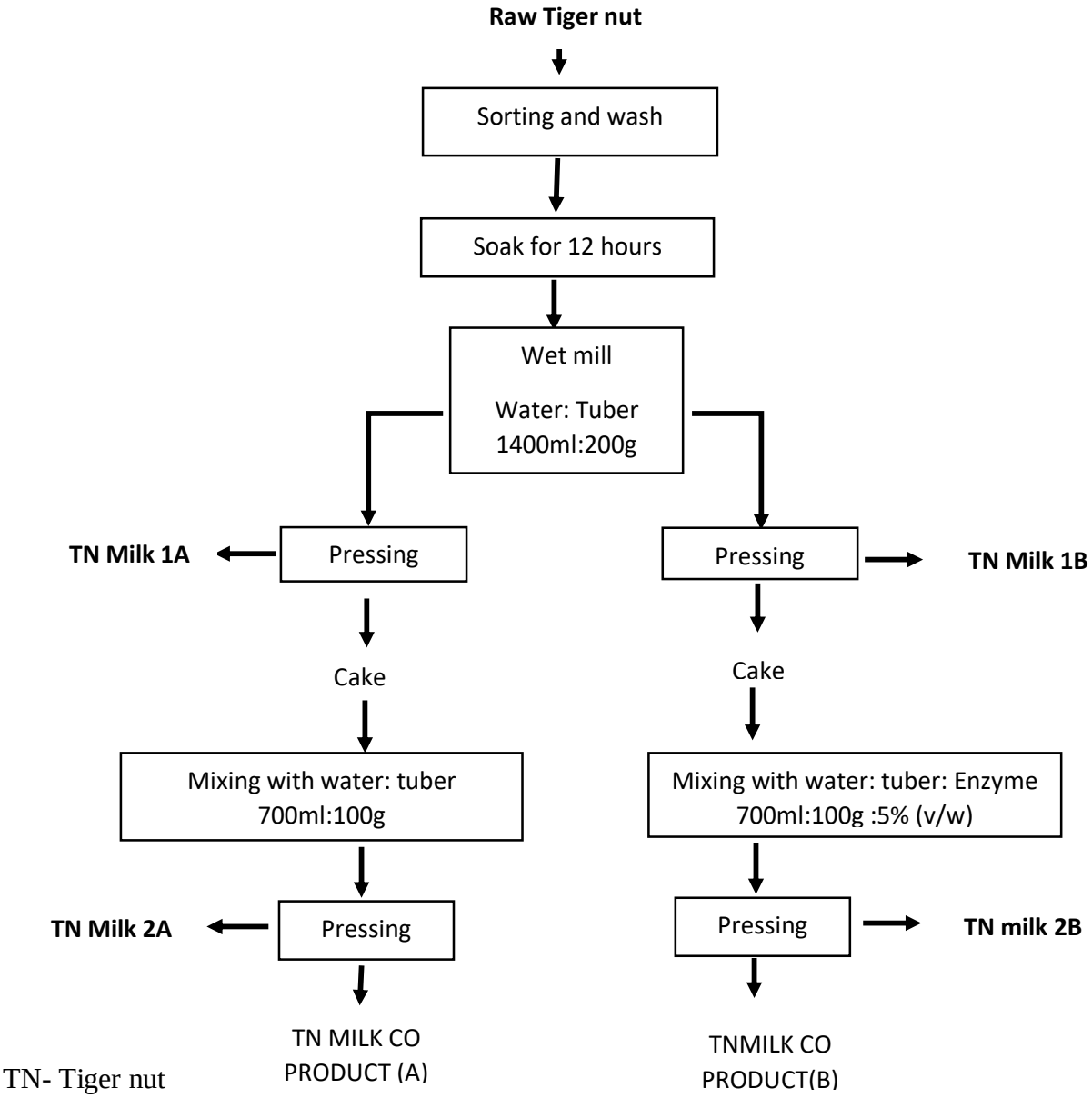


Figure 3.1: Flow diagram for the extraction of tiger nut milk

3.3 Formulation for agglomerate millet and tiger nut co-product agglomerate for synbiotic *Brukina*

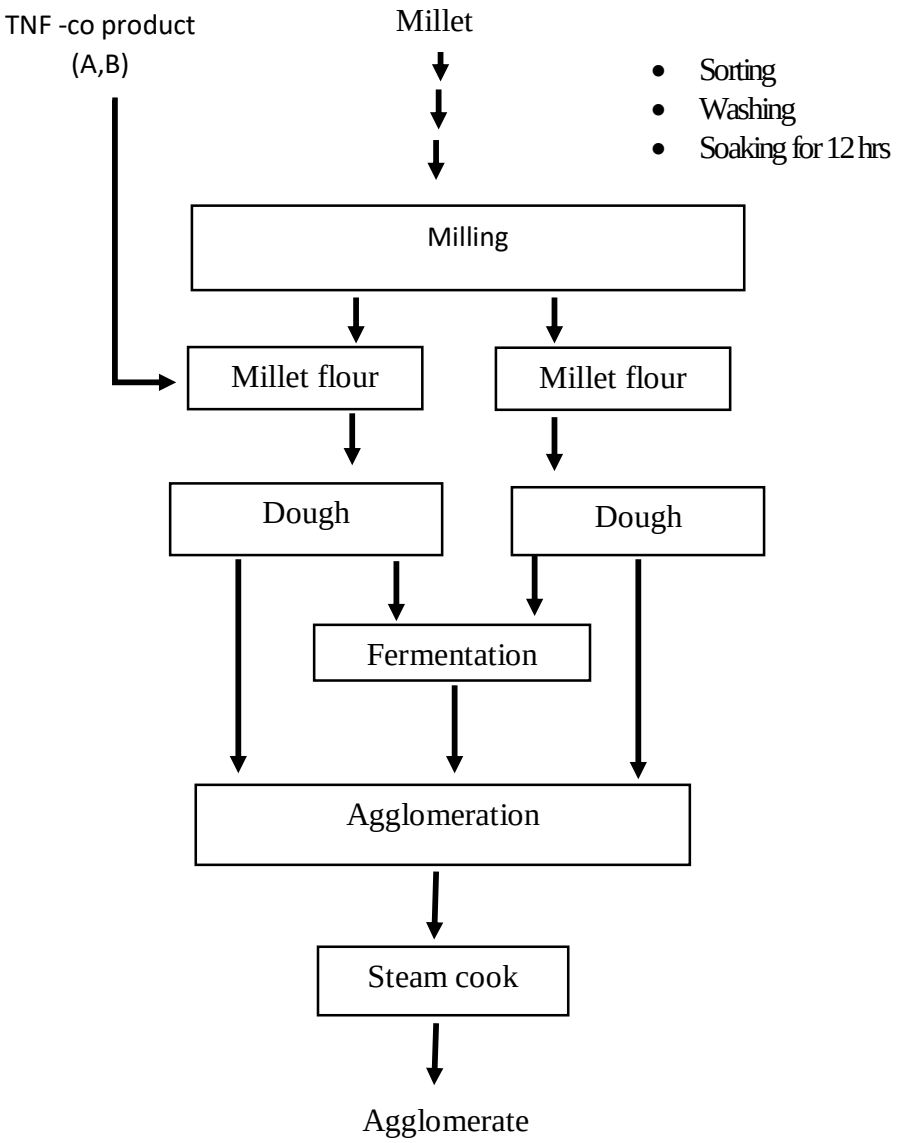
Using Minitab version 17. The define custom mixture design function was used to create a mixture design for data generated from the available literature. The Fibrous composition in fiber-based food product was used as the constraint setting it at 1.5 to 3g/100 kcal which is the fiber nutritional requirement for processed food (Mann & Cummings, 2009; Howlett et al., 2010). Two process variables, enzyme treatment of tiger nut and Fermentation for 12 and 24 hours Nine (9) formulations were generated to optimize the agglomerate development process.

Table 3.1: Formulation of millet and tiger nut co-product agglomerate for synbiotic *brukina*

SAMPLE	FERMENTATION TIME	TIGER NUT CO-PRODUCT	MILLET	ENZYME TREATMENT
A1	12	0	100	No
B1	24	10	90	No
C1	24	15	85	No
D	12	10	90	Yes
E	12	15	85	Yes
A2	12	0	100	No
B2	12	10	90	No
C2	12	15	85	No
A3	24	0	100	No

3.3.1 Preparation of millet and tiger nut co-product agglomerate

The millet (500g) was washed and sorted. It was then soaked for 12 hours to remove anti-nutrients and to aid digestion. Using the disc attrition mill, the millet was milled to obtain millet flour. The flour was divided into two equal parts and labeled A and B. Sample A was mixed with the Tiger nut coproduct (TNF) in a ratio of 10:90 and 15:85. Both samples were mixed with 1% water and knead to form a compact dough. The dough was divided into two, half was allowed to spontaneously ferment overnight, the other half was then rolled by hand on a sieve with a pore size of 2 mm to form uniform balls (agglomerate). The fermented sample was also rolled by hand to form a fermented agglomerate (Nyarko-Mensah, 2018). Both the fermented and not fermented agglomerate was steam-cooked for 30 mins.



TNF- Tiger nut fibrous co-product

Figure 3.2: Flow diagram for the preparation of millet Agglomerate and TN Co-product based Millet Agglomerate

3.4. Preparation of composite milk for synbiotic *brukina*

The fermented milk was processed using the method proposed by Sanful, (2009) with slight modifications. The powdered milk was reconstituted, (140. g) was added to 540 mL water and stirred to obtain 600 mL and added to the tiger nut milk in the ratio of 60:40. The mixture was pasteurized for 15 mins at 62ºC. The pasteurized milk was allowed to cool to 43°C, fermentation was performed under no pH control in 1000 mL Erlenmeyer flasks containing 800 mL of fermenting media and incubated at 37 °C for 10 h. The fermented milk was then pasteurized at 72°C for 30 mins and allowed to cool to 42°C to create a conducive environment for the probiotic microorganism. Samples were aseptically taken for probiotic inoculation

3.4.2 Probiotic inoculum preparation

The frozen *Lactobacillus casei* in vials were streaked unto MRS agar and subsequently cultured into MRS broth. Inoculated MRS broth was incubated at 37 °C for 12 hours in an anaerobic jar with an AnaeroGen sachet (an atmosphere generation system, Oxoid, Basingstoke, England), to achieve anaerobic conditions. Stocks were prepared routinely in the MRS medium. Before use, the lactobacilli culture was activated in sterile dairy milk media (prepared from water, and milk powder) at 37 °C for 20 hours. The culture was used as inocula at 1% (v/v) in each media during the preparation of the fermented beverages (Rozada-Sánchez, et al., 2008).

3.5 Preparation of cereal-based fermented synbiotic drink

The Synbiotic drink was prepared by adding the fermented tiger nut-dairy milk to the agglomerate millet- tiger nut co-product. 10 % of sucrose was added to taste.

The formed product at a temperature of 43°C was inoculated with 1% of probiotic *lactobacillus casie* inocula at 7 log10 CFU/ml, and bottled forming a synbiotic drink.

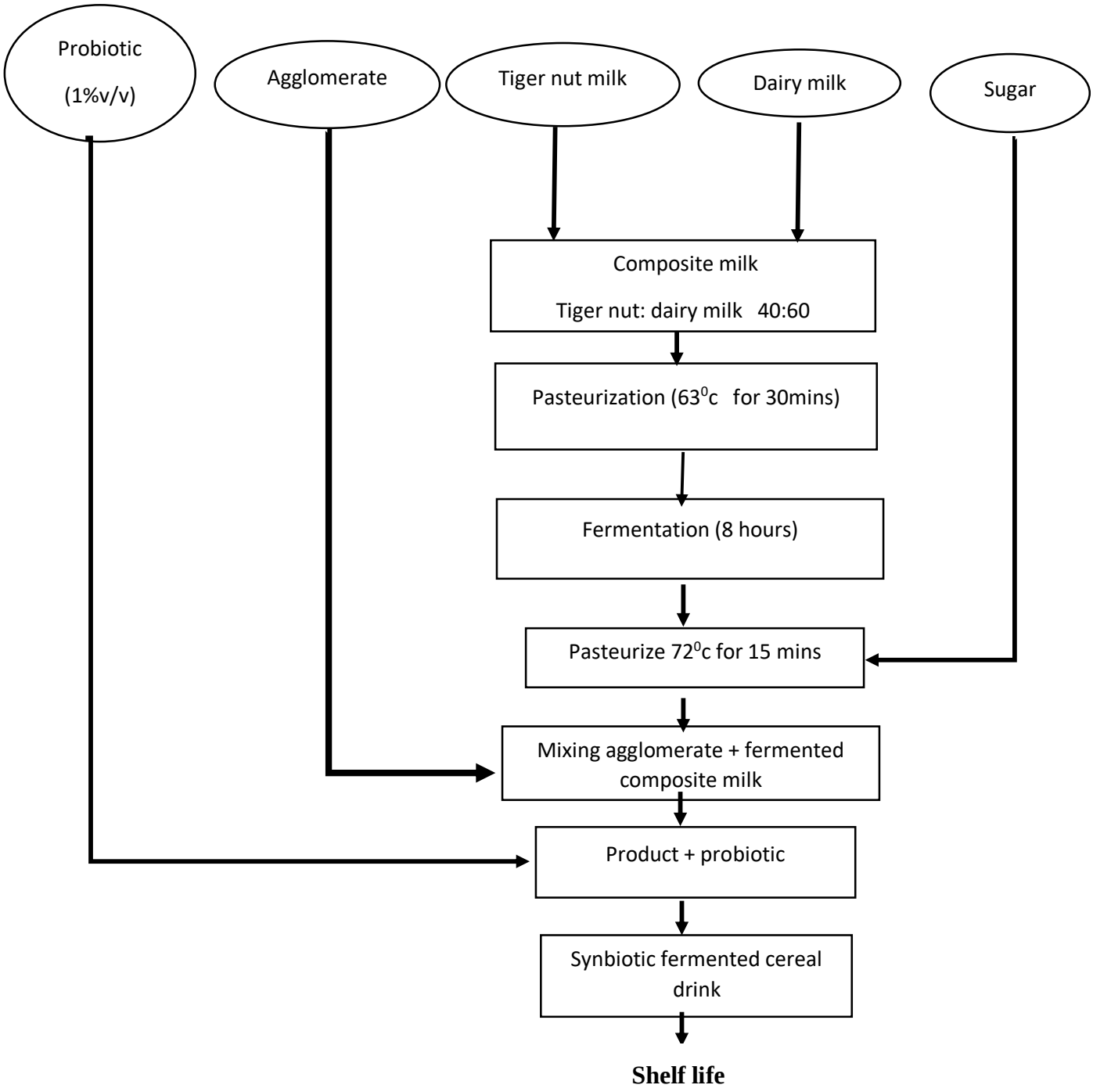


Figure 3.3: Flow diagram for the processing of fermented synbiotic cereal-tiger nut based dairy drink

3.6.0 Functional properties of agglomerate analysis

3.6.1 Texture analysis of the agglomerate

Texture measurements were obtained for individual samples using texture profile analysis (TPA) with a texture analyzer (TA-XT2i, Stable Micro Systems Ltd., UK) with compression probe and exponent software, A 1-g aliquot of soaked agglomerate was arranged in a single-grain layer on the base plate. The textural parameters of hardness (N), maximum force required for the initial compression of the sample, gumminess, the strength of internal bonds making up the body of the product, adhesiveness (N × mm), area under the abscissa post initial compression; and chewiness (N × mm), the required work to masticate the agglomerate and resilience were calculated . Force versus distance program was used to allow the plate to travel 9.9 mm, return, and repeat. The test speed was 1 mm/sec. The 1- aliquot of the agglomerate was subjected to a two-cycle compression test with a 75 mm in diameter cylindrical probe. A distance (rather than a percent) compression test was used for the TPA because of concerns that height or placement of single grains in the 1-g aliquots might unduly influence the sensed contact height that would account for the beginning of measurement used in percent compression tests . All analyses were performed in triplicate at 25ºC and the mean and standard deviation was calculated (Champagne et al., 1999).

3.6.2 Bulk density

The bulk density was determined in triplicate. The flour sample was put into 10 ml measuring cylinder. The cylinder was gently agitated continuously until a constant volume was obtained (Ocloo et al., 2014).

The bulk density (g/cm^3) = $\frac{weight\ of\ the\ flour\ (g)}{volume\ of\ flour\ (cm3)}$

3.6.3 Water absorption capacity

The water absorption capacity was determined in triplicate according to the method described by Ocloo et al. (2014), 5.0 g of the sample was weighed and mixed with 20 ml distilled water and allowed to stand for 30 min. at a room temperature of 28℃. The mixture was then centrifuged. (Centurion scientific centrifuge K3 Series) at 1512 relative centrifugal force (RCF) for 20 min. The excess water was decanted from the centrifuge tubes into a measuring cylinder and the volume determined. The water absorption capacity was calculated as (ml) of water absorbed per gram of flour.

3.6.4 Swelling index determination

About 5 grams of each sample was weighed and placed in 100ml of distilled water at room temperature in a graduated measuring cylinder. It was tapped gently to eliminate air and the volume was noted in Cylinder. The mixture was allowed to swell. It was subsequently swirled around and then it was allowed to stand for 5hour and final volume was noted.

Calculation: Swelling index = $\frac{final\ volume - initial\ volume}{sample\ weight}$

3.7.0 Physicochemical proximate analysis

3.7.1 Determination of pH and titrable acidity

The pH meter was calibrated with standard buffer solutions of pH 4, 7 and 10. About 30ml of the drink was aseptically poured into three different 50ml beakers and the pH determined using a digital pH meter (Mettler Toledo Seven Compact). The pH meter was dipped into the sample and the recording taken after about four minutes of stability. The pH meter was recalibrated after readings were taken (Salmerón et al., 2015).

Titratable acidity was determined using the (Darias-Martín et al., 2003) method. 10ml of the sample was measured into a conical flask and 90ml of distilled water would then be added. 0.1N sodium hydroxide was titrated against the solution using phenolphthalein as an indicator. The titratable acidity was calculated as percentage malic acid using the formula given below.

3.7.2 Determination of direct reducing sugars of tiger nut slurry during cellulase hydrolysis

The Lane-Eynon method was used to determine the reducing sugar content of the extracted milk 5 g of the test sample was weighed into 200 mL Erlenmeyer flask. 100 mL of water is added and the sample is shaken to extract all the sugar into the solution and finally topped up to 200mL mark. 5.0 mL each of Fehling's Solution A and B were added in a 200 mL Erlenmeyer flask containing few glass beads. From 50 mL burette, 15 mL of the sample was added. After boiling it on an electric stove for two minutes, four drops of the methylene blue solution were added. The titration was completed within a total boiling time of three minutes by the dropwise addition of the sample from the burette without preventing boiling until the blue color disappears. The titration was repeated and the mean of three parallel titrations. X (mL) was calculated .

The concentration of the direct reducing sugars, D (mg/100mL) is obtained from the titer, X (mL), by reference to the appended Lane-Eynon's table

% of Reducing sugar = $\frac{D}{S} * \frac{200}{100} * 100$

Where

D: Concentration (mg/100mL) of direct reducing sugar in the test solution, obtained by reference to the appended Lane-Eynon Table (dextrose).

S: Weight (mg) of sample collected

3.7.3 Determination of moisture content of the synbiotic drink

In determining the percentage moisture content of the samples, the method by (Aishwarya & Anisha, 2014) was used with modification by applying the sand pan technique. Clean, dry sand and a short glass stirring rod were pre-weighed into a moisture pan. The moisture pan and the sand were conditioned at 105 ºC for 30 minutes in Gallenkamp (Sanyo/Weiss) Hot Box Size 2 oven and cooled in a desiccator to room temperature. The weight of the metal dish was taken to the nearest 0.01g. Subsequently, about 5g of the test sample and the sand were mixed with the stirring rod left in the pan the total weight of the pan and the content taken. Using Gallenkamp (Sanyo/Weiss) Hot Box Size 2 oven, the metal pan with its content was heated at 105 ºC for about 5 hours. The metal dish with its content was covered and placed in a desiccator to cool for about 30-45 minutes after which it was removed and the final weight was taken. The moisture content was expressed as a percentage by mass of the product received

received; Loss of weight ×100

This implies that $\frac{M2 - M3}{M2 - M1} \times 100$

Where; M1 = Initial weight of empty pan + sand.

M2 = weight of empty glass crucible + wet sample.

M3 = weight of empty glass crucible + dry sample.

3.7.4 Determination of total ash content of the synbiotic drink

In determining the percentage Total Ash content, (AOAC, 1997) method was used. A the crucible was conditioned in a high-Temperature Furnace 2 Liter Capacity with CAL9400 Controller (Vecster) at 550 ºC for 30 minutes, then cooled in a desiccator to room temperature. The weight of the crucible was taken to the nearest 0.01g. About three grams (3g) of the test sample were weighed into the crucible and heated for about 20 minutes over boiling water bath until they were visibly dry. The total weight of crucible with its content was taken in grams. In a furnace, the crucible was heated at 660 ºC for 2 hours, then together with its content placed in a desiccator to cool for about 30-45 minutes. It was then removed and the final weight was taken. The Total Ash was expressed as a percentage (%) by mass of the product received;

This implies $\frac{M3 - M1}{M2 - M1} * 100$

Where;

M1 = Initial weight of empty crucible.

M2 = weight of empty g crucible + wet sample.

M3 = weight of empty glass crucible + ash

3.7.5 Determination of crude protein content of the synbiotic drink

In determining the percentage of protein content, (AOAC , 2012) method was used. About 0.25g of the samples were placed in a Kjeldahl digestion flask also containing a Selenium-based catalyst

and 25ml of concentrated sulphuric acid (H_2SO_4) added in a fume chamber. The flask was swirled gently to effect proper mixing and heated in a digestion chamber until digestion was completed after 5 hours. The digest was allowed to cool and transferred into a 100ml volumetric flask and topped up to the mark using distilled water. About 10ml of the diluted digest was put in the steam distillation unit, which was previously flushed with distilled water. About 18ml of 40% Sodium Hydroxide (NaOH) was then added to the solution in the steam distiller after which about 25ml of 2% boric acid was pipetted into a conical flask and two drops of bromocresol green-methyl red mixed indicator added. This mixture was placed under the condenser outlet of the distillation system, with the tip of the condenser completely immersed in it. The distillation was carried out until the boric acid solution turned from pink to yellow-green. The solution in the conical flask was titrated against 0.1M Hydrochloric acids (HCl) solutions and the endpoint recorded. The distillation and titration processes were done in triplicate . A blank was taken through the same procedure using distilled water in place of the sample. The crude protein content was then calculated using a factor of 6.25.

$$\text{Nitrogen \%} = \frac{(VS - Vb)\ 1.407 \times 100 \times NA \times 100}{Ms \times 10}$$

Where; Protein = % Total Nitrogen × 6.25

Vs= Titre value of acid titration against digested sample solution Vb = Titre value of acid titration against the digested blank NA= Normality of acid (0.1N HCl)

Ms = Initial mass of sample= Density of sample × volume. 6.25= General Protein conversion factor

3.7.6 Determination of total fat content synbiotic drink

In determining the Total fat content,(AOAC, 2000) (AOAC, 2000) method was used. About 100g of the sample was poured into a previously weighed glass Petri dish and dried over a water bath till most of the water had evaporated. The sample was then transferred to an oven and further dried at 105 ^{0}c till constant weight is obtained. The weights of water lost and dried solids obtained were determined by subtracting and later used to calculate the total amount of fat on the wet weight basis.Five grams (5g) of the dried sample was weighed into each two paper thimbles. The thimbles were sealed and placed in soxhlet extractors. About 150 ml of petroleum ether was poured into each of the two previously dried and weighed round bottom flask attached to the extractor. Extraction was carried out for 16 hours. After this period, the petroleum ether was recovered and placed in an oven (with the door partially closed) for the ether to completely evaporate. The flasks were cooled in a desiccator, weighed and the fat content calculated on the weight basis using the water content after drying the wet sample .

$$\text{Fat}= \frac{MF - WD}{MS - WT} * 100$$

Where; MF = mass of the fat extracted WD = mass of the total dried sample

MS = mass of the dried sample taken for extraction WT = mass of wet sample originally taken and weighed

3.7.7 Carbohydrate determination by difference method for synbiotic drink

The percentage of carbohydrate was determined using (Pattee et al., 1981), carbohydrate the percentage was expressed in percentage (%) by mass of the product, as the amount obtained after subtracting the moisture content, total ash, protein content and fat content of the test synbiotic drink (tiger nut-based *brukina*) from 100.

That is, %Carbohydrate = 100 - (Moisture content + Total Ash + Protein content + Fat content)

3.8 Mineral analysis of synbiotic drink

A wet digestion method using closed-vessel microwave digester (ETHOSEZ milestone) was used for the synbiotic *brukina* digestion during the mineral analysis. About 0.3g of the sample was placed in vessels with the 10 ml nitric acid and 5ml sulfuric acid. The vessels were then sealed and set on a carousel where the temperature and pressure sensors were then connected to a control vessel. The carousels were then placed in the microwave cavity, and the sensors were connected to the instrument. Time, temperature, pressure, and power parameters were chosen and the unit set to operate. The digestion was about 30 min, but because of the pressure generated by raising the temperature of the reaction, the vessels were allowed to cool for about 1 hour before opening. The solution was then cooled slightly after the process, and about 30 ml distilled water was used to rinse the vessel after the content has been transferred into a measuring cylinder it was topped with distilled water to the 50ml mark. The content was then transferred into a volumetric flask and cooked. This solution was used for all the mineral analysis.

The following minerals; Sodium (Na), Potassium (K), Magnesium (Mg), Calcium (Ca), Zinc (Zn), Iron (Fe), Phosphorus (P), Manganese (Mn) and Copper (Cu) were all determined in triplicate with

the use of the Perkin Elmer Atomic Absorption Spectrophotometer (Model AA 220FS, Massachusetts, USA).

3.7.9 Determination of vitamin E in the synbiotic *brukina*

3.7.9.1 Saponification

The sample was mixed and 2g were put into a round-bottom flask, 10ml of the methanolic ascorbic acid solution was added and 2ml of KOH solution was also added. The mixture was refluxed after 35 minutes the flask was removed from water-bath and kept in the dark until cooling.

3.7.9.2 Extraction

After cooling, the test material was put into a separating funnel, rinsed two times with 5ml water and successively with 30ml ether. The funnel was closed and mixed several times. The aqueous phase was recovered in the round-bottom flask and the ether phase was put into a flask. The extraction procedure was repeated 2 times with 30ml ether. Ether phases were combined and transferred in the separating funnel, rinsed 6 times with 50ml water, and recovered in a round-bottom flask. Separating funnel was rinsed with 10ml ether recovered in the round-bottom flask. Then, the test material was evaporated to dryness in a rotary evaporator under partial vacuum at water-bath. The dried extract was then solubilized with methanol and injected in HPLC (C18 column, reversed-phase). The quantitative determination of vitamin E was carried out by a UV detector settled at 294 nm.

3.8. Microbial analysis on the synbiotic *brukina*

3.8.1 Sample Preparation

Ten grams of each sample was weighed aseptically into a sterile stomacher bag, 90ml of sterilized 0.1% buffered peptone water (Oxoid CM0009) was added and homogenized for one minute using Stomacher® 400 CIRCULATOR. Serial dilutions were prepared from homogenates.

3.8.2 Total Plate Count (TPC) Determination:

Aerobic bacteria were enumerated by pour plating, the mesophilic bacteria were determined in duplicate using the plate count agar (pH 7.0 from Oxoid Ltd., Basingstoke, Hampshire - England) method of (Baxter & Holzapfel, 1982). Using micropipettes and sterilized pipette tips, 1ml of the dilutions was transferred into sterilized Petri-dishes in duplicates. About 15ml of a molten plate count agar was added and swirled thoroughly for 1 minute and allowed to solidify. The plates were incubated at 35°C for 48hrs. The number of colonies was counted and recorded as colony-forming units/ml

3.8.3 Yeasts and molds determination

Malt Extract Agar (pH 5.4 from Oxoid Ltd., Basingstoke, Hampshire - England) method (Baxter & Holzapfel, 1982) was used to determine the yeasts and molds population in the samples in duplicate. Sterilized Petri-dishes were inoculated with 1 ml of the sample solution after which 15ml of the malt extract agar were added and swirled to mix. The plates were incubated at 25 °C for how long? The number of colonies developed was counted and recorded as colony-forming units per gram of sample (CFU/g).

3.8.4 *Staphylococcus* species

S. aureus was enumerated by pour plating on Mannitol Salt Agar (MSA, Oxoid CM0085). Using micropipettes and sterilized pipette tips, 1ml of the dilutions was transferred into sterilized Petri-dishes in duplicates. About 15ml of a sterilized Mannitol Salt Agar was added and swirled thoroughly for 1 minute and allowed to solidify. The plates were incubated at 35 ℃ for 48hrs. The number of black colonies with hollow rings was counted and recorded as colony-forming units per gram of sample (CFU/ml).

3.8.5 Enumeration of *Lactobacillus casei*

Enumeration of viable cells was performed by estimating colony-forming units on De Man, Rogosa and Sharpe (MRS Oxoid CM036). Probiotic Bacteria counts were determined by the pour plate overlay method after incubation at 37 ℃ for 48 hours (Maselli & Hekmat, 2016).

3.9 Shelf life determination based on accelerated shelf-life testing method (Arrhenius model)

Fermented cereal-tiger nut beverage in plastic bottles with a net weight of 350 mL stored at critical temperature variations of 4℃ and 25℃ and 35℃. The analysis was done periodically every 24 hours from day 0 to day 5, to get 5 points of observation. Two parameters were used in the shelf life study, the Log CFU/ml of *Lactobacillus casei* and the pH value of the product.

The result data of each parameter is plotted against time (day) and the linear equation is obtained, so that the three equations obtained for three conditions of product storage temperature with the following equation:

$y = mx + c$ (1)

The choice of reaction order for a parameter is done by comparing the regression value (R^2) of each linear equation at the same temperature. The reaction order with a larger R^2 value is the order of reactions used by that parameter.

Ln (k) = ln (k0) − (EaR)/(1T⁄) (2)

From equation (2) Ko represents the deteriorating constant. It the factor that determines the quality of a product kept at a constant temperature. :

$k=k0 \cdot e^{-EaR/T}$ (3)

Based on the Arrhenius equation (equation 3) and the calculation of k , the shelf life of fermented cereal-tiger nut based drink using the first order kinetics

Ln(shelf life)= (EaR)/(1/T) +ln(K)

Shelf life = e - (EaR)/(1/T) +ln(K)

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Texture of composite agglomerate

The parameters measured for the textural analysis included hardness, resilience, cohesiveness, adhesiveness, and chewiness for the tiger nut and millet agglomerates as shown in figures 4.1-4.6. These parameters for samples B (15% TNF, fermented for 24 hours), C (10% TNF, fermented for 24 hours), D (15% TNF, fermented for 12 hours), and E (10% TNF, fermented for 12 hours) were compared to sample A (control), which was not fermented and non-tiger nut fibre-based agglomerate. Otwey, (2015) and Nyarko-Mensah, (2018) reported on the usage of millet without fermentation in the preparation of agglomerate with high consumer acceptability based on their textural characteristics. This informed the choice of control sample formulation since it has high consumer acceptability. The inclusion of TNF necessitated the fermentation and enzyme hydrolysis.

Hardness, defined as the peak force of the first compression cycle and in this context represents how firm the agglomerate behaves when compressed. This hardness is an objective representation of how the agglomerate responses to the first bite in the mouth (Mochizuki, 2005). For soft solid foods, the harder the food, the more significant force is required to compress the food, and this helps to provide the needed mouthfeel when eating. Figure 4.1 shows the effect of the compression test performed on the agglomerate. The hardness ranged from 27.21N to 38.21N. The control agglomerate compared to fermented agglomerate for 12 and 24 hours, tiger nut fibre inclusion and the cellulase hydrolysis did not have any significant effect (P=0.142) on the hardness of the agglomerate. This was an ideal trend and it favoured the production of agglomerate with desired hardness comparable to the control. This could be explained based on the following: the

agglomerate preparation, which involves steam cooking for 30 minutes initiated pregellatinization of the agglomerate starch. The amylose and amylopectin in the starch granules gelatinize and swell in the formation of soft solid food when heated and cooled; this formed polymeric network that defined the textural characteristics of the food.(Cagiao et al., 2004).

Also, the observed similarities in hardness of the agglomerates compared to the control could be due to the formation of complex long-chain polysaccharides, proteins and the starch component of the TNF and the millet which are capable of holding a high proportion of liquid that helps contribute to the textural characteristics, making the agglomerate firmer.

Extensive spontaneous fermentation leads to a reduction in pH by activities of amylolytic bacteria. Amylolactic bacteria at pH of 6 produce lactic acid and amylase for hydrolysis of starch which is attributed to a lowering number of high molecular weight starch molecules. This could have a result on the removal of adverse effects of the branching structure of intact amylopectin on gel formation. In starch hydrolysis, free regions are hydrolyzed preferentially and are composed of mainly amylopectin branches that are known to have effect on the texture (Wang et al., 2003;Srichuwong et al., 2017).

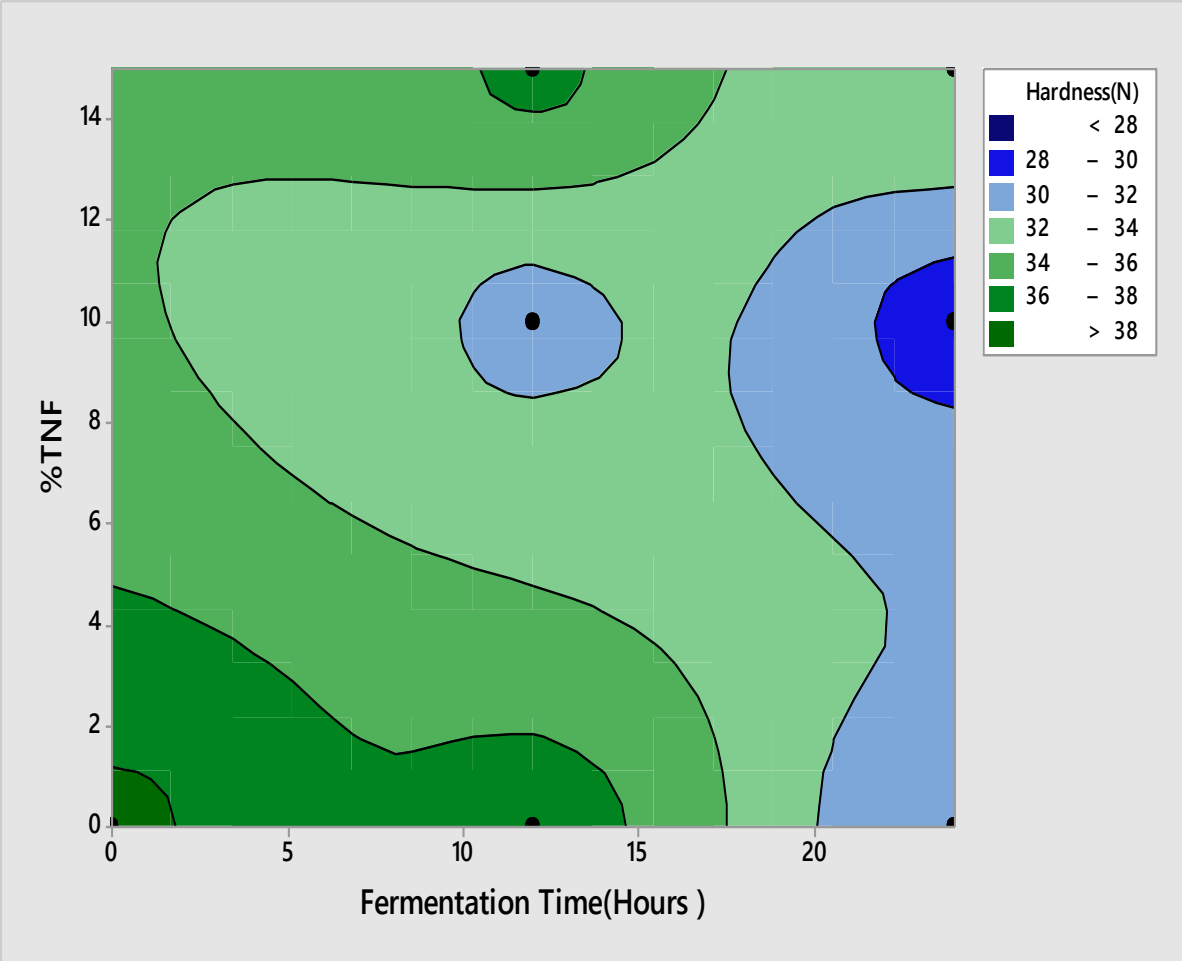


Figure 4.1: Contour plot showing the effect of tiger nut fibrous product inclusion and Fermentation time (hours) on the hardness(N) of composite agglomerate for synbiotic tiger nut-based *brukina* preparation

For soft solid food, adhesiveness and cohesiveness are objective depictions of the needed internal force to execute consistent break down of food during mastication. Adhesiveness is the negative force area for the first bite into food. It represents the work required to overcome the attractive forces between the surface of food and other materials with which the food comes into contact. Cohesiveness indicates the strength of internal bonds making up the body of food with respect to

the degree to which a food can be deformed. Cohesiveness and adhesiveness, as observed in this study, showed a similar trend as can be observed in figures 4.2 and 4.3. It was observed that 15% and 10% TNF inclusion, regardless of cellulase hydrolysis, and 12 hours' fermentation time significantly (P=0.001) increased cohesiveness of the TNF-millet agglomerate. Similarly, adhesiveness of TNF-millet agglomerate significantly increased (P=0.000) in samples with 15% TNF inclusion and fermented for 12 hours regardless of cellulase hydrolysis, compared to the control (no TNF and not fermented agglomerate). This observed trend could be related to the structural bonding and networking of large structure-forming molecules of the starch (amylose and amylopectin). Tiger nut starch and millet starch have A-type crystalline structure (Neto et al., 2018). Enzymes easily hydrolyze the A-type structure due to the absence of structural obstruction to amylase hydrolysis during the process of starch fermentation (Suma P & Urooj, 2015; Neto et al., 2018). The hydrolysis of TNF with cellulase and fermentation of the starch component in the TNF-millet samples led to increased tightly packed crystalline structure due to the preferential cellulase hydrolysis of amorphous regions within the biopolymers of the TNF and millet. The tight-packing of the crystal structure formed defined the internal bonding for the agglomerate, which was responsible for the cohesive and adhesive nature of the agglomerate as observed in the figures 4.2 and 4.3. The hydrogen bonds formed within the crystalline starch molecule prevented water absorption into the formed gel during steam cooking, giving the food it sticky nature (adhesiveness) and stronger internal bonds (cohesiveness) that will provide the needed mouthfeel during mastication.

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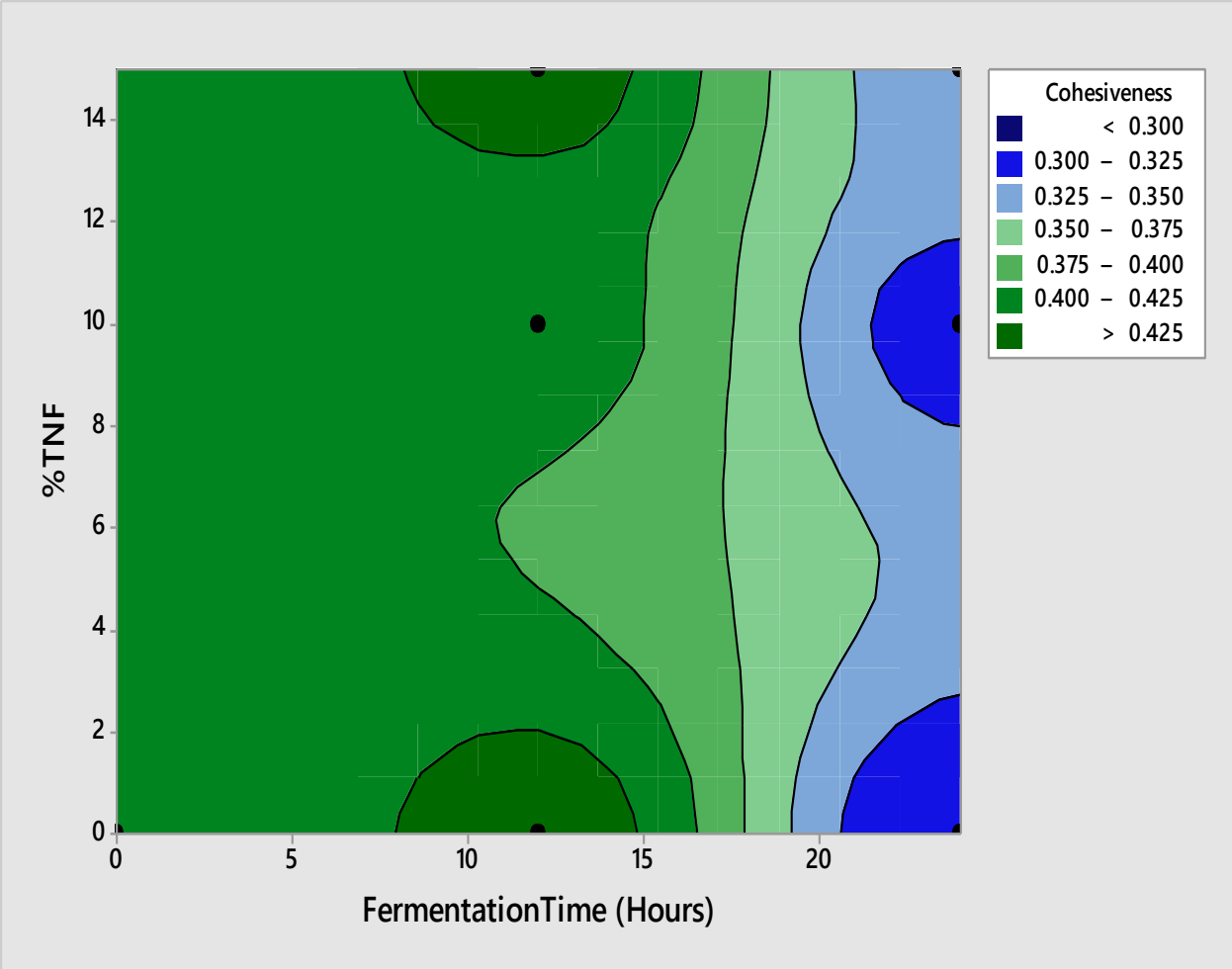

Figure 4.2: Contour plot showing the effect of tiger nut fibrous inclusion and fermentation time(hours) on the cohesiveness of composite agglomerate for synbiotic tiger nut based *brukina* preparation

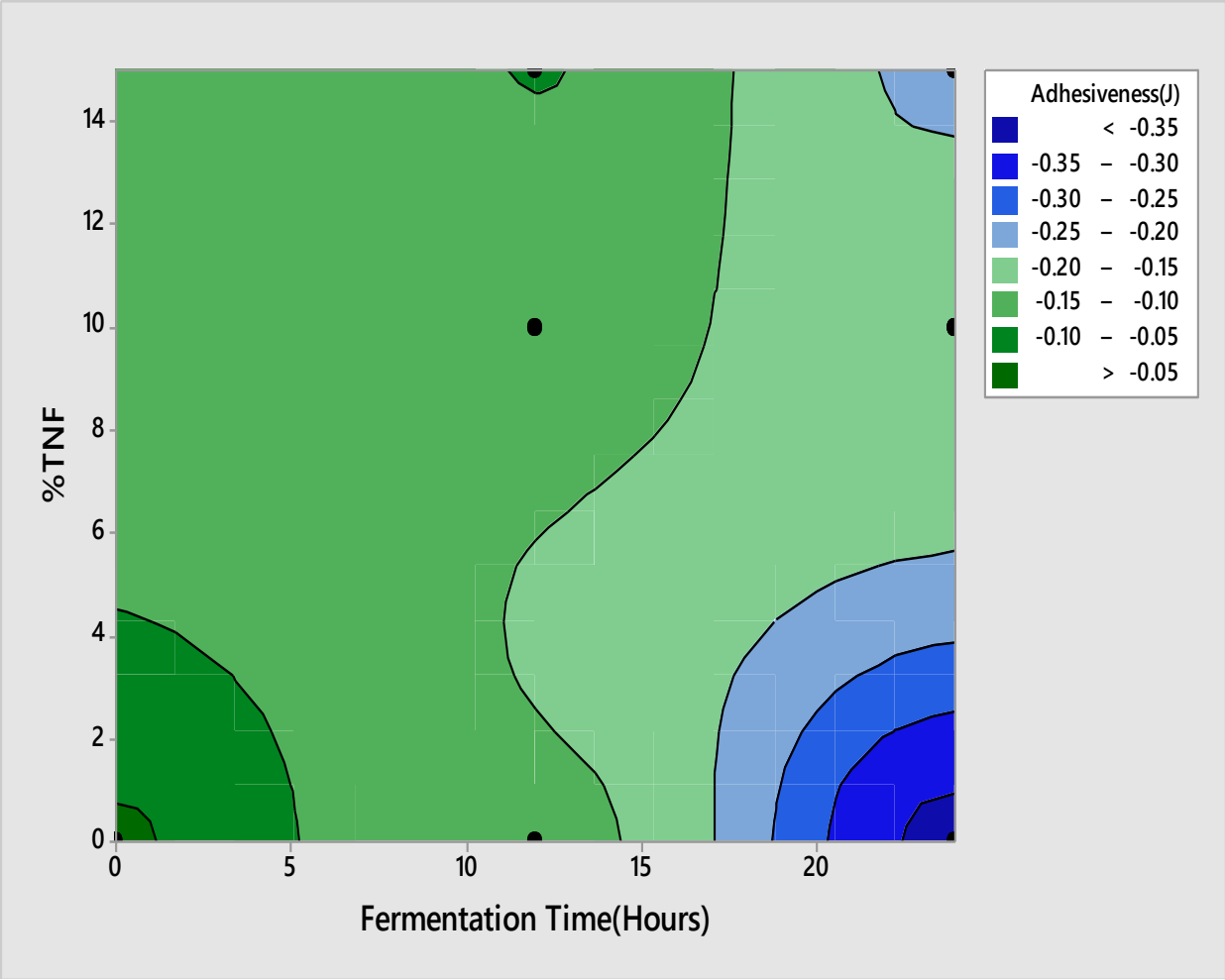

Figure 4.3: Contour plot showing the effect of tiger nut fibrous inclusion and Fermentation time (hours) on the adhesiveness of composite agglomerate for synbiotic tiger nut based *brukina* preparation

Resilience, gumminess, and chewiness are textural parameters of soft solid food. They are related to ease with which food breaks down during simulated mastication observed in TPA analysis. These parameters objectively relate to the needed force and speed with which soft solid food will be masticated. Resilience is a measure of how well a product fights to regain its original position, similar to elasticity (Patil & Arya, 2019). Chewiness, in the case of soft solid food, relates to gumminess and elasticity of the food as the product of the food hardness, cohesiveness, and

springiness. In this study, as can be observed in figure 4.3-4.6, cellulase hydrolysis, 15% TNF inclusion and fermentation time (12 hours) significantly increased (P=0.000) resilience of TNF-millet agglomerate samples compared to the control (no TNF, unfermented agglomerate).

Also, 12 hours' fermented agglomerates containing 10% and 15% TNF, regardless of cellulase hydrolysis, were significantly gummier (P=0.001) compared to the control. The mean gumminess of these samples (10% and 15% TNF, 12 hours fermentation) were 13 J and 15J respectively, while the mean chewiness of same samples (10% and 15% TNF, 12 hours fermentation) recoded as 5.6 N to 9.2 N respectively, were significantly higher (P=0.000) than the control.

The following characteristics are force and bonding related. The spontaneous fermentation will lead to changes in bonding and influence the crystalline structure of the starch in the TNF and millet. Fermentation led to increased crystallinity during starch hydrolysis due to the abundance of high amylose A-type crystal structures (Song & Jane, 2000) as explained above. The cleavage of some of the amylose chains running through the unstructured regions may allow reordering of the newly released chain ends into a more crystalline structure. This, with time, will result in increased crystallinity and partially fill all the water channels with double helices, rearrange it into the more crystalline regions (Song & Jane, 2000). In effect, there will more hydrogen bonds forming, less water absorption by the agglomerate. The hydrogen bonding will contribute to the resilience of the agglomerate. The gel formed during the hydrothermal process (steam cooking) made the food gummy and chewable due to the reduced influx of water to make it less hard (Suma P & Urooj, 2015).

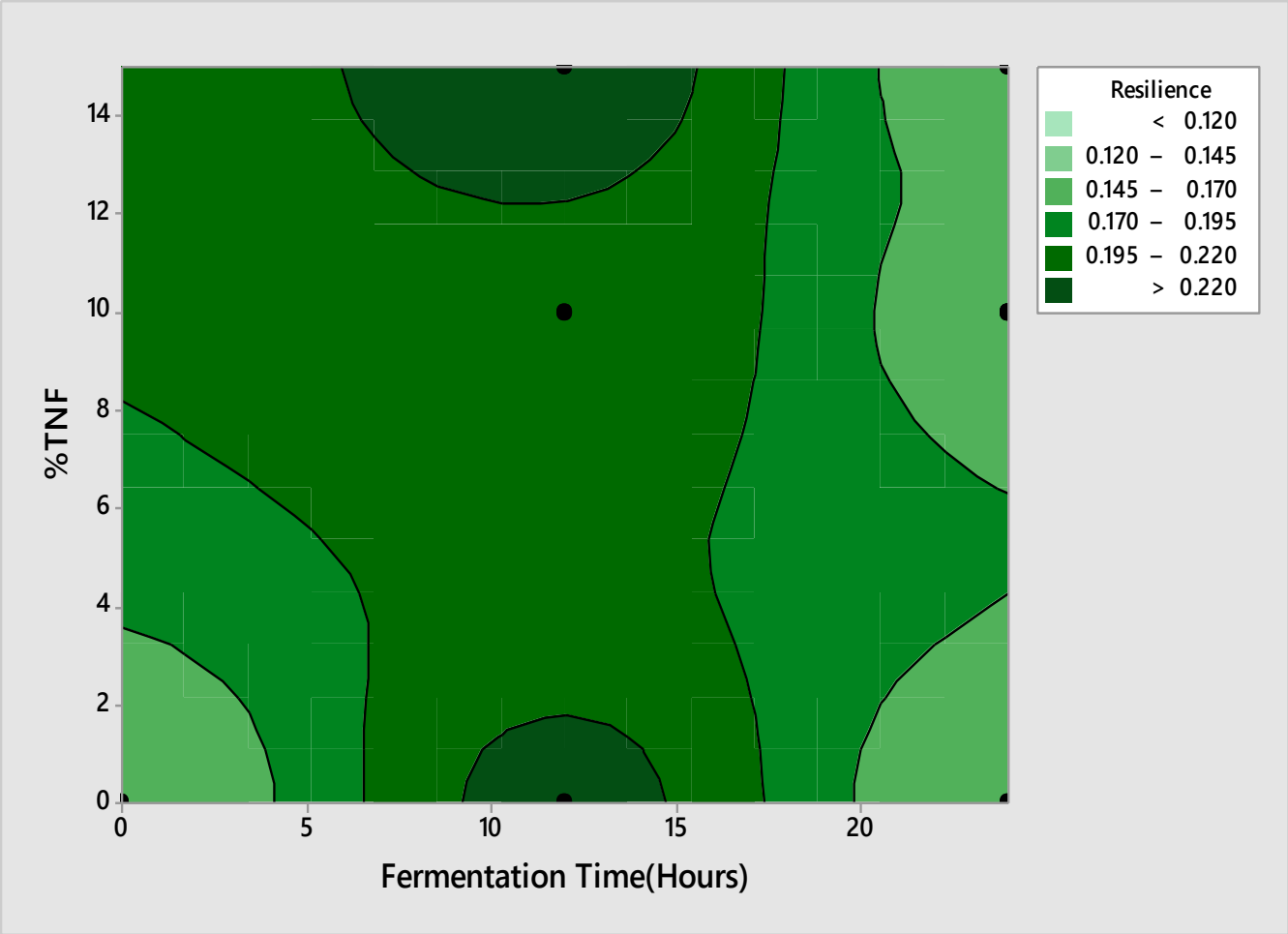

Figure 4.4: Contour plot showing the effect of tiger nut fibrous inclusion and fermentation time on the resilience of composite agglomerate for synbiotic tiger Nut based *brukina* preparation

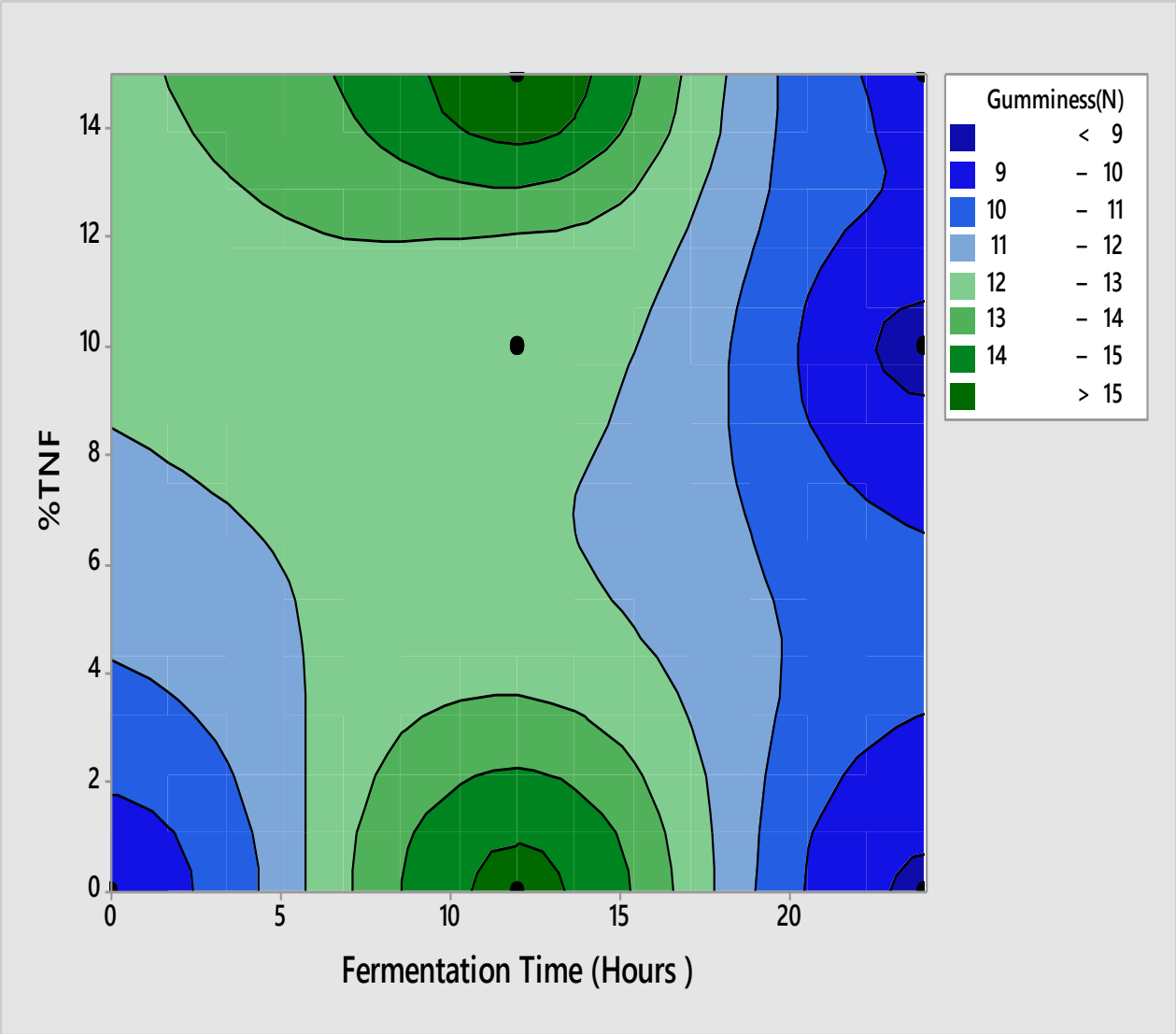

Figure 4.5: Contour plot showing the effect of tiger nut fibrous inclusion and fermentation time(hours) on the gumminess of composite agglomerate for symbiotic tiger nut-based *brukina* preparation

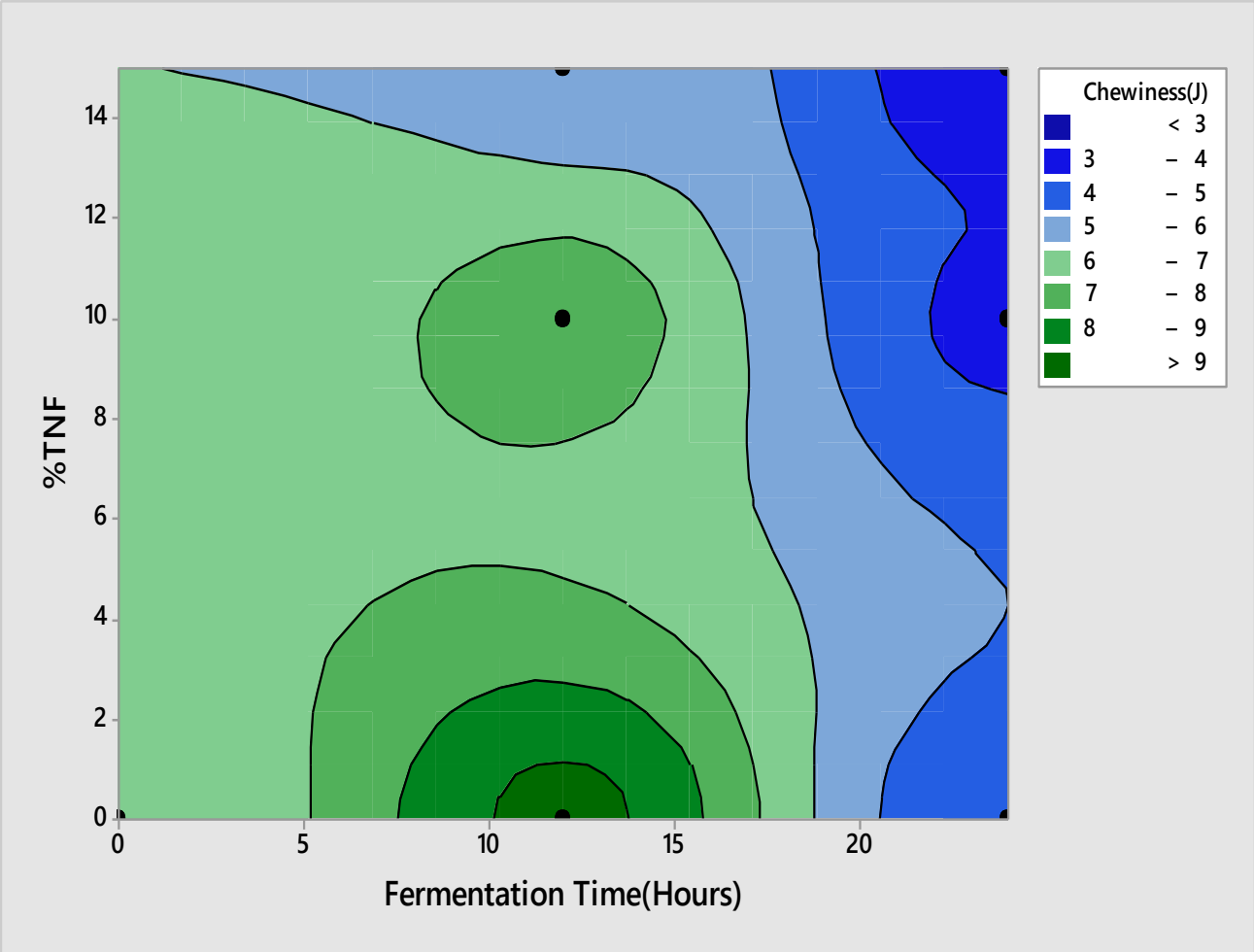

Figure 4.6: Contour plot showing the effect tiger nut fibrous inclusion and fermentation time (hours) on the chewiness of composite agglomerate for synbiotic tiger Nut based *brukina* preparation

4.2 Functional properties of composite agglomerate

4.2.1 Swell index, water absorption capacity, and bulk density

The compositional blend of 15% and 10% TNF agglomerate for the the synbiotic *brukina* production did not have any significant effect on the swell index and water absorption capacity (P= 0.422) and (P=0.640) respectively (Table 4.1).

Bulk density (BD) values for 10% and 15% TNF agglomerate were 0.55 - 0.63g/ml and 0.53-0.63g/ml for non-enzymatic hydrolyzed and enzymatic hydrolyzed fibre respectively (Table 4.1). The observed difference in BD was significantly higher (P=0.009) than the control. Similar bulk density values of 0.59g/ml (Adejuyitan et al., 2009) and 0.56g/ml (Chinma et al, 2009) have been reported for TNF based products. This observed difference in the bulk density is associated with the varying starch composition of the agglomerate from varying sources which influences structural deference.

Steam cooking at atmospheric pressure leads to the absorption of water by the agglomerate until gel formation. The high-water environment and heating is an ideal condition for starch pasting and gelatinization, leading to starch granules swelling, and losing crystallinity through the absorption of water. The overall effect is the creation of a gelatinized starch product. The linear amylose and available amylopectin align to form hydrogen bonding which expels water when cooled. For this reason, the formed agglomerate does not absorb much moisture and has little capability to swell further. The similarity in swell index and water absorption among all samples was expected as

these functional properties are characteristic of starches found in all the agglomerate ingredients, as can be observed in Table 4.1.

Also, as explained by Wang et al. (2015), hydrolysis has been found to cause changes in the swelling power of starch morsels. Amylopectin plays a critical role in starch granule swelling and water-holding ability once the amylopectin structure is disrupted, an intact linkage cannot be formed and the damaged chains tend to dissolve as they no longer can entrap water. Hence the observed trend in Table 4.1. Thus low water absorption and swelling capacity since the morsels in the hydrolysis of TNF and fermentation of the agglomerate loses its amylopectin structure water holding capacity as a resulting of enzymatic hydrolysis.

Table 4.1: Functional properties of cellulase and non-cellulase hydrolysed tiger nut fibrous co-product based millet agglomerate fermented for 12 hours

Sample ID(FT) (M: TNF) %	swell index	Bulk density g/l	Water Absorption l/g
A(0) (100:0)%	1.81 ± 0.20^{a}	0.54 ± 0.02^{b}	0.86 ± 0.12^{a}
B(12) (85:15)%	1.78 ± 0.16^{a}	0.63 ± 0.04^{a}	0.89 ± 0.17^{a}
C(12) (90:10)%	1.62 ± 0.08^{a}	0.59 ± 0.04^{ab}	0.70 ± 0.17^{a}
D(12E)(85:15)%	1.80 ± 0.06^{a}	0.55 ± 0.04^{b}	0.80 ± 0.17^{a}
E(12E) (90:10)%	1.66 ± 0.16^{a}	0.63 ± 0.00^{a}	0.79 ± 0.18^{a}

For every parameter, values in the same column with different superscripts are statistically different at $\alpha=0.05$. Sample A(0) (100:0)% is the control sample.

FT- Fermentation time for agglomerate in hours

M-Millet

TNF-Tiger nut fibre A-E- sample ID

4.2.2 Enzymatic activity of cellulase

The enzymatic activity of cellulase was determined by measuring the reducing sugar content of tiger nut slurry over time. The tiger nut slurry was obtained after adding warm water to the tiger nut co-product after tiger nut milk extraction. The analysis of the reducing sugar content in the tiger nut slurry, as shown in figure 4.7, revealed an initial reducing sugar content of 4.20 g/100g. Cortés et al., (2005) and Pascual et al, (2000) reported that starch content of tiger nut milk is within the range of 2.2-4.0 g/100g . The reducing sugar content increased in the milk during hydrolysis. The highest increase in reducing sugar content was within the time range of 6 hrs to 10hrs with reducing sugar content in the range of 10.5g/100g to 10.8g/100g. The results showed that cellulase was active and its activity was optimum at 6 hour treatment. The reducing sugar content of the tiger nut tubers, according to Adejuyitan, (2011) increased during storage through some processes related to residual enzymes inherent in the tiger nut. This gives credence to the observed reducing sugar concentration as observed with time since the slurry was enzymatically treated as a cellulose source. The increasing reducing sugar content stabilized after 6 hours and therefore, cellulose treatment of TNF was done for 6 hours at room temperature.

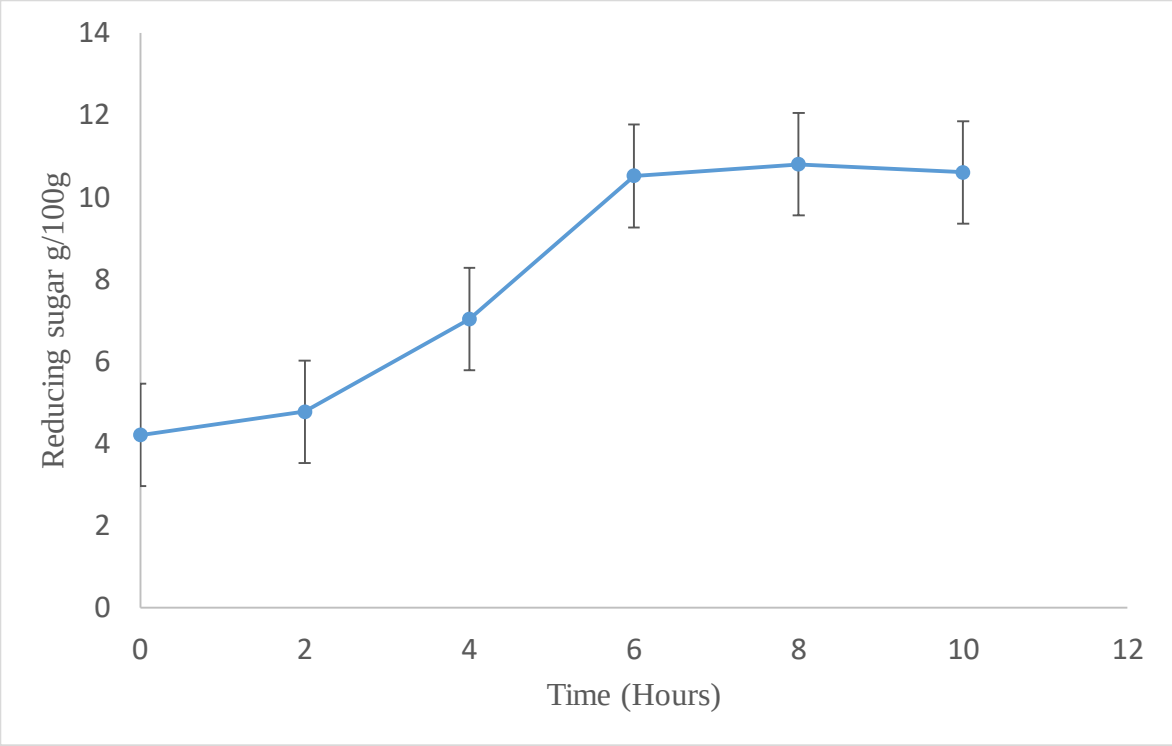

Figure 4.7: Reducing sugar content during cellulase hydrolysis of tiger nut slurry

4.3 Mineral Composition of synbiotic *Burkina* Samples

The mineral composition determined included sodium, potassium, magnesium, calcium, zinc, phosphorus and iron. Table 4.2 shows the results obtained from the analyses. It is observed that the mineral content of the fermented synbiotic *brukina* samples was high. They showed significant differences across all the minerals examined ($P \leq 0.05$) except for potassium and iron. Despite the observed high mineral content, none exceeded the daily recommended mineral intake so taking a bottle or two in a day can be recommended. In as much as no nutritional claims can be made on the product in terms of mineral composition since this project did not focus on the bioavailability of the minerals present, the product can be said to have a functional property of supporting the growth of the probiotic bacteria, *L. casei* as a prebiotic source to promote healthy gut microbiota.

The observed high mineral content is not shocking as both animal's dairy products, and tiger nut milk is notable for their high mineral content (Sanful, 2009).

The differences in the minerals examined for the various synbiotic *brukina* can be attributed factors such as the processing method employed in the product development and incorporation of whole tiger nut in the product. Tiger nut is known to be rich in Zinc. It is therefore not surprising that the tiger nut incorporated samples (B, D, E) had higher values than sample A made without the tiger nut. (Table 4.2). Minerals in food are known for their dietary and physiological roles; their importance cannot be overlooked during food processing (Yonemori et al., 2009).

Table 4.2: Mineral composition of synbiotic composite tiger nut-based cereal *brukina* with 10% and 15% of cellulase and non-cellulase treated

Sample ID(FT)(M: TNF)%	Na (mg/100g)	K (Mg/100g)	Mg (mg/100g)	Ca (mg/100g)	Zn (Mg/100g)	P (mg/100g)	Fe (mg/100g)
A(12) (100:0)%	141.82±6.96^{b}	131.33±3.12^{a}	5.26±0.90^{ab}	10.38±0.82^{a}	1.18±0.10^{b}	20.60±0.08^{c}	12.71±0.55^{a}
B(12) (85:15)%	187.34±8.18^{a}	147.81±9.99^{a}	8.26±0.98^{a}	10.80±0.40^{a}	2.21±0.42^{ab}	36.80±0.80^{a}	10.81±2.33^{a}
D(12E)(85:15)%	110.42±2.65^{c}	137.73±1.07^{a}	6.06±1.22^{ab}	7.24±0.00^{b}	1.66±0.09^{ab}	16.56±0.32^{c}	12.57±0.06^{a}
E(12E) (90:10)%	163.36±0.85^{b}	149.63±0.73^{a}	3.64±0.24^{b}	10.80±0.40^{a}	2.28±0.29^{a}	30.12±2.92^{a}	10.87±1.49^{a}

For every parameter, values in the same column with different superscripts are statistically different at α=0.05

FT- fermentation time for agglomerate in hours

M-Millet

TNF-tiger nut fibrous co-product

A-E- sample ID

4.4 Proximate analysis of the synbiotic *brukina*

The proximate composition of the fermented synbiotic *brukina* samples determined included moisture, ash, protein, fat, carbohydrate and the caloric content. The values were reported as a percentage dry-matter basis except for moisture content values. Table 4.3 shows the proximate composition of the fermented synbiotic *brukina* samples.

4.4.1 Moisture of synbiotic *brukina*

The moisture content of fermented synbiotic *brukina* ranged from 70.45% -78.85%.The values obtained were comparatively lower than 81.71–86.45% reported in the studies of Awonorin et al., (2014) and 92.44% reported by Badau et al., (2015), respectively but higher than 62.8–73.5% reported by Musa & A, (2013) for tiger-nut milk. It was a little lower to tiger nut-based *brukina* recorded by Nyarko-Mensah, (2018), which ranged from 75.42 % - 86.29 %. Synbiotic *brukina* from tiger nut incorporated fibre without cellulase hydrolysis for both 15% and 10% recorded lower moisture content ($p = 0.000$) compared to the hydrolyzed fibre 10% and 15% incorporated drinks as observed in (Table 4.3). The hydrolysis of the tiger nut starch and the gelling of the agglomerate might have increased the available, total solid content in the product leading to low moisture compared to the available literature. The observed moisture for a beverage is good since moisture around 70 %t will provide enough free water to support the probiotic *Lactobacillus casei*.

4.4.3 Carbohydrate and protein content of synbiotic *brukina*

Synbiotic samples with tiger nuts had higher carbohydrate contents (P=0.000) compared with control samples without tiger nuts. The carbohydrate content of the samples ranged from 9.15% to 18.85%, which did not fall within range reported by (Frimpong, 2016)but was much higher. The observed starch content can be explained from the processing point of view, the temperature range for which starch granules crystalizes is dependent on the available water content and the type of starch. Blending two starches sources in the presence of enough moisture and temperature causes gelatinization to increases the availability of starch (Eliasson, 2017b). The incorporation of tiger nuts into synbiotic *brukina* resulted in a detectable level of protein content in the product. For both cellulase and non-cellulase hydrolyzed composite milk, protein levels were higher in 10% than the 15% TNF incorporation (P= 0.000) when compared. The synbiotic *brukina* made without tiger nuts had higher protein levels than for 15% TNF incorporated (Table 4.3). This reduction in crude protein contents of the samples incorporated with TNF could be due to the binding and screening effects of the anti-nutritional factors such as tannins present in the millet (Babatunde, 2016)The increase in the protein and carbohydrate content observed in the synbiotic product affirms the fact that the usage of tiger nut is an excellent composite material to be used in combination with cereal for beverage development (Eliasson, 2017a). Carbohydrates contribute to the taste and flavour of the products (Eliasson, 2017a) and it also serves as source of carbon for probiotic microorganism *Lactobacillus casei* in the product.

4.4.5 Fat content

The synbiotic samples with tiger nuts incorporation had higher fat levels (P =0.000) compared with a synbiotic *brukina* without tiger nuts (the control). It was observed from (Table 4.3) that the incorporation of 40 % tiger nut milk and the 10-15% fibre into the synbiotic samples increased the fat content of the product. This characteristic can be attributed to the fact that tiger nut is rich in fatty acids (Liu et al., 2019). The fat levels were within the range of 3.2 % – 4.8 % as observed in (Table 4.3). Tiger nut fats most of which are oleic acid, and monounsaturated fats (Eteshola & Oraedu, 1996), are known help with weight loss, reduce the risk of heart disease and decrease inflammation(Adeniyi et al., 2013). Hence the product will be ideal health beneficial drink coupled with its probiotic potential of it improving gut microbiota when consumed.

4.4.6 Total ash

The total ash content determined for the five samples did not show any significant difference as observed in Table 4.3. The incorporation of tiger nuts into synbiotic *brukina* did not affect the ash (Table 4.3). Earlier researchers had reported comparable values (0.20–0.50%) for the ash content of tiger-nut milk (Belewu & Belewu, 2007; Bristone et al., 2015). All synbiotic samples with tiger nuts did not show any significant difference in ash levels (P= 0.144). both tiger nut and millet are high in minerals such as magnesium, potassium and phosphorous (Abraham, 2013) which would contribute to the high similarities observed in ash levels of all the samples.

4.4.7 Crude fibre

The crude fibre of the samples except for sample B and E did not show any significant difference (P=0.144) from one another. However, the value range (0.21–0.62%) was higher than 0.20% reported by Belewu and Abodunrin (2008) in tiger nut-based products. Fibre in food means carbohydrate not digested nor absorbed in the human small intestine (Codex, 2010). fibres have been put into the following categories: edible carbohydrate polymers naturally occurring in the food as consumed, edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic, or chemical means (codex, 2010). The high crude fibre observed can be attributed to the processing and the tiger nut and millet component. Fibre increases faecal output (digestion), reduces the faecal pH, incidence of colon cancer, diabetes, heart diseases, obesity and certain degenerative diseases (Cummings et al., 1996; Ingabire and Vasanthakaalam, 2011).

Table 4.3: The proximate of synbiotic tiger nut-based cereal *brukina* with varying composition of cellulase and no- cellulase treated TNF (10%,15%)

Sample ID (FT)(M: TNF)%	Moisture g/100g	Carbohydrate g/100g	Fat g/100g	Crude Fibre g/100g	ASH g/100g	Protein g/100g	Energy KCAL
A(12) (100:0)%	78.850±0.636^{a}	12.585±0.304^{c}	3.205±0.063^{d}	0.280±0.014^{b}	0.635±0.021^{ab}	4.510±0.014^{c}	97.220±1.730^{d}
B(12) (85:15)%	70.685±0.276^{c}	19.240±0.170^{a}	4.895±0.106^{a}	0.565±0.035^{a}	0.665±0.021^{a}	3.940±0.042^{d}	136.780±1.460^{a}
C(12 (90:10)%	70.455±0.361^{c}	18.800±0.311^{a}	4.205±0.063^{c}	0.255±0.007^{b}	0.625±0.049^{ab}	5.660±0.056^{b}	135.690±1.590^{a}
D(12E)(85:15)%	75.240±0.070^{b}	15.365±0.191^{b}	4.660±0.084^{b}	0.210±0.014^{b}	0.585±0.007^{b}	3.940±0.042^{d}	119.160±0.170^{b}
E(12E) (90:15)%	78.825±0.276^{a}	9.155±0.247^{d}	4.260±0.042^{c}	0.615±0.092^{a}	0.670±0.028^{a}	6.475±0.049^{a}	100.860±0.410^{c}

For every parameter, values in the same column with different superscripts are statistically different at α=0.05

FT- fermentation time for agglomerate in hours

M-Millet

TNF-tiger nut fibrous co-product

A-E- sample ID

The vitamin E content of samples A, B, D, and E, ranged from 9.9mg/100g to 11.28 g/100g, while vitamin A was non detected in all the samples. The samples all have an appreciable amount of vitamin E. but the amount in all the products was below the WHO daily vitamin E recommended level for all age groups, which is 300mg to 1000 mg per daily requirement. The product can be consumed without restraint for all age categories, but no nutritional claim can be made on it concerning vitamin E since the bioavailability of the vitamins is not known for this product.

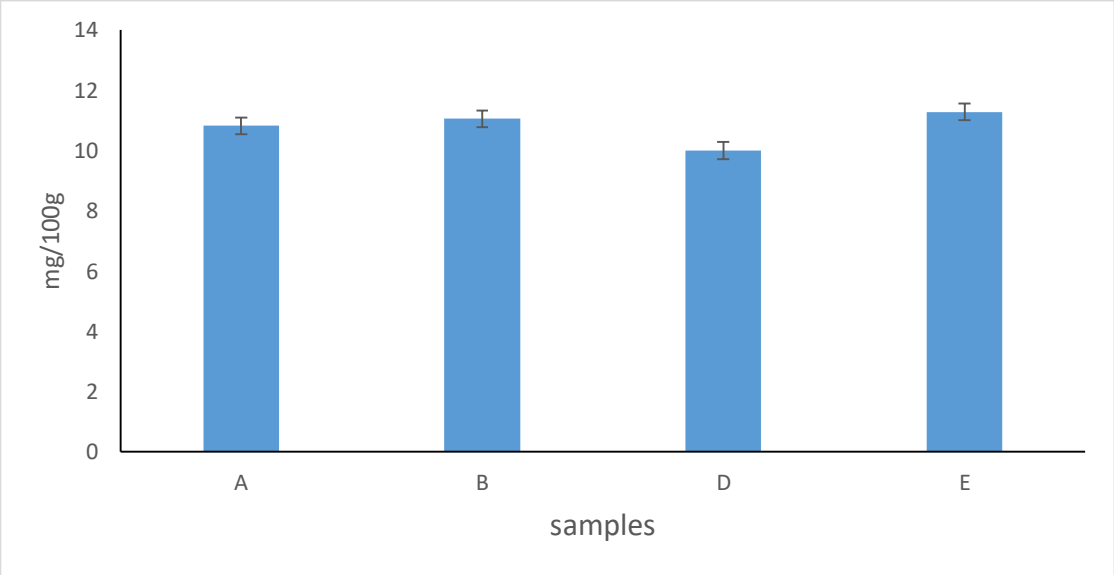

Figure 4.7 Vitamin E content of fermented tiger nut-millet based synbiotic *brukina*

A- *Brukina* without tiger nut and tiger nut fibre

B- B- Synbiotic *brukina* with 15% TNF

C- Synbiotic *brukina* with 10% cellulase hydrolyzed TNF

D- Synbiotic *brukina* with 15% cellulase hydrolyzed TNF

4.5 The viability of *Lactobacillus casei*

The changes in the *L. casie* population in the synbiotic products during five days of refrigerated storage and room temperature are given in Table 4.4. *L. casei* attained 10 log count in the fermented products made with fermented dairy milk, tiger nut, millet (A, B, D, and E) after four days of storage for all the storage conditions of 5℃ and 25℃.

After 4 days of storage, products A, B, D, and E for refrigeration temperature recorded final pH of 3.54, 3.56,3.39, and 3.53, respectively which were the most considerable reduction in pH of all the products, with corresponding concentration of 11.48, 11.52, 11.35 and 11.15 log CFU/mL, respectively (Table 4.4). The same trend was observed in the sample kept at room temperature, and where the growth of *L. casei* in products A, B, D, and E was resulted in high production of lactic acid leading to low final pH as can be observed in figures 4.8 t0 4.11. An excellent probiotic culture should contain not less than 6 to 8 log CFU/g viable cells. According to to the Food and Agriculture Organization and the World Health Organization, there is the need for an appropriate count of probiotic bacteria needed to exert the preferred benefits.

In this study, products A, B, D, and E for both storage conditions had levels around 11 log CFU/ml. All the products made in our study were initially inoculated with *L. casei* at 7 log CFU/g. This probiotic microorganism grew rapidly during the first two days (P =0.000) and reached a maximum of 8 and 10 log CFU/g in products stored at refrigeration and room temperature respectively (Table 4.4). The peak growth of *L. casei* was observed after day four, which recorded 11 log CFU/mL for all the samples in the two storage conditions. In this study, the final products had high levels of *L. casei*, and this makes it an excellent functional product.

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This observed trend of log increase in *L. casei* at refrigeration temperature is consistent with the result obtained by Parra et al., (2013)in cereal blend where the viability of *L. casei* during 28 days of refrigeration storage survived and attained the highest populations after seven days of storage in a cereal-based diet. Also, higher survival of *L. casei* compared with *L. acidophilus* has been found in varying functional fermented products and non-fermented products that do not contain milk (Yoon et al., 2006). *L. casei shirota* was also found reducing in the count of around 1 log cycle each day for 21 days in a milk dessert. the product after the 21 days recorded a viable cell count of 10^7 CFU/g in the milk-based dessert (Magariños et al., 2008)

The growth of *L. casei* depended upon its ability to utilize the nutrients available in the growth medium for its survival. For lactobacilli to survive in any media aside from the fermentable carbohydrate, there is the need for amino acids, vitamins, and related growth factors. The 60% dairy milk provided lactose, which is known to activate the production of the inducible enzyme beta-galactosidase, which is known to improve the growth of *L. sp* (Magariños et al., 2008).

Corcoran et al. (2005) observed that the survival of lactobacilli in acidic environments is enhanced in the presence of metabolizable sugars that allow cell membrane proton pumps to operate and prevent the lowering of intracellular pH. The heterofermentative nature of *L casei* in this product, kept in an airtight bottle with enough metabolizable sugar from the tiger nut and millet is the right condition for fermentation, hence the observed reduction in pH of the product and survival *L casie*.

Table 4.4: Mean growth of probiotic *lactobacillus casei* in fermented tiger nut-based *brukina* stored at two varying temperatures 5℃ and 25℃ for 4 days

Sample ID(FT)(M: TNF)%	**Temp**	**Mean ± SD (Log CFU/ml) *Lactobacillus casei***				
		DAY 0	**DAY 1**	**DAY 2**	**DAY 3**	**DAY 4**
A(0) (100:0)%	5℃	7.16 ± 0.06^{a}	7.52±0.09^{b}	8.71 ±0.39^{de}	10.74±0.01^{a}	11.48±0.22 ^{a}
B(12) (85:15)%		6.89±0.01^{a}	7.47±0.02^{b}	8.84 ±0.02^{cde}	10.6±0.09^{a}	11.52±0.00 ^{a}
D(12E)(85:15)%		7.07±0.14^{a}	7.18± 0.08^{c}	8.48 ± 0.68^{e}	10.09±0.01^{c}	11.35±0.01^{a}
E(12E) (90:10)%		6.52±0.44 ^{a}	7.38±0.05^{bc}	9.10±0.30^{bcde}	10.60±0.02^{ab}	11.15±0.05^{a}
A(0) (100:0)%	25℃	7.16±0.06^{a}	8.80±0.05^{a}	10.23±0.01^{abcd}	10.78±0.06^{a}	11.41±0.45 ^{a}
B(12) (85:15)%		6.89±0.01^{a}	8.73±0.10^{a}	10.84±0.85^{a}	10.33±0.09^{bc}	11.14±0.03^{a}
D(12E)(85:15)%		7.07 ± 0.14^{a}	8.70±0.00^{a}	10.45± 0.19^{abc}	10.27±0.14^{c}	11.56±0.26^{a}
E(12E) (90:10)%		6.52 ± 0.4^{a}	8.65±0.03^{a}	10.74 ± 0.03^{ab}	10.68±0.10^{a}	11.17±0.02^{a}

For every parameter, values in the same column with different superscripts are statistically different at α=0.05

FT- Fermentation time for agglomerate in hours

M-millet TNF-tiger nut fibrous co-product A-E- sample

4.5.1 pH and titrable acidity(TTA) of the synbiotic beverage

Figure 4.8 -4.11 shows changes in pH and lactic acid production in the synbiotic product A, B, D, and E during the five days of storage. Both samples were kept at 5℃ and 25℃ showed a similar trend of pH reduction with a corresponding increase in lactic acid production. The initial pH values in the products depended on the initial fermentation of both the agglomerate and the composite milk used in the preparation, and it ranged from 4.41 to 4.75. *L. casei* is a known microorganism, which is more resistant to acidic conditions in a grain diet than other lactobacilli. (Charalampopoulos et al., 2002) The *L. casei* presence leads to a considerable decrease in pH within the first three days of storage with a corresponding increase in the production of lactic acid as can be observed in (Figures. 4.8-4.11) for all samples kept at room and refrigerated temperature. The reduction in pH caused by *L. casei* during refrigerated storage was very similar to that of room temperature storage, However, comparing the *L. casei* in the product kept at 5℃ and 25℃, there was significant initial pH lowering effect for the 25℃ sample as observed in figures 4.8- 4.11. Despite this observations, there was same log count for *L. casie* in both products after 4 days of storage. This observation is due to a stress response mechanism initiated by the probiotic under refrigeration condition of 5℃, under this condition, metabolic activity slows down, the organism channel its energy into generation of cold-shock protein (CSPs). CSPs are able to bind RNA and it is believed that these proteins act as RNA chaperones, thereby reducing the increased secondary folding of RNA at low temperatures,(Wouters et al., 2000). This leads to initially observed reduction in pH till the organism created the necessary conditions for cold survival. These results confirm the intrinsic resistance of *L. casei* to low pH values. *L. casei* is well adapted to adverse environmental pH conditions, and it supported and grew at even lower pH values of 3.5 to 3.2. These drastic changes in pH and lactic acid production of probiotic drinks are due to hydrolysis of

starch into sugars during fermentation which is readily utilized by the organisms and converted to lactic acid (Sharma et al., 2017)

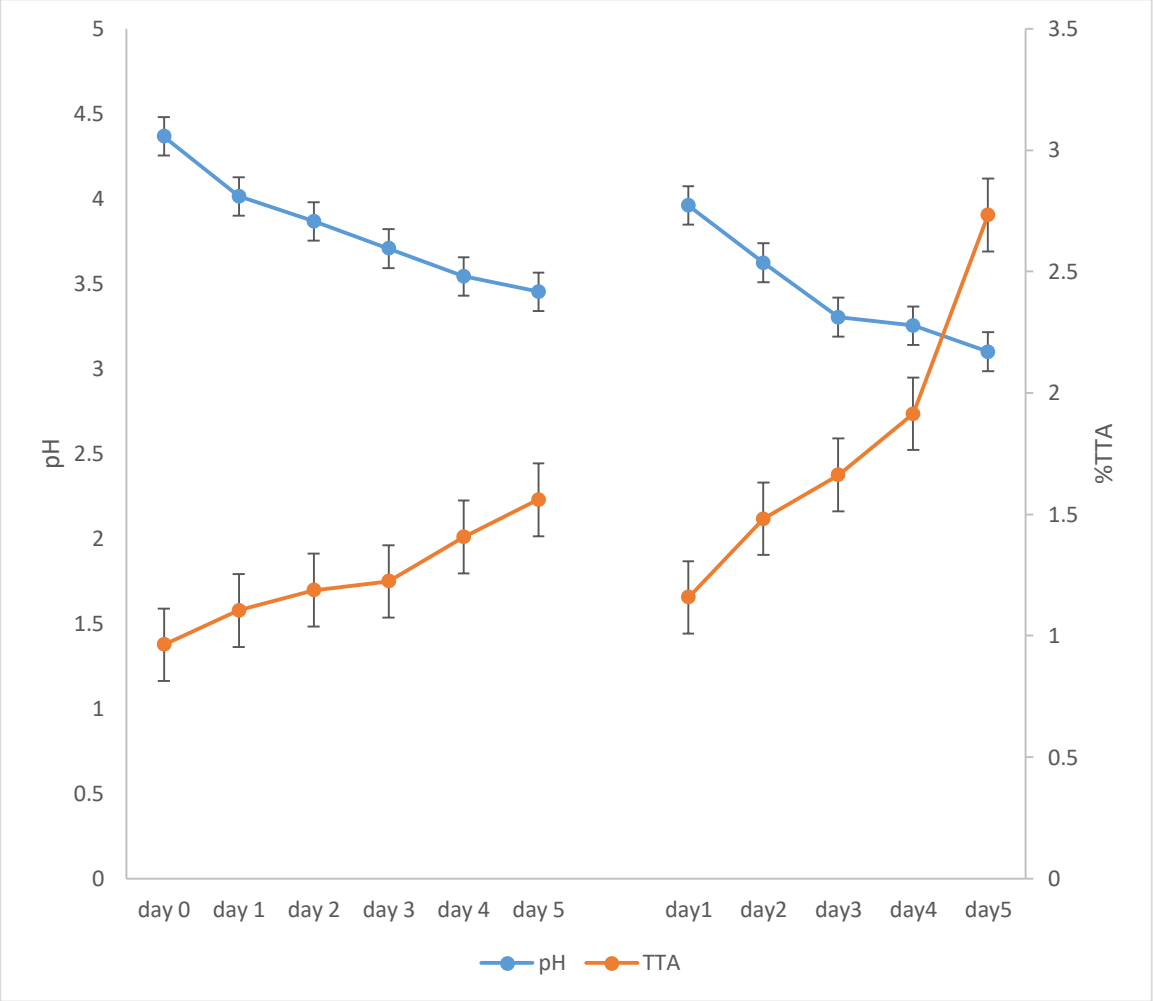

Figure 4.8: pH and percentage lactic acid content of synbiotic *brukina*, without tiger nut stored at **5°C,** and **25°C** for 5 days

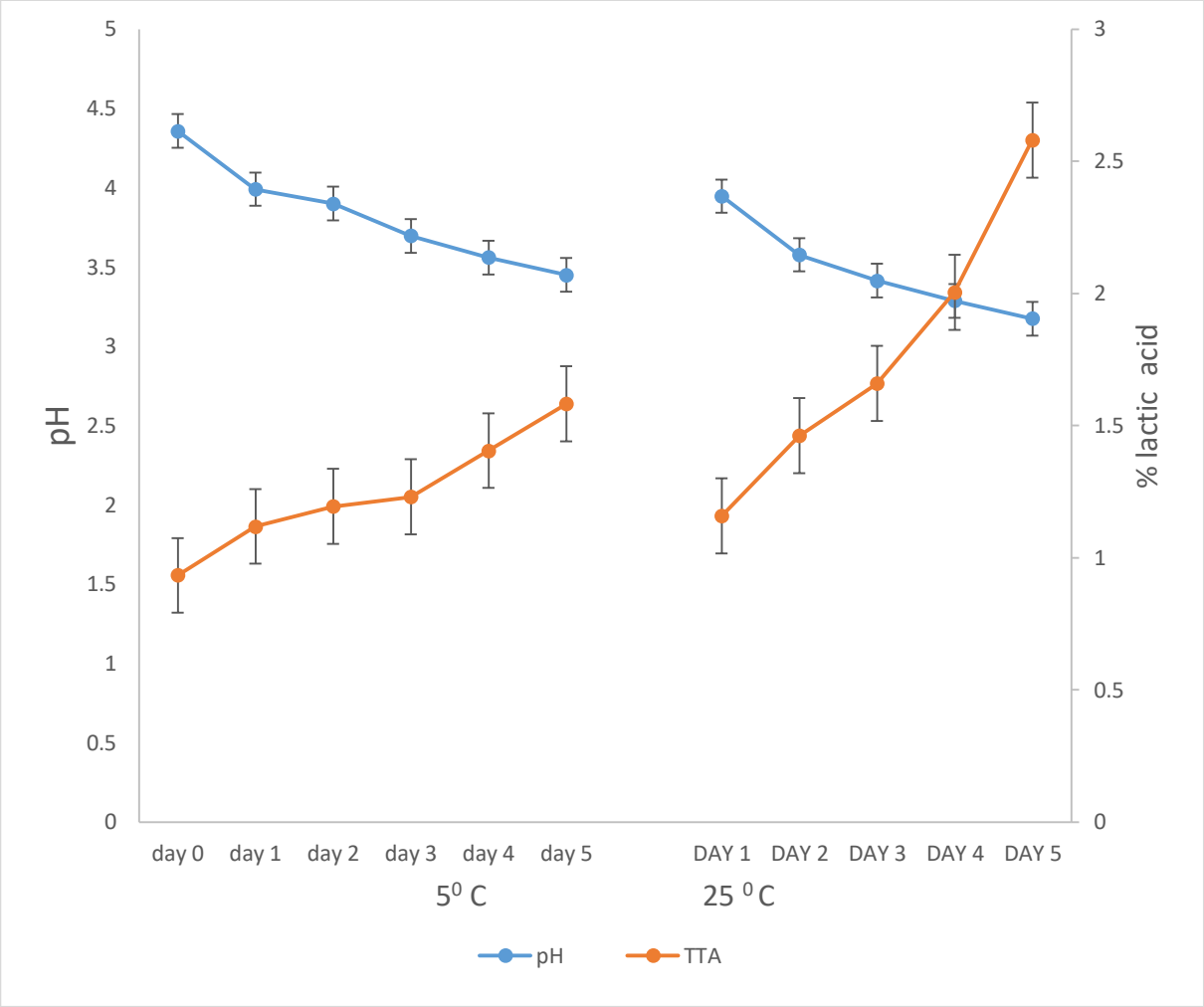

Figure 4.9: pH and lactic percentage lactic acid content of the synbiotic *brukina* with 15% TNF-millet agglomerate, stored at **5°C,** and **25°C** for 5 days

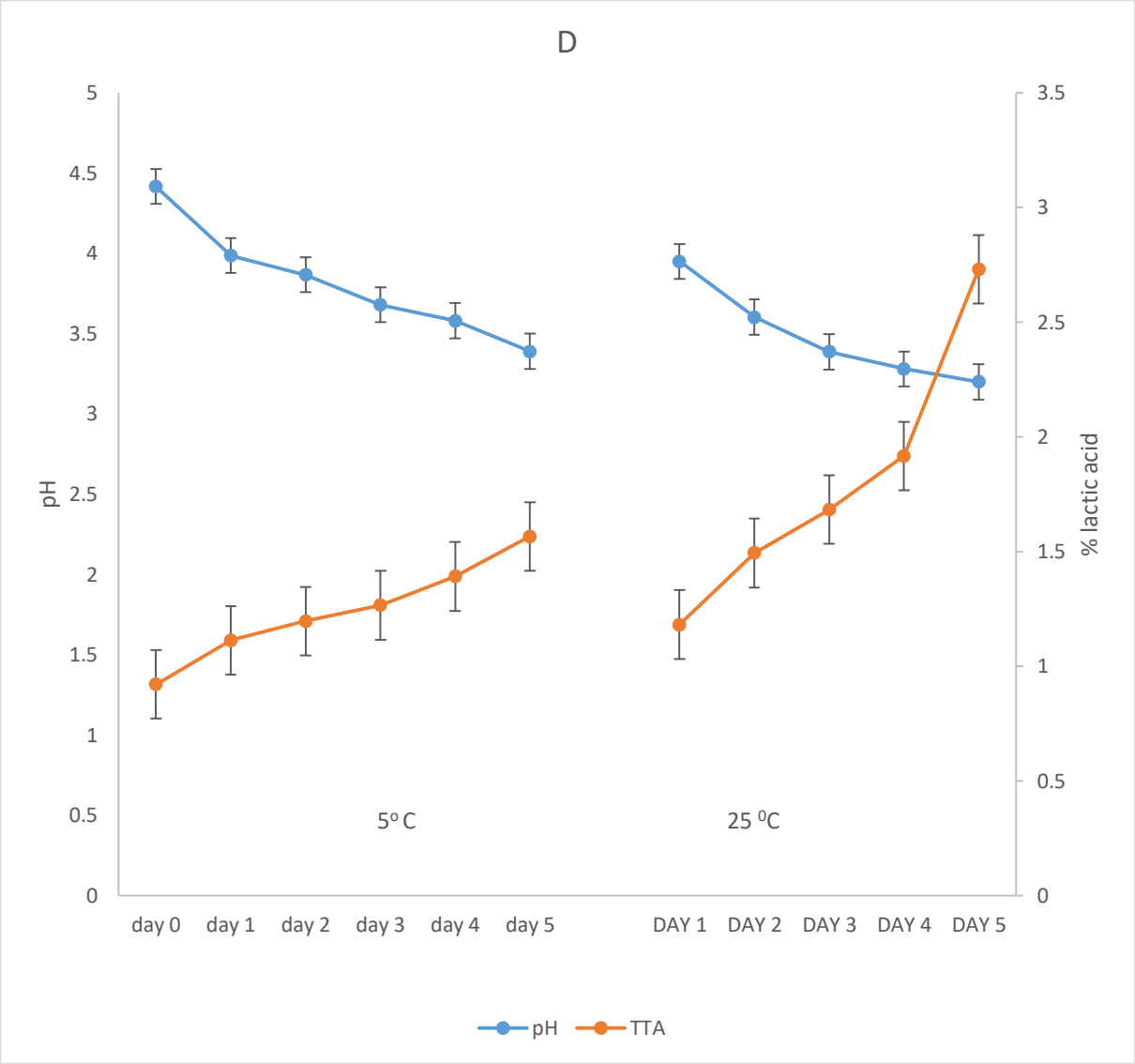

Figure 4.10: pH and lactic percentage lactic acid content of synbiotic *brukina* with cellulase hydrolysed 15% TNF- millet agglomerate, stored at **5°C**, and **25°C** for 5 days

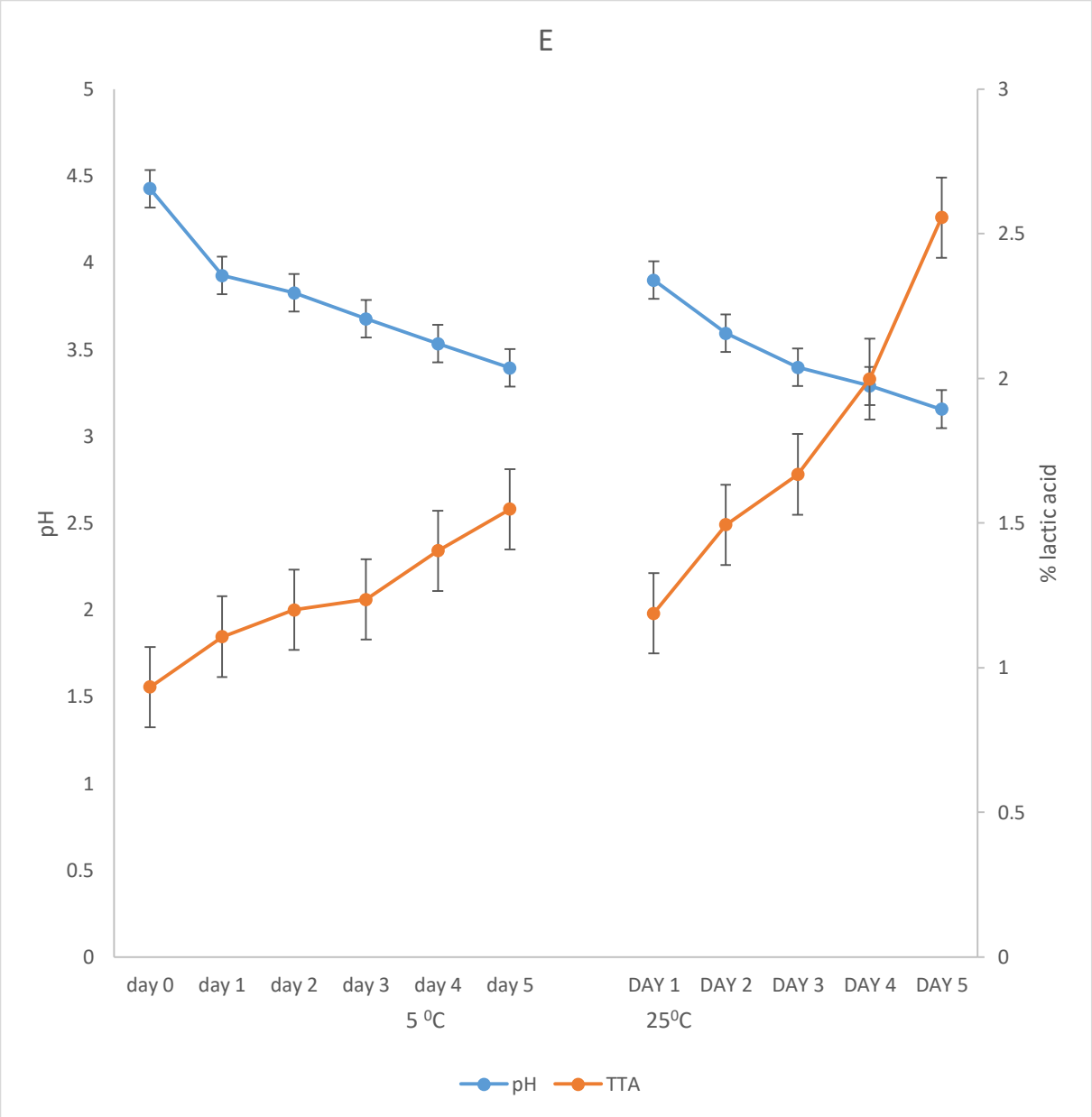

Figure 4. 11: pH and lactic percentage lactic acid content of synbiotic *brukina* cellulase hydrolysed 10% TNF-millet agglomerate stored at **5°C**, and **25°C** for 5 days

4.6 Shelf life studies

4.6.1 Shelf life and microbial quality

The shelf-life was determined based on the products' chemical characteristics (pH) and microbiological characteristics. They are mainly the factors that are of concerned and when affected by environmental temperature cause products to deteriorate. The temperature range for this work was 5℃, 25℃ and 35℃ as this is the temperature range that the product would be exposed to during distribution and storage. For a probiotic-based ready to eat dairy-based product, the shelf-life is defined by either pH changes (because of the live probiotic activity) or by the probiotic count in the product with varying environmental temperature conditions. (Tano-Debrah et al., 2019)

The shelf-life of fermented synbiotic tiger nut cereal-based drink was estimated by transforming the pH using the regression of log shelf-life versus temperature to obtain the following equations from the figure that can be obtained in Appendix 1

Log (self-life A) = -1346.2 (T) + 3.10088 R^2 = 0.9916……. 1

Log (shelf-life B) = -1394.4(T) + 3.2423 R^2= 0.9850…..... 2

Log (shelf-life D) = -1014.4(T) + 1.9703 R^2 = 0.9995……3

Log (shelf-life E) = -1218(T) + 2.693 R^2 =0.997 4

where T in the equations represent the storage temperature (in kelvin) of the

synbiotic *brukina* The equations 1-4 as contained in Figures A1.5-A1,58 obtained had a high regression coefficient (R^2) which fit the model and was used to estimate the shelf-life of the product at different storage conditions within the temperature in Table 4.5

The microbiological quality of the product after the estimated shelf life of 5 days under refrigeration temperature was still microbiological safe for both the cellulase treated and none cellulase treated sample kept at different temperatures.

Table 4.5: Shelf life of synbiotic *brukina* stored for 5 days at of 5℃, 25℃ and 3℃5

sample id	T	K	R(2)	1/T	LN(K)	R	Ea/R	Ea	shelf life
A	278	0.1753	0.9571	0.003597	-1.74126	-1.986	-1346.2	2673.553	5.704507
	298	0.2507	0.9349	0.003356	-1.3835	-1.986			3.988831
	308	0.2783	0.9252	0.003247	-1.27906	-1.986			3.593245
B	278	0.1722	0.9558	0.003597	-1.7591	-1.986	-1393.4	2767.292	5.807201
	298	0.2303	0.924	0.003356	-1.46837	-1.986			4.342162
	308	0.2843	0.9553	0.003247	-1.25773	-1.986			3.517411
D	278	0.1869	0.9428	0.003597	-1.67718	-1.986	-1014.4	2014.598	5.350455
	298	0.2374	0.9067	0.003356	-1.43801	-1.986			4.2123
	308	0.2671	0.8996	0.003247	-1.32013	-1.986			3.743916
E	278	0.1856	0.912	0.003597	-1.68416	-1.986			5.387931
	298	0.2448	0.8999	0.003356	-1.40731	-1.986	-1218	2418.948	4.084967
	308	0.2858	0.9549	0.003247	-1.25246	-1.986			3.49895

4.6.2 Microbial quality

Synbiotic samples were analyzed to ascertain microbiological wholesomeness. This was to ensure that the samples solely contain the inoculated probiotic and also to ensure that the probiotic microorganism growth and condition do not favour the presence of harmful microorganisms in the ‘‘synbiotic sample" Table 4.6-4.10 shows the mean count of microorganisms monitored in the sample where no growth was observed for *E. coli S. aureu*s and yeast and moulds, except for total plate count, the obtained result is consistent with work done by Baidoo and Kunadu, (2019) where optimized *brukina* sample, for coliform and *S. aureus* did not show any count but for total Plate Count(TPC).

Microbiological counts were observed in the synbiotic samples (Table 4.7) for only TPC. However, these counts are a reflection of the presence of lactic acid bacteria, *lactobacillus casei* used as the probiotic microorganism in the product. PCA count served as a check on the probiotic to be sure it is the sole bacterial residing in the product since any other bacterial would have caused the total plate count log readings to have been higher than that of the MRS reading observed in Table 4.6 for *Lactobacillus casei.*

The observation in Table 4.6-4.10 for *E coli,* and yeast and mould and staphylococcus sp is as a result of the fact that samples had a live probiotic that created an unfavorable environment for their existence. Pasteurization of the composite milk for 15 minutes at 70℃ after fermentation eliminated all other viable competitive microorganisms, making the product the sole hub for the introduced health beneficial microorganism.

Table 4.6: Mean pH and bacteriological quality of fermented tiger nut-millet based *brukina* before and after probiotic inoculation

	MEAN ±SD (log cfu/ ml)				
Sample ID (FT)(M: TNF)%	Total plate count	*E. coli*	*Staphylococcus sp.*	Y&M	pH
B(12) (85:15)%	0.0 ±0.000[b]	0.0	0.0	0	4.41
D(12E)(85:15)%	0.0±0.000[b]	0.0	0.0	0	4.45
B1(12)(85:15)%	7.98218±0.01280[a]	0.0	0.0	0	4.35
D1(12E)(85:15)%	7.9563±0.0238[a]	0.0	0.0	0	4.36

B and D = before inoculation with probiotic; B1 and D1= After inoculation with probiotics.

Table 4.7: Mean total bacterial count of synbiotic *brukina* stored at 5℃ and 25℃ for 5 days

		Total Plate Count				
Sample ID(FT)(M:TNF)%	Temp	day 1	day 2	day 3	day 4	day 5
B(12)(85:15)%	5℃	8.04±0.06[a]	8.72±0.05[b]	9.20±0.17[b]	9.80±0.07[b]	11.50±0.03[a]
D(12E)(85:15)%		7.85±0.24[b]	8.19±0.03[b]	9.31±0.08[b]	9.79±0.04[b]	11.50±0.05[a]
B(12)(85:15)%	25℃	8.16±0.11[a]	10.23±0.17[a]	10.57±0.08[a]	11.49±0.01[b]	11.56±0.23[a]
D(12E)(85:15)%		8.21±0.02[a]	10.28 ±0.05[a]	10.76±0.00[a]	11.53±0.08[a]	11.59±0.03[a]

Define samples B and D and 12E

Table 4.8: Mean *E. coli* count of synbiotic *brukina* for 5 days

E. COLI					
Sample ID(FT)(M:TNF)%	day 1	day 2	day 3	day 4	day 5
B(12)(85:15)%	0.00	0.00	0.00	0.00	0.00
D(12E)(85:15)%	0.00	0.00	0.00	0.00	0.00
B(12)(85:15)%	0.00	0.00	0.00	0.00	0.00
D(12E)(85:15)%	0 .00	0 .00	0 .00	0 .00	0 .00

Table 4.9: Mean total *S. aureus* count synbiotic *brukina* stored for 5 days

Staphylococcus aureus					
	day 1	day 2	day 3	day 4	day 5
B(12)(85:15)%	0.00	0.00	0.00	0.00	0.00
D(12E)(85:15)%	0.00	0.00	0.00	0.00	0.00
B(12)(85:15)%	0.00	0.00	0.00	0.00	0.00
D(12E)(85:15)%	0.00	0.00	0.00	0.00	0.00

Table 4.10: Mean yeast and mould count in synbiotic *brukina* stored for 5 days

Yeast and Moulds					
	day 1	day 2	day 3	day 4	day 5
B(12)(85:15)%	0.00	0.00	0.00	0.00	0.00
D(12E)(85:15)%	0.00	0.00	0.00	0.00	0.00
B(12)(85:15)%	0.00	0.00	0.00	0.00	0.00
D(12E)(85:15)%	0.00	0.00	0.00	0.00	0.00

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.2 Conclusion

The whole tiger nut can be used in the development of wholesome synbiotic beverage. The developed agglomerate from TNF and millet had improved objective textural characteristics when fermented for 12 hours. Results from the proximate and mineral analyses of the synbiotic beverage containing whole tiger nut indicated an increase in the protein, carbohydrate, fat, and other mineral composition. The developed product supported the growth of *Lactobacillus casei* with 1 log increase in the probiotic count of both refrigerated and room temperature for five days. Incorporation of whole tiger nut and a known probiotic into dairy cereal-based beverage will increase the nutritional content of the product as a functional food providing consumers with the health benefits associated with the consumption of the whole tiger nuts based probiotic products.

5.3 Recommendations

1. This research focused on the full incorporation of tiger nut and probiotic into a cereal-based fermented dairy beverage in developing functional food. The physicochemical and nutritional profiling observed require further studies focused on the sensorial properties of this product and consumer acceptance.

2. This research observed that after five days, the product was still microbiologically safe. It is therefore recommended that further studies on this product should dive more into regulating the pH of the product to help extend the shelf life of the product.

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APPENDICES

Appendix:1 Shelf-life calculation

The influence of storage time on the pH of the synbiotic *brukina* may be described using a linear plot below Figure A1 to Figure A4

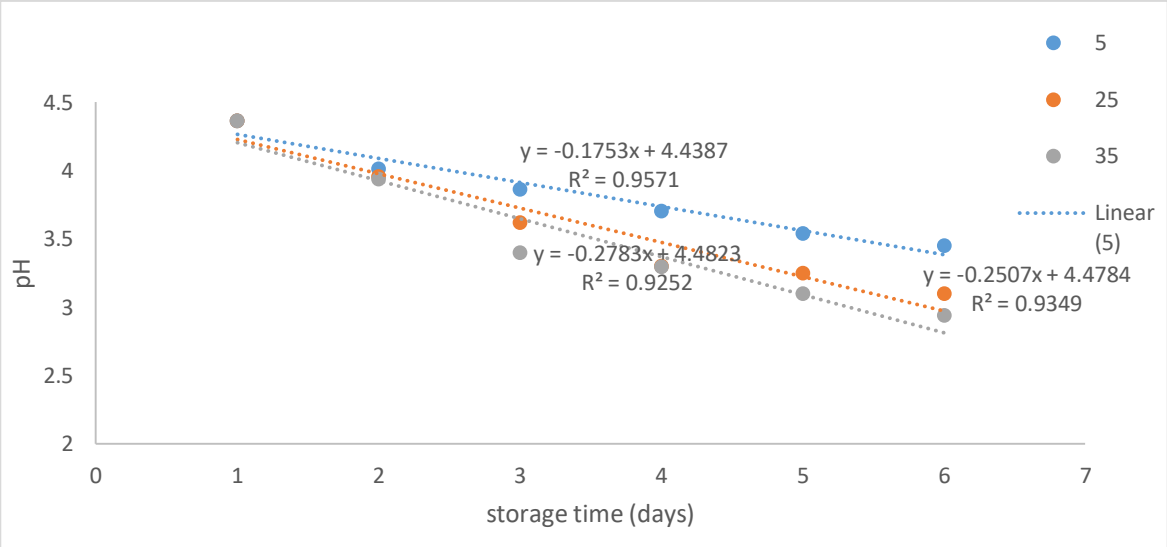

Figure A1.1: pH against storage time of sample synbiotic without tiger nut fibrous and tiger nut milk stored at three different temperatures 5℃, 25℃ and 35℃. For five days

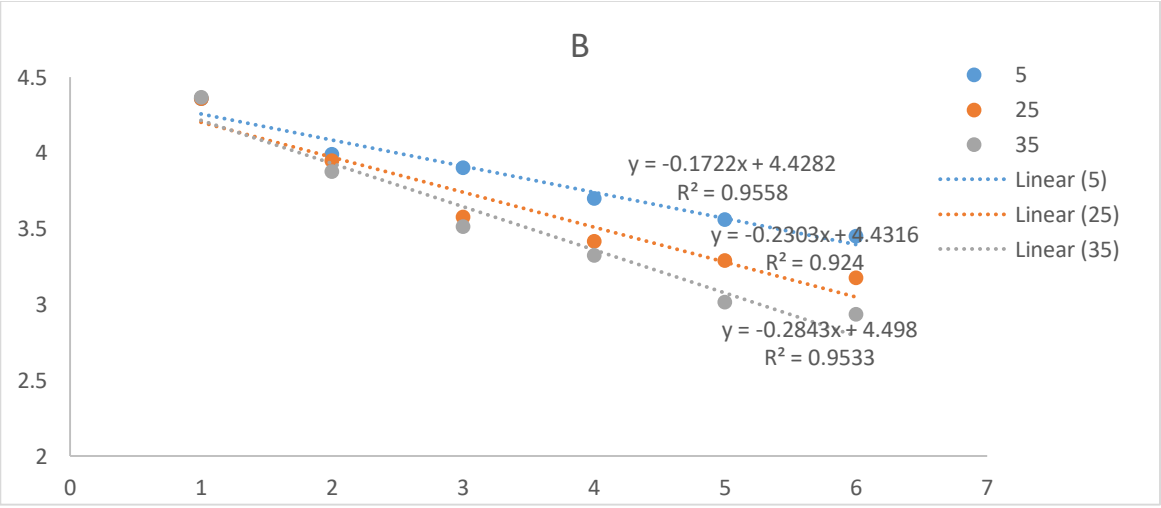

Figure A1.2: pH against storage time of symbiotic *brukina* with 15 percent tiger nut fibrous co-product in the agglomerate stored at three different temperatures of 5℃, 25℃and 35 ℃. For five days

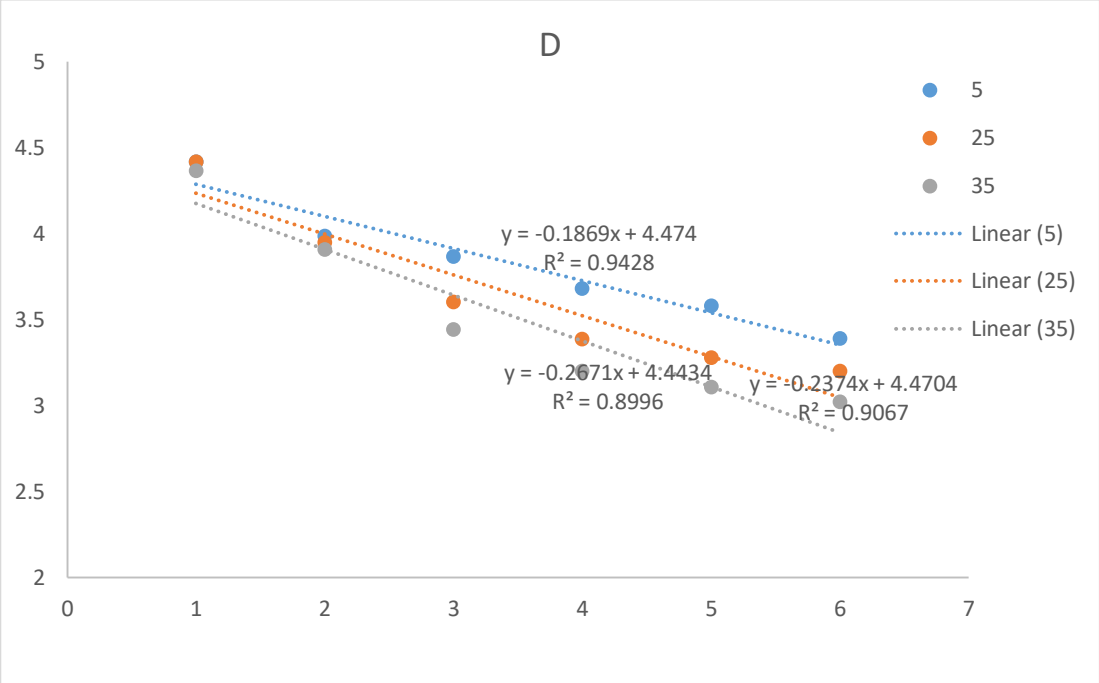

Figure A1.3: pH against storage time of synbiotic *brukina* with enzymatically hydrolyzed 15 percent tiger nut fibrous co-product in the agglomerate stored at three different temperatures 5℃, 25℃ and 35℃. for five days

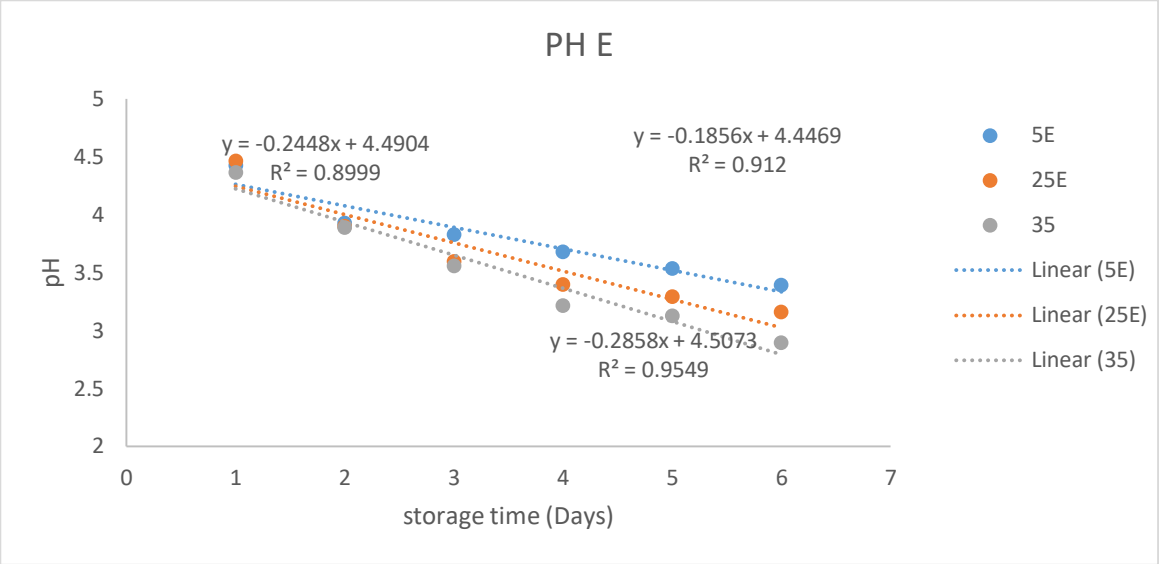

Figure A1.4: pH against storage time of synbiotic *brukina* with enzymatically hydrolyzed 10 percent tiger nut fibrous co-product in the agglomerate stored at three different temperatures 5°C, 25°C and 35°C. for five days

The influence of temperature on the reaction rate may be described by using the Arrhenius relationship as follows k = ko exp Ea/RT

Where;

Ko is the pre-exponential factor,

EA is the activation energy,

R is the ideal gas constant and

T is the temperature

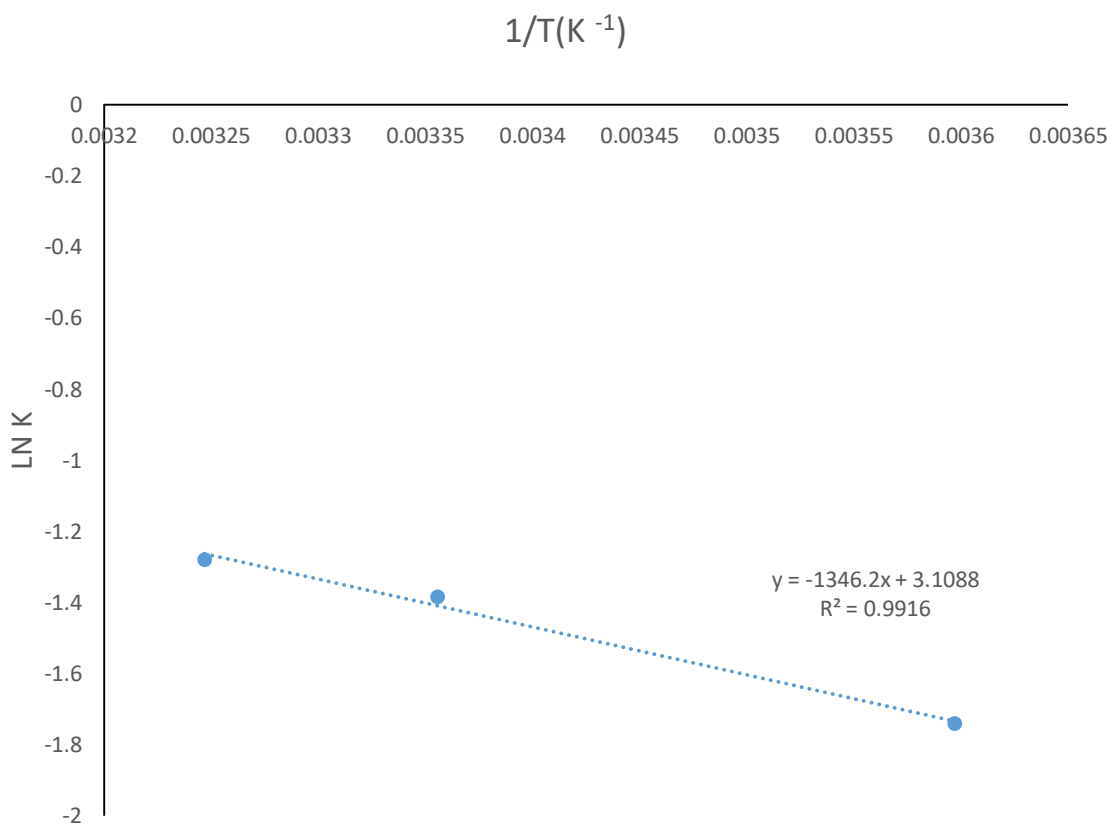

Figure A1.5: Arrhenius logarithmic plot for synbiotic *brukina* without tiger nut fibre inclusion in the agglomerate

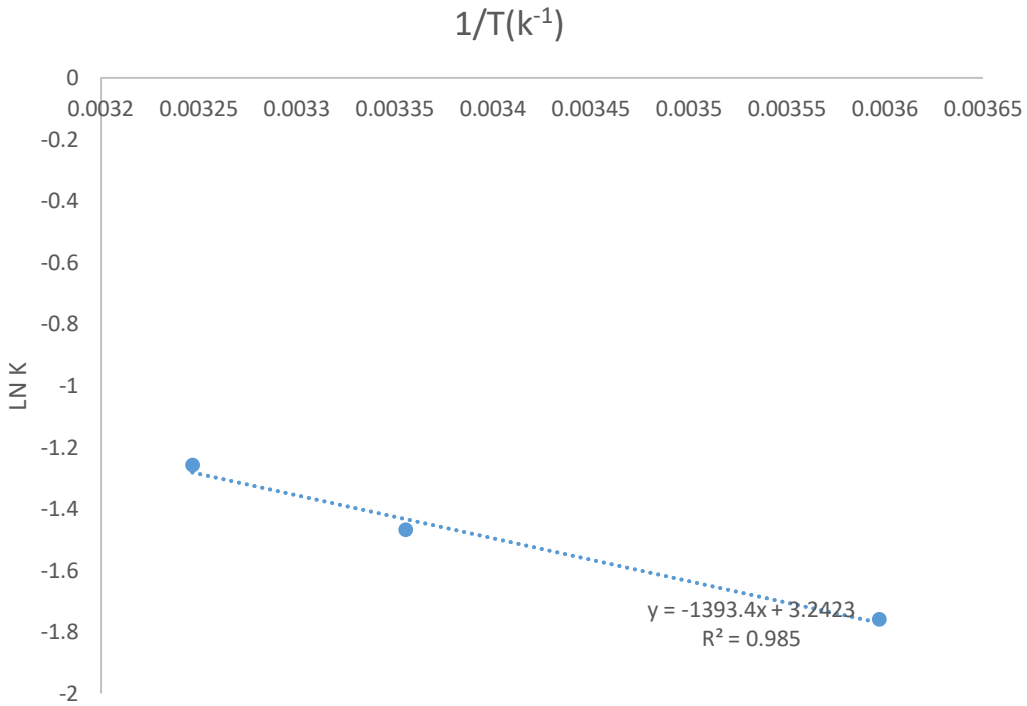

Figure A1.6: Arrhenius logarithmic plot for synbiotic *brukina* with 15 percent tiger nut fibre inclusion in the agglomerate

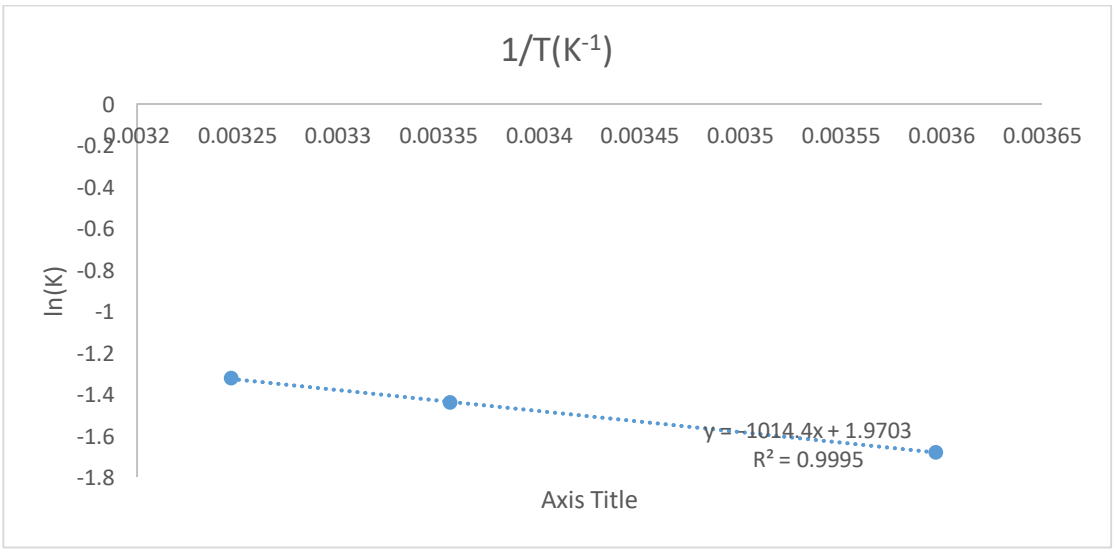

Figure A 1.7: Arrhenius logarithmic plot for synbiotic *brukina* with 15 percent cellulose hydrolysed tiger nut fibre inclusion in the agglomerate

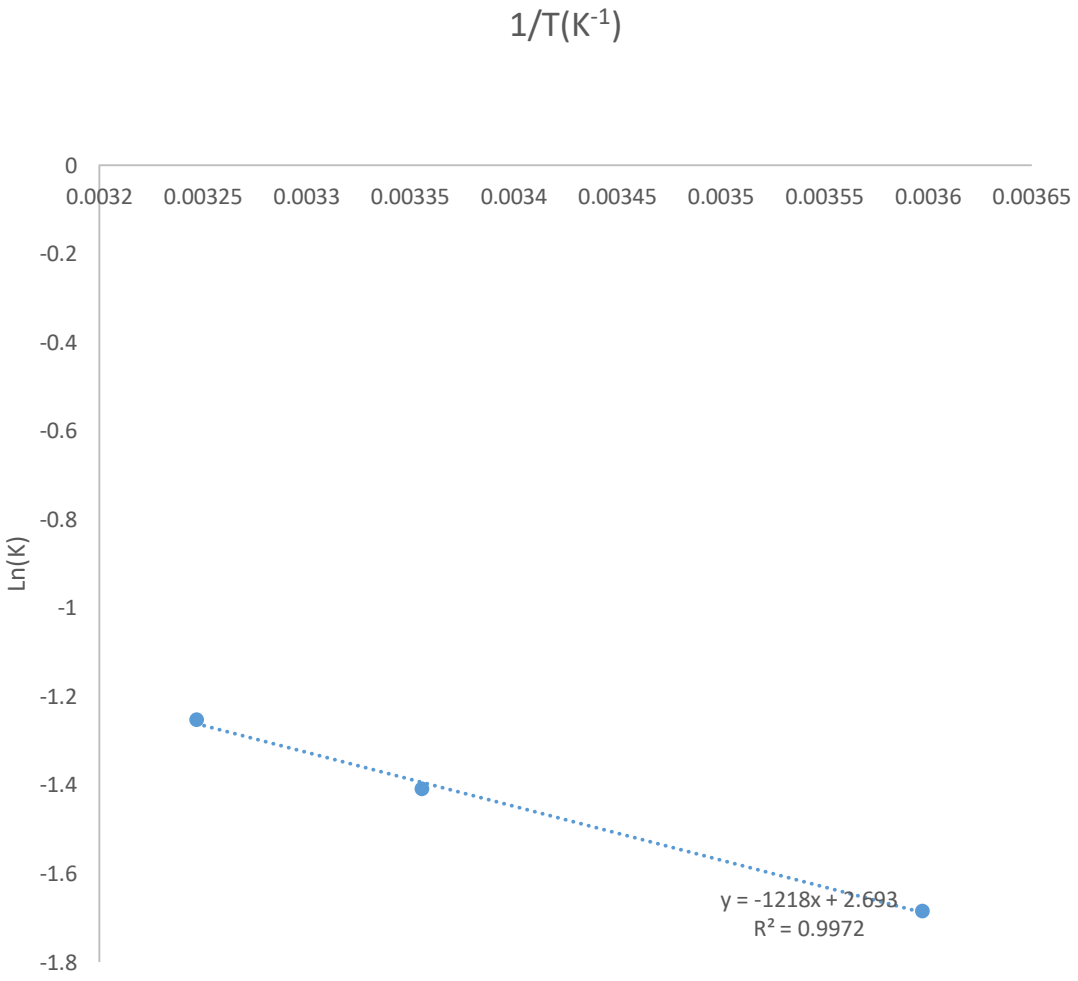

Figure A1.7: Arrhenius logarithmic plot for synbiotic *brukina* with 10 percent cellulose hydrolysed tiger nut fibre inclusion in the agglomerate

Appendix 2 ANOVA result for functional properties of the agglomerate

Hardness

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Test ID	8	3037677	379710	1.80	0.142
Error	18	3788708	210484		
Total	26	6826386			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
458.785	44.50%	19.83%	0.00%

Means

Test ID	N	Mean	stdev	95% CI
1	3	3071	521	(2515, 3628)
2	3	3184	675	(2628, 3741)
3	3	3129	328	(2573, 3686)
4	3	3550	245	(2993, 4106)
5	3	2722	208	(2165, 3278)
6	3	3821	851	(3265, 4378)
7	3	3625.8	91.8	(3069.3, 4182.3)
8	3	3161	430	(2605, 3718)
9	3	3660	198	(3103, 4216)

Pooled stdev = 458.785

One-way ANOVA: Gumminess

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Test ID	8	1900251	237531	3.97	0.007
Error	18	1077308	59850		

Total 26 2977559

Model Summary

S R-sq R-sq(adj) R-sq(pred)

244.643 63.82% 47.74% 18.59%

Means

Test

ID N Mean stdev 95% CI

1 3 893.2 116.0 (596.4, 1189.9)

2 3 1109 179 (812, 1406)

3 3 871.6 97.3 (574.8, 1168.3)

4 3 850 640 (553, 1147)

5 3 893.6 56.6 (596.9, 1190.4)

6 3 961 243 (664, 1258)

7 3 1572.5 41.3 (1275.8, 1869.3)

8 3 1215.3 100.6 (918.6, 1512.1)

9 3 1521.0 26.0 (1224.3, 1817.8)

Pooled stdev = 244.643

One-way ANOVA: Chewiness versus Test ID

Analysis of Variance

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

Test ID 8 1125799 140725 5.49 0.001

Error 18 461550 25642

Total 26 1587349

Model Summary

S R-sq R-sq(adj) R-sq(pred)

160.130 70.92% 58.00% 34.58%

Means

Test

ID N Mean stdev 95% CI

1 3 422.6 108.0 (228.4, 616.9)

2 3 266.8 118.5 (72.6, 461.1)

3 3 331.1 48.2 (136.9, 525.4)

4 3 385 286 (191, 579)

5 3 417.9 42.1 (223.6, 612.1)

6 3 616.2 164.7 (421.9, 810.4)

7 3 562 270 (368, 756)

8 3 761.7 126.5 (567.5, 956.0)

9 3 929.8 56.8 (735.6, 1124.1)

Pooled stdev = 160.130

One-way ANOVA: Resilience

Analysis of Variance

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

Test ID 8 0.050320 0.006290 35.66 0.000

Error 18 0.003175 0.000176

Total 26 0.053495

Model Summary

S R-sq R-sq(adj) R-sq(pred)

0.0132813 94.06% 91.43% 86.65%

Means

Test

ID N Mean stdev 95% CI

1 3 0.15243 0.00935 (0.13632, 0.16854)

2 3 0.10817 0.00486 (0.09206, 0.12428)

3 3 0.13274 0.00506 (0.11663, 0.14885)

4 3 0.19505 0.00354 (0.17894, 0.21116)

5 3 0.17988 0.01015 (0.16377, 0.19599)

6 3 0.1524 0.0292 (0.1363, 0.1685)

7 3 0.23795 0.01041 (0.22184, 0.25406)

8 3 0.2219 0.0176 (0.2058, 0.2380)

9 3 0.23027 0.00805 (0.21416, 0.24638)

Pooled stdev = 0.0132813

One-way ANOVA: Adhesiveness

Analysis of Variance

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

Test ID 8 3120.9 390.11 28.27 0.000

Error 18 248.4 13.80

Total 26 3369.3

Model Summary

S R-sq R-sq(adj) R-sq(pred)

3.71501 92.63% 89.35% 83.41%

Means

Test

ID N Mean stdev 95% CI

1 3 -35.96 3.83 (-40.46, -31.45)

2 3 -35.40 9.86 (-39.90, -30.89)

3 3 -17.862 1.291 (-22.369, -13.356)

4 3 -6.595 0.543 (-11.102, -2.089)

5 3 -13.436 1.411 (-17.943, -8.930)

6 3 -4.75 2.40 (-9.25, -0.24)

7 3 -9.868 1.117 (-14.374, -5.362)

8 3 -13.643 0.794 (-18.149, -9.137)

9 3 -14.031 0.809 (-18.537, -9.525)

Pooled stdev = 3.71501

Analysis of Variance

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

Test ID 8 0.09707 0.012133 6.11 0.001

Error 18 0.03577 0.001987

Total 26 0.13283

Model Summary

S R-sq R-sq(adj) R-sq(pred)

0.0445775 73.07% 61.10% 39.41%

Means

Test

ID N Mean stdev 95% CI

1 3 0.3042 0.0186 (0.2502, 0.3583)

2 3 0.2887 0.0684 (0.2346, 0.3428)

3 3 0.28723 0.00423 (0.23316, 0.34130)

4 3 0.38966 0.00811 (0.33559, 0.44374)

5 3 0.3296 0.0328 (0.2755, 0.3837)

6 3 0.4153 0.0998 (0.3613, 0.4694)

7 3 0.4358 0.0193 (0.3817, 0.4898)

8 3 0.4080 0.0246 (0.3539, 0.4620)

9 3 0.4396 0.0278 (0.3855, 0.4936)

Pooled stdev = 0.0445775

One-way ANOVA: swell index

Analysis of Variance

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

Sample id 4 0.08740 0.02185 1.07 0.422

Error 10 0.20471 0.02047

Total 14 0.29211

Model Summary

S R-sq R-sq(adj) R-sq(pred)

0.143076 29.92% 1.89% 0.00%

Means

Sample

Id N Mean stdev 95% CI

1 3 1.812 0.203 (1.627, 1.996)

2 3 1.7775 0.1594 (1.5935, 1.9616)

3 3 1.6246 0.0840 (1.4405, 1.8086)

4 3 1.7979 0.0550 (1.6139, 1.9820)

5 3 1.6621 0.1605 (1.4780, 1.8462)

Pooled stdev = 0.143076

One-way ANOVA: bulk density

Analysis of Variance

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

Sample id 4 0.022534 0.005634 6.20 0.009

Error 10 0.009080 0.000908

Total 14 0.031614

Model Summary

S R-sq R-sq(adj) R-sq(pred)

0.0301328 71.28% 59.79% 35.38%

Means

Sample

Id N Mean stdev 95% CI

1 3 0.54021 0.01638 (0.50145, 0.57897)

2 3 0.6304 0.0406 (0.5916, 0.6691)

3 3 0.5931 0.0358 (0.5544, 0.6319)

4 3 0.5480 0.0364 (0.5093, 0.5868)

5 3 0.63053 0.00379 (0.59176, 0.66929)

Pooled stdev = 0.0301328

One-way ANOVA: Water absorption

Analysis of Variance

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

Sample id 4 0.06924 0.01731 0.65 0.640

Error 10 0.26641 0.02664

Total 14 0.33565

Model Summary

S R-sq R-sq(adj) R-sq(pred)

0.163221 20.63% 0.00% 0.00%

Means

Sample

Id N Mean stdev 95% CI

1 3 0.8601 0.1156 (0.6501, 1.0701)

2 3 0.8946 0.1712 (0.6846, 1.1045)

3 3 0.6959 0.1722 (0.4859, 0.9058)

4 3 0.7983 0.1720 (0.5884, 1.0083)

5 3 0.794 0.177 (0.584, 1.004)

Pooled stdev = 0.163221

Appendix 3 ANOVA result for proximate and vitamin E

One-way ANOVA: Vitamin E(mg/100g)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	3	1.8854	0.6285	2.77	0.175
Error	4	0.9074	0.2269		
Total	7	2.7929			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.476291	67.51%	43.14%	0.00%

Means

Sample	N	Mean	stdev	95% CI
1	2	10.822	0.794	(9.887, 11.757)
2	2	11.057	0.410	(10.122, 11.992)
3	2	9.997	0.194	(9.062, 10.932)
4	2	11.281	0.266	(10.346, 12.216)

Pooled stdev = 0.476291

Appendix 3

One-way ANOVA: Vitamin E(mg/100g)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	3	1.8854	0.6285	2.77	0.175
Error	4	0.9074	0.2269		
Total	7	2.7929			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.476291	67.51%	43.14%	0.00%

Means

Sample N Mean stdev 95% CI

1 2 10.822 0.794 (9.887, 11.757)

2 2 11.057 0.410 (10.122, 11.992)

3 2 9.997 0.194 (9.062, 10.932)

4 2 11.281 0.266 (10.346, 12.216)

Pooled stdev = 0.476291

One-way ANOVA: % Moisture

Analysis of Variance

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

SAMPLE ID 4 137.217 34.3042 247.81 0.000

Error 5 0.692 0.1384

Total 9 137.909

Model Summary

S R-sq R-sq(adj) R-sq(pred)

0.372062 99.50% 99.10% 97.99%

Means

SAMPLE

ID N Mean stdev 95% CI

1 2 78.850 0.636 (78.174, 79.526)

2 2 70.685 0.276 (70.009, 71.361)

3 2 70.455 0.361 (69.779, 71.131)

4 2 75.2400 0.0707 (74.5637, 75.9163)

5 2 78.825 0.276 (78.149, 79.501)

Pooled stdev = 0.372062

One-way ANOVA: % Fat

Analysis of Variance

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

SAMPLE ID 4 3.35630 0.839075 147.99 0.000

Error 5 0.02835 0.005670

Total 9 3.38465

Model Summary

S R-sq R-sq(adj) R-sq(pred)

0.0752994 99.16% 98.49% 96.65%

Means

SAMPLE

ID N Mean stdev 95% CI

1 2 3.2050 0.0636 (3.0681, 3.3419)

2 2 4.8950 0.1061 (4.7581, 5.0319)

3 2 4.2050 0.0636 (4.0681, 4.3419)

4 2 4.6600 0.0849 (4.5231, 4.7969)

5 2 4.2600 0.0424 (4.1231, 4.3969)

Pooled stdev = 0.0752994

One-way ANOVA: crude fibre

Analysis of Variance

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

SAMPLE ID 4 0.28770 0.071925 35.43 0.001

Error 5 0.01015 0.002030

Total 9 0.29785

Model Summary

S R-sq R-sq(adj) R-sq(pred)

0.0450555 96.59% 93.87% 86.37%

Means

SAMPLE

ID N Mean stdev 95% CI

1 2 0.2800 0.0141 (0.1981, 0.3619)

2 2 0.5650 0.0354 (0.4831, 0.6469)

3 2 0.25500 0.00707 (0.17310, 0.33690)

4 2 0.2100 0.0141 (0.1281, 0.2919)

5 2 0.6150 0.0919 (0.5331, 0.6969)

Pooled stdev = 0.0450555

One-way ANOVA: % Ash

Analysis of Variance

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

SAMPLE ID 4 0.009440 0.002360 2.81 0.144

Error 5 0.004200 0.000840

Total 9 0.013640

Model Summary

S R-sq R-sq(adj) R-sq(pred)

0.0289828 69.21% 44.57% 0.00%

Means

SAMPLE

ID N Mean stdev 95% CI

1 2 0.6350 0.0212 (0.5823, 0.6877)

2 2 0.6650 0.0212 (0.6123, 0.7177)

3 2 0.6250 0.0495 (0.5723, 0.6777)

4 2 0.58500 0.00707 (0.53232, 0.63768)

5 2 0.6700 0.0283 (0.6173, 0.7227)

Pooled stdev = 0.0289828

One-way ANOVA: % Protein

Analysis of Variance

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

SAMPLE ID 4 10.1068 2.52670 1336.88 0.000

Error 5 0.0094 0.00189

Total 9 10.1162

Model Summary

S R-sq R-sq(adj) R-sq(pred)

0.0434741 99.91% 99.83% 99.63%

Means

SAMPLE

ID N Mean stdev 95% CI

1 2 4.5100 0.0141 (4.4310, 4.5890)

2 2 3.9400 0.0424 (3.8610, 4.0190)

3 2 5.6600 0.0566 (5.5810, 5.7390)

4 2 3.9400 0.0424 (3.8610, 4.0190)

5 2 6.4750 0.0495 (6.3960, 6.5540)

Pooled stdev = 0.0434741

One-way ANOVA: % Total carbohydrate

Analysis of Variance

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

SAMPLE ID 4 145.086 36.2714 574.37 0.000

Error 5 0.316 0.0631

Total 9 145.401

Model Summary

S R-sq R-sq(adj) R-sq(pred)

0.251297 99.78% 99.61% 99.13%

Means

SAMPLE

ID N Mean stdev 95% CI

1 2 12.585 0.304 (12.128, 13.042)

2 2 19.240 0.170 (18.783, 19.697)

3 2 18.800 0.311 (18.343, 19.257)

4 2 15.365 0.191 (14.908, 15.822)

5 2 9.155 0.247 (8.698, 9.612)

Pooled stdev = 0.251297

One-way ANOVA: ENERGY KCAL versus SAMPLE ID

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SAMPLE ID	4	2783.94	695.984	442.07	0.000
Error	5	7.87	1.574		
Total	9	2791.81			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.25475	99.72%	99.49%	98.87%

Means

SAMPLE ID	N	Mean	stdev	95% CI
1	2	97.22	1.73	(94.94, 99.51)
2	2	136.78	1.46	(134.49, 139.06)
3	2	135.69	1.59	(133.40, 137.97)
4	2	119.160	0.170	(116.879, 121.441)
5	2	100.860	0.410	(98.579, 103.141)

Pooled stdev = 1.25475

Appendix 4 ANOVA result of minerals content of synbiotic *brukina*

One-way ANOVA: Na (mg/100g)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SAMPLE ID	3	6407.5	2135.84	34.71	0.003
Error	4	246.2	61.54		
Total	7	6653.7			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
7.84486	96.30%	93.53%	85.20%

Means

SAMPLE ID	N	Mean	stdev	95% CI
1	2	141.82	9.85	(126.42, 157.22)
2	2	187.34	11.56	(171.93, 202.74)
3	2	110.42	3.75	(95.02, 125.82)
4	2	163.362	1.197	(147.960, 178.763)

Pooled stdev = 7.84486

One-way ANOVA: K (Mg/100g)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SAMPLE ID	3	447.0	149.02	2.68	0.182
Error	4	222.2	55.56		
Total	7	669.3			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)

7.45391 66.79% 41.89% 0.00%

Means

SAMPLE

ID N Mean stdev 95% CI

1 2 131.33 4.41 (116.69, 145.96)

2 2 147.81 14.12 (133.17, 162.44)

3 2 137.73 1.51 (123.10, 152.37)

4 2 149.633 1.035 (134.999, 164.266)

Pooled stdev = 7.45391

One-way ANOVA: Mg (Mg/100g)

Analysis of Variance

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

SAMPLE ID 3 22.153 7.384 4.45 0.092

Error 4 6.633 1.658

Total 7 28.785

Model Summary

S R-sq R-sq(adj) R-sq(pred)

1.28771 76.96% 59.68% 7.83%

Means

SAMPLE

ID N Mean stdev 95% CI

1 2 5.260 1.273 (2.732, 7.788)

2 2 8.260 1.386 (5.732, 10.788)

3 2 6.06 1.73 (3.53, 8.59)

4 2 3.640 0.339 (1.112, 6.168)

Pooled stdev = 1.28771

One-way ANOVA: Ca (mg/100g)

Analysis of Variance

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

SAMPLE ID 3 17.780 5.9266 11.94 0.018

Error 4 1.985 0.4962

Total 7 19.765

Model Summary

S R-sq R-sq(adj) R-sq(pred)

0.704415 89.96% 82.43% 59.83%

Means

SAMPLE

ID N Mean stdev 95% CI

1 2 10.380 1.160 (8.997, 11.763)

2 2 10.800 0.566 (9.417, 12.183)

3 2 7.240 0.000 (5.857, 8.623)

4 2 10.800 0.566 (9.417, 12.183)

Pooled stdev = 0.704415

One-way ANOVA: Zn (Mg/100g)

Analysis of Variance

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

SAMPLE ID 3 1.6022 0.5341 3.82 0.114

Error 4 0.5596 0.1399

Total 7 2.1618

Model Summary

S R-sq R-sq(adj) R-sq(pred)

0.374045 74.11% 54.70% 0.00%

Means

SAMPLE

ID N Mean stdev 95% CI

1 2 1.181 0.147 (0.446, 1.915)

2 2 2.210 0.592 (1.476, 2.944)

3 2 1.6551 0.1303 (0.9208, 2.3895)

4 2 2.282 0.414 (1.548, 3.016)

Pooled stdev = 0.374045

One-way ANOVA: P (mg/100g)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SAMPLE ID	3	503.77	167.924	36.21	0.002
Error	4	18.55	4.638		
Total	7	522.32			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.15351	96.45%	93.78%	85.79%

Means

SAMPLE ID	N	Mean	stdev	95% CI
1	2	20.6000	0.1131	(16.3721, 24.8279)
2	2	36.800	1.131	(32.572, 41.028)
3	2	16.560	0.453	(12.332, 20.788)
4	2	30.12	4.13	(25.89, 34.35)

Pooled stdev = 2.15351

One-way ANOVA: Fe (mg/100g)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SAMPLE ID	3	6.511	2.170	0.55	0.677
Error	4	15.926	3.981		
Total	7	22.437			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.99536	29.02%	0.00%	0.00%

Means

SAMPLE

ID	N	Mean	stdev	95% CI
1	2	12.712	0.780	(8.795, 16.629)
2	2	10.81	3.29	(6.89, 14.73)
3	2	12.5727	0.0912	(8.6553, 16.4901)
4	2	10.87	2.11	(6.95, 14.79)

Pooled stdev = 1.99536

Appendix:5 ANOVA result of microbial analysis of synbiotic *brukina*

One-way ANOVA: Day 0 PCA

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SM ID	3	127.019	42.3395	232461.72	0.000
Error	4	0.001	0.0002		
Total	7	127.019			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0134958	100.00%	100.00%	100.00%

Means

SM ID	N	Mean	stdev	95% CI
1	2	0.000000	0.000000	(-0.026495, 0.026495)
2	2	0.000000	0.000000	(-0.026495, 0.026495)
3	2	7.98218	0.01280	(7.95568, 8.00867)
4	2	7.9563	0.0238	(7.9298, 7.9828)

Pooled stdev = 0.0134958

One-way ANOVA: D1 PCA

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample id	3	0.03360	0.011202	2.70	0.181
Error	4	0.01662	0.004154		
Total	7	0.05022			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)

0.0644507 66.91% 42.10% 0.00%

Means

Sample

Id N Mean stdev 95% CI

1 2 8.0414 0.0585 (7.9149, 8.1679)

2 2 8.1574 0.1106 (8.0308, 8.2839)

3 2 8.1857 0.0260 (8.0592, 8.3123)

4 2 8.2107 0.0170 (8.0842, 8.3372)

Pooled stdev = 0.0644507

One-way ANOVA: Day 2 PCA

Analysis of Variance

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

Sample id 3 8.52624 2.84208 125.13 0.000

Error 4 0.09085 0.02271

Total 7 8.61709

Model Summary

S R-sq R-sq(adj) R-sq(pred)

0.150709 98.95% 98.15% 95.78%

Means

Sample

Id N Mean stdev 95% CI

1 2 8.7230 0.0464 (8.4272, 9.0189)

2 2 10.230 0.173 (9.934, 10.526)

3 2 7.849 0.237 (7.553, 8.145)

4 2 10.2820 0.0481 (9.9861, 10.5778)

Pooled stdev = 0.150709

One-way ANOVA: Day 3 PCA

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample id	3	4.00070	1.33357	124.74	0.000
Error	4	0.04276	0.01069		
Total	7	4.04346			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.103395	98.94%	98.15%	95.77%

Means

Sample Id	N	Mean	stdev	95% CI
1	2	9.201	0.172	(8.998, 9.404)
2	2	10.5649	0.0826	(10.3619, 10.7679)
3	2	9.3070	0.0800	(9.1040, 9.5100)
4	2	10.7547	0.0027	(10.5517, 10.9577)

Pooled stdev = 0.103395

One-way ANOVA: Day 4 PCA

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample id	3	5.85914	1.95305	584.38	0.000
Error	4	0.01337	0.00334		
Total	7	5.87251			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0578107	99.77%	99.60%	99.09%

Means

Sample

Id	N	Mean	stdev	95% CI
1	2	9.7966	0.0685	(9.6831, 9.9101)
2	2	11.4842	0.0141	(11.3707, 11.5977)
3	2	9.7910	0.0496	(9.6775, 9.9045)
4	2	11.5261	0.0775	(11.4126, 11.6396)

Pooled stdev = 0.0578107

One-way ANOVA: day 5 TPC

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample id	3	0.01813	0.006042	0.44	0.738
Error	4	0.05507	0.013766		
Total	7	0.07319			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.117330	24.77%	0.00%	0.00%

Means

Sample

Id	N	Mean	stdev	95% CI
1	2	11.4972	0.0283	(11.2668, 11.7275)
2	2	11.463	0.226	(11.233, 11.694)
3	2	11.4983	0.0487	(11.2680, 11.7287)
4	2	11.5912	0.0260	(11.3609, 11.8216)

Pooled stdev = 0.117330

One-way ANOVA: DAY 0 *Lactobacillus casei*

Analysis of variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SAMPLE ID	3	0.4869	0.16231	2.95	0.161
Error	4	0.2199	0.05498		
Total	7	0.7068			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.234481	68.89%	45.55%	0.00%

Means

SAMPLE ID	N	Mean	stdev	95% CI
1	2	7.1623	0.0591	(6.7020, 7.6227)
2	2	6.88358	0.01204	(6.42323, 7.34392)
3	2	7.0702	0.1373	(6.6099, 7.5306)
4	2	6.518	0.444	(6.058, 6.979)

Pooled stdev = 0.234481

One-way ANOVA: Day1 *L.casei*

Analysis of variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SAMPLE ID	3	0.13591	0.045304	11.82	0.019
Error	4	0.01533	0.003831		
Total	7	0.15124			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0618985	89.87%	82.27%	59.47%

Means

SAMPLE

ID	N	Mean	stdev	95% CI
1	2	7.5241	0.0852	(7.4026, 7.6456)
2	2	7.4659	0.0179	(7.3444, 7.5875)
3	2	7.1801	0.0749	(7.0585, 7.3016)
4	2	7.3772	0.0463	(7.2556, 7.4987)

Pooled stdev = 0.0618985

One-way ANOVA: day*2 L, casei*

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SAMPLE ID	3	0.4034	0.1345	0.78	0.566
Error	4	0.6937	0.1734		
Total	7	1.0971			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.416430	36.77%	0.00%	0.00%

Means

SAMPLE

ID	N	Mean	stdev	95% CI
1	2	8.705	0.386	(7.887, 9.522)
2	2	8.8417	0.0221	(8.0242, 9.6593)
3	2	8.477	0.675	(7.660, 9.295)
4	2	9.097	0.298	(8.279, 9.915)

Pooled stdev = 0.416430

One-way ANOVA: DAY3 *L. casei*

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SAMPLE ID	3	0.509790	0.169930	85.47	0.000

Error 4 0.007953 0.001988

Total 7 0.517743

Model Summary

S R-sq R-sq(adj) R-sq(pred)

0.0445888 98.46% 97.31% 93.86%

Mean

SAMPLE

ID N Mean stdev 95% CI

1 2 10.7347 0.0124 (10.6472, 10.8222)

2 2 10.6546 0.0868 (10.5671, 10.7422)

3 2 10.0899 0.0050 (10.0024, 10.1774)

4 2 10.5954 0.0156 (10.5078, 10.6829)

Pooled stdev = 0.0445888

One-way ANOVA: day 4 *L.casei*

Analysis of Variance

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

SAMPLE ID 3 0.16362 0.05454 4.46 0.091

Error 4 0.04897 0.01224

Total 7 0.21259

Model Summary

S R-sq R-sq(adj) R-sq(pred)

0.110640 76.97% 59.69% 7.87%

Means

SAMPLE

ID N Mean stdev 95% CI

1 2 11.481 0.216 (11.264, 11.698)

2 2 11.52 0.00 (11.30, 11.74)

3 2 11.3540 0.0136 (11.1368, 11.5712)

4 2 11.1540 0.0473 (10.9368, 11.3713)

Pooled stdev = 0.11064

Appendix 6 ANOVA: reducing sugar of tiger nut slurry

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample ID	3	73.7672	24.5891	388.29	0.000
Error	8	0.5066	0.0633		
Total	11	74.2738			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.251648	99.32%	99.06%	98.47%

Means

Sample ID	N	Mean	stdev	95% CI
1	3	10.513	0.289	(10.178, 10.848)
2	3	7.0253	0.1363	(6.6903, 7.3604)
3	3	4.768	0.389	(4.433, 5.103)
4	3	4.204	0.000	(3.869, 4.539)

Pooled stdev = 0.251648