EFFECT OF FERMENTATION TIME ON THE QUALITY OF OPTIMIZED YOGHURT PRODUCED FROM BLENDS OF SOYBEAN, OAT, AND COCONUT MILK

BY

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DECLARATION

I, Orieruo Akpezi Ufuoma, do hereby declare that this project is entirely my own work and composition. The work embodied in this project has not been submitted in a candidature or any degree and is not currently being submitted for any other degree. All references to works of other persons have been duly acknowledged.

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CERTIFICATION

This is to certify that this dissertation report on the topic "EFFECT OF FERMENTATION TIME ON THE QUALITY OF OPTIMIZED YOGHURT PRODUCED FROM BLENDS OF SOYBEAN, OAT, AND COCONUT MILK" was completed and is being submitted by ORIERUO, AKPEZI UFUOMA, with matriculation number PG/MCB/130301, carried out under the supervision of PROF. STEPHEN. A. ENABULELE, to The Department of Biological Sciences (Food and Industrial Microbiology option) Faculty of Science, Benson Idahosa University, Benin City, Nigeria.

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DEDICATION

This work is dedicated to God Almighty for His Immense love and to my loving family, whose unwavering support, prayers and encouragement have been my greatest source of strength.

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ABSTRACT

Yoghurt is one of the most extensively consumed fermented foods worldwide. It is conventionally produced by the metabolic activity of lactic acid bacteria such as *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*. This work was designed, optimized, and evaluated plant-based yoghurts produced from composite milk blends of soybean, oat, and coconut. Optimization was performed using a mixture design in order to find the blend ratios at which sensory properties are maximized. The superior formulations from optimization were selected based on their overall acceptability following the criteria of texture, aroma, and appearance. They were then subjected to full physicochemical, nutritional, antioxidant, and microbiological characterization. The optimized yoghurts displayed some major nutritional and functional characteristics, such as the total phenolic content of 1.14 to 4.37 mg GAE/g and flavonoids of 0.08 to 0.55 mg QE/g. Different Formulations exhibited varying degrees of antioxidant activities, and S27_CO was among them, which had the highest antioxidant activity (50.64%) that was almost the same as that of commercial yoghurt. The analytical technique unequivocally demonstrated the presence of the major vitamins A and C their concentrations were in the range of 365.17 to 848.28 µg RAE/g and 1.91 to 3.10 mg/g, respectively. The physicochemical parameters were determined for the samples, revealing pH values of 3.68 to 4.93 and °Brix values of 6.00 to 12.25%, thus confirming the attainment of good fermentation and product stability. The microbiological study indicated the presence of beneficial lactic acid bacteria and the absence of any pathogenic organisms. Sensory evaluation of optimized blends confirmed their suitability, showing a great preference for the formulations with higher coconut milk proportions. Optimized yoghurts displayed a desirable nutritional profile, functional antioxidant properties, microbial safety, and consumer acceptability, and such yoghurts represent feasible alternatives to traditional dairy yoghurts.

CHAPTER ONE

INTRODUCTION

1.1. Background of the Study

Fermentation is a conventional and highly varied biotechnological operation used for the conversion of raw food materials into a stable, safe, and nutritionally enhanced product. Yoghurt is one of the most extensively consumed fermented foods worldwide (Béal and Helinck, 2014). It is conventionally produced by the metabolic activity of lactic acid bacteria such as *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* (Olodu and Enabulele, 2025). These microorganisms ferment lactose to lactic acid to create typical gelation, acidity, and flavouring (Dan *et al*., 2023; Bai *et al*., 2024). Although yoghurt is widely accepted, lactose intolerance, milk allergy, and vegetarianism necessitate the need to seek alternatives to dairy yoghurt. Plant milk alternatives have also received much attention because of the potential that the sensorial and nutritional characteristics of milk can be replicated, along with other environmental advantages and uses (Chetachukwu *et al*., 2019).

Among all the substitutes, soybeans (*Glycine max*) are the most researched and widely used vegetable crop for the manufacture of non-dairy yoghurt. This development is because of its high-quality protein and essential amino acids (Modgil *et al*., 2020). Zhang *et al*. (2017) also reported that soybeans are rich in phytochemicals and bioactive substances, such as isoflavones, saponins, and phytosterols, with antioxidant properties, as well as hypocholesterolemia and hormone-modulating activities. Although these benefits are present, traditional soy yoghurts are still susceptible to flaws like their inherent "beany" flavour, proportionally decreased carb content

suitable for fermentation, and weak gel strength texture. These flaws justify the blending of soy with supporting plant material for texture, flavour, and fermentation efficiency enhancement.

Oat (*Avena sativa*) offers a natural means of addressing the majority of these flaws. Oat is a cereal grain containing the unique soluble fiber, β-glucan, with yoghurt-like nature and also cholesterol-reducing properties (Chalupa-Krebzdak *et al*., 2018). Oats are made up of merely fermentable carbohydrates that may be able to stimulate the activity of growth of lactic acid bacteria in yoghurt processing to promote acidification as well as microbial development. Other than that, their inherent water-holding abilities and viscosity contribute to enhanced mouthfeel and gel stability of fermented foods (Amiri *et al*., 2019). A blend of soybeans with oats plays the function to balance the protein-carbohydrate ratio, enhance smoother fermentation kinetics, and mask soy-induced off-flavours typical in soy-containing foods.

Coconut (*Cocos nucifera*) is one of the fruits used in a wide variety of food products due to its nutrient-richness, flavour, and suitable creamy texture. It has an indigenous lipid profile, such as medium-chain triglycerides (MCTs), which provides rapid metabolic energy and a creamy, tasty texture (Duranova *et al*., 2025). Aside from its organoleptic attributes, coconut milk is micronutrient- and antioxidant-rich, which nutritionally fortifies plant yoghurts. If coconut yoghurts are sold and consumed as monolithic products, they will not have sufficient protein and fermentable substrates to achieve acceptable gel firmness and satisfactory microbial growth. So, coconut, soy, and oat individually as well as combined, may provide a more integrated substrate that includes the soy's protein content, the oat's fermentable carbohydrate portion, and coconut's lipid-based richness. In the event this is conducted in a composite matrix, product quality has an independent factor represented by fermentation time. Fermentation time influences lactic acid

production, pH decrease, viability of the microorganisms, and structure development, all of which influence texture, flavour, and safety (Greis *et al*., 2022; Sionek *et al*., 2024).

As the plant matrices are also very different from dairy milk in terms of nutrient composition and ability to buffer, this would most probably impact growth during fermentation substantially. Optimal fermentation time, on the other hand, assumes prime significance for peak acidity, even coagulation, and added sensory attractiveness in yoghurt produced from composite plant sources. While interest in the production of nondairy yoghurt is picking up steam, no systematic data as yet are available on the effect of fermentation time on functional, microbial, and sensory properties of yoghurt produced from soybean, oat, and coconut blends. This study, therefore, seeks to examine the effect of the fermentation period on the quality of yoghurts produced using such blends and elucidate, using a simplex lattice optimization method through evidence-based, science-based research, how plant synergy and microbial kinetics can be utilized towards the production of nutritionally adequate, acceptable, and sustainable yoghurt alternatives.

1.2. Problem Statement

Global demand for dairy, lactose-free products has risen considerably, with lactose intolerance, milk allergy, nutritional concerns in terms of cholesterol content, and environmental sustainability becoming increasingly prevalent. Soy, oat, and coconut-milk-based yoghurts are gaining popularity due to their nutritional composition, but most such products now commercially available have not succeeded in textural acceptability, taste, and microbial shelf life compared to traditional dairy yoghurt. This is because, protein structural change, sugar content, and lipid content of vegetable matrices are likely to result in poor gelation, low viscosity, and abnormal acidification during fermentation (Amiri *et al*., 2019; Montemurro *et al*., 2021).

Moreover, fermentation duration, an important factor for acid production, microbial development, and sensorial properties, has not been adequately optimized in mixed vegetable substrates. Short fermentation time may cause incomplete acidification and textural defects. At the same time, over-fermentation causes over-sourness, syneresis, and microbial instability (Rahman *et al*., 2024). Although there is considerable knowledge on yoghurt produced from soybean, coconut, and oat milk, there is limited information on the synergistic effects of these milk fractions during fermentation, particularly the impact of fermentation time on the physicochemical, microbial, and sensory attributes of the final product. Solving this problem will enhance the development of stable, healthy, and consumer-acceptable plant yoghurts.

1.3. Justification of the Study

The importance lies in producing good-quality, nondairy yoghurt replacers in an attempt to offset the concerns related to health and sustainability. Soybean contains a high level of protein and limiting amino acids but poor texture and flavour when consumed alone (Ismail *et al*., 2017). Oats provide fermentable carbohydrates, β-glucan, and viscosifying agents that promote fermentation rates and mouthfeel, whereas coconut provides natural fats and flavour that ensure sensory acceptability improvement (Chetachukwu *et al*., 2018; Dhakal *et al*., 2024). A combination of all three components offers the potential to create a nutritionally similar and texturally stable product. However, the success of this system largely relies upon the optimization of the fermentation factors, particularly time. Understanding of how the fermentation time affects acidity, pH, microbial load, and sensory characteristics was the basis for the creation of acceptable, shelf-stable, and healthy yoghurt alternatives. It also complements global efforts towards sustainable food production through the application of plant materials in reducing the use of animal-derived ingredients.

This study, therefore, contributes to the growing body of plant fermentation-based research through the provision of empirical data on the production of alternative yoghurts that match the needs of lactose-intolerant, vegan, and healthy food consumers.

1.4. Aim of the Study

The aim of this study is to evaluate the effect of fermentation time on the quality of optimized yoghurt produced from blends of soybean, oat, and coconut.

The Specific Objectives of this Study are to;

(a) determine the best yoghurt formulation and fermentation time through consumer acceptability using optimal simplex lattice design;

(b) determine the nutritional composition (proximate and mineral composition) of the optimized yoghurt samples;

(c) determine the physicochemical changes (pH, titratable acidity, total solids, protein) in the optimized yoghurt samples;

(d) determine the antioxidant activities and the vitamin composition of the optimized yoghurt samples;

(e) assess the microbial quality and viability of lactic acid bacteria of the optimized yoghurt samples; and

(f) evaluate the sensory properties of the optimized yoghurt samples.

CHAPTER TWO

LITERATURE REVIEW

2.1. Overview of Fermentation

Fermentation is the oldest and most interdisciplinary biotechnological process used by man for the enrichment of foods' nutritional, storage stability, and safety properties (Admassie, 2018). Fermentation is the hydrolysis of carbohydrates to low molecular weight compounds like organic acids, alcohol, or gases due to the enzyme activity of microorganisms under anaerobic or microaerophilic conditions (Phillips *et al*., 2017; Guzmán-Hernández *et al*., 2025). It also protects food and alters its physicochemical and sensory characteristics, in many instances developing products that are more digestible and whose nutrient bioavailability increases (Terefe and Augustin, 2020). Lactic acid fermentation occupies a very special position among most food science fermentation reactions because it has enabled the manufacture of foods like yoghurt, kefir, sauerkraut, and kimchi using Lactic acid bacteria (LAB) starters.

Lactic acid bacteria are polymorphic communities of Gram-positive, catalase-negative, non-sporing bacteria, which ferment carbohydrates to lactic acid as the primary product (Negm, 2018; Zheng *et al*., 2020). They include members of genera that are most *Lactobacillus, Streptococcus*, *Lactococcus*, *Leuconostoc*, and *Pediococcus* (Säde and Björkroth, 2019). They possess acidification metabolisms that prevent the growth of spoilage microorganisms and pathogens and facilitate desirable flavour, texture, and aroma development (Okoye *et al*., 2025). During yoghurt manufacturing, symbiosis of *Lactobacillus delbrueckii subsp. Bulgaricus* and *Streptococcus thermophilus* are common. Both of these microbes are protocooperative; *S. thermophilus* initiates the rapid acidification process and yields formic acid and CO_2, while *L. Bulgaricus* yields amino acids and peptides that induce S. *thermophilus* growth (Yamauchi *et al*., 2019). Metabolic synergy

increases maximum lactic acid production, which initiates the development of the characteristic gel structure and flavour of yoghurt. Biochemically, lactic acid fermentation is glycolytic glucose breakdown to pyruvate, its reduction to lactic acid, to recycle NAD^+ to continue glycolysis (Chaudhry and Varacallo, 2018). Depending on the metabolism process, LAB is homolactic with major lactic acid production, or heterolactic with lactic acid production supplemented by ethanol, acetic acid, or CO_2 (König and Fröhlich, 2017).

These metabolic differences influence not only the acidification rate but also the textural and organoleptic properties of fermented food. Protein denaturation and gelation to achieve desired firmness and viscosity in yoghurt systems are manifestations of acidifying reaction (Dan *et al*., 2023; Bai *et al*., 2024). Fermentation conditions, especially time, temperature, and inoculum level, must be maintained to ensure maximum product quality. Fermentation time, in particular, governs the interaction of strength of flavour, acidity, and microbial viability.

Such interactions are formed well within milk systems but in the case of substrates of plant origin, i.e., soybean, oat, and coconut, fermentation activity is strongly resisted because of variability in nutritional composition, buffering, and availability of fermentable carbohydrates (Jeske *et al*., 2018). These compositional variations affect lactic acid bacteria growth kinetics and pH decline rate, hence end-product quality traits. Hypothesis and biochemical rationale of fermentation thus lie at the root of knowledge of the mechanism of production process of plant and dairy yoghurts. This is then followed by the next section, where yoghurt, its composition, manufacturing process, and quality characteristics are explained in a manner that will serve as a basis to explain how fermentation time controls its physicochemical and sensory properties.

2.2 Yoghurt and Manufacturing Process

Yoghurt is consumed daily across the world because of its functional, sensory, and nutritional attributes. It is synthesized through lactic acid fermentation of milk by specific starter cultures, the key ones being *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*, which ferment lactose to lactic acid and decrease pH and stimulate milk protein casein coagulation, primarily casein (Huppertz, 2017). The bioconversion gives yoghurt its characteristic sour taste, viscous texture, and extended shelf life. Apart from the enhanced digestibility, fermentation also enhances the bioavailability of micronutrients, in addition to enhancing the formation of bioactive peptides with positive health impacts such as enhanced gut function and immune modulation (Tachie *et al*., 2024). Excessive dependence on cow milk for yoghurt production, however, has nutritional, economic, and sustainability limitations. Lactose intolerance is prevalent in approximately 57% to 65% of the global population, they being the Asian and African populations, cutting the consumption of the conventional dairy-based yoghurt (Acharya *et al*., 2025).

Irregular milk supply, food system ethics related to animal foods, and environmental impact of dairy farming have also fueled global demand for developing plant-based yoghurt (Sethi *et al*., 2016). These others, besides attempting to match the textural and sensory characteristics of the conventional yoghurt, attempt to add improved nutrition and functional characteristics to the food by employing legumes, cereals, and nuts. Soybeans, oats, and coconut are some of the best-documented substrates of vegetable origin that have gained a lot of publicity due to their richness in protein, starch, and lipids, respectively. These foods possess nutritional and functional attributes that are prone to interact synergistically in a cohesive yoghurt matrix (Bernat *et al*., 2015).

Soybeans contain quality plant protein and bioactive isoflavones, while oats possess β-glucans, which add additional viscosity and lower serum cholesterol. Coconut, being a natural source of

emulsifiers and medium-chain triglycerides, plays a role in creamy texture and normal flavour character, thereby improving plant yoghurts' palatability (Oluwajuyitan *et al*., 2021; Le *et al*., 2025). With the addition of these three substrates, the blend is a good vehicle for the production of nutritionally adequate, functionally enhanced, and texturally comparable yoghurt equivalents. The addition of such plant matrices into a fermentation process requires a basic transition between microbial function and substrate makeup. Fermentation time, however, is responsible for developing the physicochemical and sensory nature of the final product. Soy, oat, and coconut substrate interaction with varying fermentation times controls acidity, microbial growth, nutrient transformation, and flavour development. Having such interaction in a composite plant medium forms the foundation for determining how fermentation time determines the quality and functional ability of processed yoghurt.

2.3 Soybean as a Functional Substrate for Yoghurt Production

Soybean (*Glycine max*) ranks among the most researched plant matrices to produce non-dairy yoghurt due to its high content in protein, functional versatility, and a realistic amino acid profile (Le *et al*., 2025). The 35-40% protein and 18-20% fat content of soybeans make it a nutritionally dense substrate for microbial cultures and explain the composition and nutritional content of milk (Modgil *et al*., 2020).

Glycinin and β-conglycinin soy proteins exhibit improved gelation, water retention, and emulsification potential, which can provide the texture and mouthfeel requirements in fermented foods (Yang *et al*., 2024). Soy proteins undergo proteolytic hydrolysis to produce peptides and amino acids during fermentation that affect flavour as well as demonstrate bioactive properties such as antioxidant, antihypertensive, and cholesterol-lowering activity (Duong *et al*., 2019). Fermentation improves the digestibility and sensorial acceptance of soy milk by removing

interference otherwise caused by inherent beany flavour and trypsin inhibitor activity of raw soy (Sanjukta and Rai, 2016). LAB fermentation metabolism hydrolyzes raffinose and stachyose, which are causative oligosaccharides of flatulence, therefore enhancing consumer tolerance (Shori, 2024). Soy yoghurt is also lactose-, cholesterol-, and low saturated fat-free by default, therefore a functional food for lactose intolerance, hyperlipidemia, and cardiovascular disease patients (Munir *et al*., 2018). Despite these advantages, unflavoured soy yoghurt possesses textural flaws like syneresis and mild chalky mouthfeel. These are consequences of the structural difference between milk and soy proteins in their poor ability to develop casein-like micelles (Bernat *et al*., 2015).

To supplement texture, consistency, and flavour, researchers have been attempting to mix soy with other vegetable matrices that are starch or fiber-rich to create additional gel structure and viscosity. This activity not only masks the structural disadvantage in soy but also differs in nutrients. Among these additives, oat was also identified as being of untapped potential because of its soluble fiber, β-glucan, which gives water-holding capacity and texture smoothness to plant-based fermented foods (Chalupa-Krebzdak *et al*., 2018). Soybean (Plate 2.1) and oat substrates mixture, therefore, offers a balanced substrate for microbial fermentation with the potential of producing yoghurt-like food with improved rheological and nutritional profile.

2.4 Oat as Plant-Based Yoghurt Fermentation Medium

Avena sativa L., oat, is among the functional food materials of choice for plant-based yoghurt fermentation because of its pharmaceutically potent bioactive molecules and balanced macronutrients. It contains approximately 60-65% carbohydrate, 10-17% protein, and 4-7% lipid content, and rich dietary fiber, i.e., β-glucan, a water-soluble polysaccharide that has water-soluble viscosity and cholesterol-lowering activity (Biel *et al*., 2020).



Plate 2.1: Soybean

Source: https://www.markets.com/

Adding oats (Plate 2.2) to yoghurt food not only turns out to be useful in texture but also in the sense of offering a smooth and creamy mouthfeel, as with dairy yoghurt (Brückner-Gühmann *et al*., 2019; Kosterina *et al*., 2020; Nehaa *et al*., 2022). The natural sweetness and non-toxic flavour of oat substrates further improve plant yoghurts' sensory acceptability, overtaking the stringent, comparatively beany or insipid flavour of soy (Nehaa *et al*., 2022). Fermentation is associated with the enzymatic hydrolysis of oat starch and lactic acid bacteria's physiological metabolism to produce organic acids, exopolysaccharides, flavour volatile compounds, and rheological properties (Zare *et al*., 2012). Oats' carbohydrate content is sufficient to act as a substrate for efficient microbial metabolism, leading to acid formation and fermentation.

The β-glucan level also provides a yoghurt fiber that encourages the growth of healthy microbes, hence justifying the end product's probiotic nature (Suchecka *et al*., 2017). This dual purpose of enhancing microbial function and product texture modification makes oat a critical base supplement when combined with protein-rich legumes such as soy. Apart from nutritional and functional quality, oat yoghurt has also been reported to play a significant role in glycemia regulation and lipid metabolism. β-glucan possesses gel-like viscousness in the intestine that slows down glucose absorption and generates satiety (Suchecka *et al*., 2017). All these attributes make oat-supplemented fermented foods a suitable functional food for the treatment of obesity, type 2 diabetes, and hypercholesterolemia.

In addition, blending the oat carbohydrate with soy protein increases the matrix stability during fermentation with more consistency, water retention ability, and reduced syneresis of composite yoghurts (Bernat *et al*., 2015; Saeed *et al*., 2024). But although oats contribute to mouthfeel and viscosity, they contain relatively low-fat levels, and this may reduce the overall thickness and creaminess of the final product below that of dairy yoghurt.



Plate 2.2: Oats

Source: https://www.bbcgoodfood.com/

2.5 Coconut as a Lipid-Rich Substrate in Yoghurt Production

Coconut (*Cocos nucifera L.*) is a promising plant substrate in yoghurt cultivation since it possesses high lipid, excellent sensory characteristics, and a unique fatty acid profile. As opposed to most vegetable fats that are primarily made up of long-chain triglycerides, coconut fats are primarily made up of MCTs, such as lauric, capric, and caprylic acids, which are more easily metabolized and provide an easily accessible source of energy (Duranova *et al*., 2025). Not only does this contribute to the nutritional benefit of coconut-containing products, but also to product texture and smoothness. Coconut milk's natural emulsifying agents, including phospholipids and certain proteins, stabilize emulsions and improve mouthfeel by inhibiting phase separation (Phakthawat *et al*., 2023).

When coconut milk or cream is added in the process of yoghurt making, it balances the low-fat content of legume- or cereal-based products. Its fat globules mimic milk fat's structural role in conventional yoghurt, enabling gelation and contribution of the smooth, creamy mouthfeel of dairy (Patil and Benjakul, 2018). Furthermore, coconut's natural sweetness and flavour can mask soy-based types' natural earthy or beany flavour, which is most intensely experienced, rendering it marketable to consumers (Moss *et al*., 2023). Coconut also contains bioactive phenolics, tocopherols, and phytosterols possessing antioxidant and antimicrobial activities, which can further increase the shelf stability and functional quality of the resultant fermented product (Ngampeerapong *et al*., 2018).

There is a physico-chemical interaction among coconut lipids with the gelling polysaccharide-protein matrix of other plant-based substrates, including soy and oat, in the regulation of the rheological and sensory properties of composite yoghurts. Coconut fat can be added to the protein-

starch gel structure formed during fermentation for the production of a coherent gel structure of good balanced viscosity and low syneresis (Maghazechi, 2023).

In addition to the functional process, coconut's unique lipid structure also influences microbial equilibrium during fermentation (Zhang *et al*., 2017). Certain lactic acid bacteria are resistant to MCT, with this capability enabling them to ferment them to produce short-chain fatty acids and flavour-active compounds that influence the sensory acceptability of the product (Blaszczak *et al*., 2022; Monteiro *et al*., 2022). Concentrations of lipids too high, however, inhibit the growth of the bacteria or retard acidification, indicating the necessity to get to a balance of fermentation conditions such as temperature and inoculum size, but particularly fermentation time. This must be achieved in an attempt to get to a balance between microbial metabolism and product quality. The synergy of substrate composition and fermentation time is thus the cornerstone of optimizing yoghurt formation from composite plant sources. The typical image of coconut is presented in Plate 2.3.

2.6 Fermentation Time as a Factor in Yoghurt Quality Formation

Fermentation time is among the most significant parameters to control the quality of yoghurt, with effects on the extent of microbial growth, rate of acidification, nutrient conversion, and structure development in the matrix. The process typically involves the metabolic conversion of carbohydrates, such as lactose or other fermentable carbohydrates, to lactic acid through LAB, leading to a gradual lowering of pH that favors casein or plant protein coagulation and gelation (Huppertz, 2017). The length of fermentation directly affects such biochemical reactions, not only sensory and rheological characteristics but also nutritional and functional characteristics of the yoghurt (Maghazechi, 2023).



Plate 2.3: Coconuts

Source: https://www.webmd.com/

Microbial growth and acid development are slow in the early stages of fermentation, and this gives a poor or incomplete network of gelation. During subsequent fermentation, lactic acid production decreases pH in the direction of proteins' isoelectric point, and this supports gelation and aggregation (Shah *et al*., 2018; Qamar *et al*., 2019). The ideal fermentation time results in a stabilized gel matrix with maximum viscosity, minimum syneresis, and the most balanced flavour profile. Over-fermentation may cause over-acidification, hydrolysis of proteins, and textural defects such as graininess or undue whey separation (Tang *et al*., 2024). Structural balance, viability of microorganisms, and sensory attractiveness, therefore, require an ideal fermentation time. Plant-based yoghurt systems, particularly those based on soy, oat, and coconut matrices, have remarkably distinct fermentation kinetics compared with dairy milk because of differences in substrate content and buffering capacities.

Soy proteins, on the other hand, exhibit reduced coagulation rates, while oat carbohydrates yield readily fermentable carbohydrates that facilitate increased acid formation (Chalupa-Krebzdak *et al*., 2018). Coconut lipids, on the other hand, influence fermentation kinetics by controlling oxygen penetration, in addition to microbial metabolism (Monteiro *et al*., 2022). These interactions would imply that fermentation time should be optimized relative to the composite matrix in order to produce homogeneous acidity, a stable gel structure, and the required flavour development. Besides, fermentation time affects bioavailability and bioactive compounds transformation in plant yoghurts.

Proteolysis and excretion of short-chain peptides with potential antioxidant and antihypertensive activity are enhanced by greater fermentation time (Duong *et al*., 2019). Similarly, enzyme hydrolysis of starch and fiber with extended fermentation will increase amounts of yoghurt oligosaccharides, organic acids, and reducing sugars, that will increase functional and nutritional

performance (Tachie *et al*., 2024). Over-fermentation, however, will ruin sensory quality by creating too much acidity and volatile off-flavours. Optimization of fermentation time, thus, is not just a technological necessity but also a factor influencing the general product quality and acceptability. Optimization of fermentation time is highly important as far as the development of stable, acceptable, and functionally dense vegetable yoghurts is concerned. Substrate composition, microbial metabolism, and time collectively determine the physicochemical, microbial, and sensory quality of the product. It is on this background of fermentation's time dimension that a platform is set for the exploration of how different times of fermentation control the quality attributes of yoghurt, like texture, acidity, microbial viability, and bioactive capacity.

Through the regulation of fermentation time, acidification kinetics, onset and strength of gelation (viscosity/firmness), microbial growth and metabolism (and therefore viability and metabolite formation), nutrient transformation (proteolysis, anti-nutrient degradation, bioactive peptide formation), and flavour/odor development (volatile compounds, off-flavour masking) are controlled. For instance:

- Shorter fermentation may yield flavour neutrality, reduced gel network strength, increased syneresis, lower viscosity, and insufficient microbial and nutritional conversions.
- Optimum fermentation time achieves target pH/TA, good gel network with WHC, high viability of LAB, enhanced bioavailability of nutrients, balanced flavour/texture, negligible syneresis, and excellent consumer acceptability.
- Over-fermentation can lead to over-acidification, rigid gel texture, excessive syneresis, low probiotic viability, extremely sour or off-flavour, and probable compromised quality of nutrients by degradation or oxidation.

2.7 Quality Attributes and the Effect of Fermentation Time on Plant-Based Yoghurt

The dairy and plant yoghurt attributes are regulated by a complex set of physicochemical, microbiological, nutritional, and sensorial parameters, which, as a whole, decide product traits and acceptability by the consumer.

These factors, especially in plant systems, are highly sensitive to substrate composition, fermentation time, microbial dynamics, and process operation (Huang *et al*., 2022). Natural variation in protein content, carbohydrate content, and lipid composition of plant- and dairy-matrices regulates gel network formation, viscosity properties, acidification rate, flavour development, and nutrient bio accessibility (Mäkinen *et al*., 2016). ‘

2.7.1. Physicochemical properties

Major determinants of yoghurt stability and quality of fermentation include pH, titratable acidity (TA), viscosity, syneresis (separation of whey), water-holding capacity (WHC), gel firmness, and rheological behavior (Arab *et al*., 2023). Commercial yoghurts sold as plant-based yoghurts made from soy, coconut, cashew, almond, and hemp, the authors concluded that among certain soy- and coconut-based types, texture and viscosity levels were close to dairy control, partly because they contained hydrocolloid/gelling agents.

In soy yoghurts compared to oat-based, microstructural analysis revealed that the soy samples formed a denser, finer network that was measured as greater gel firmness, better WHC, while the oat-based system formed a looser, coarser network with lower mechanical stability.

Absence of casein micelles (in milk from dairy animals) renders plant-protein gels alone less strong and more susceptible to destruction of structure or phase separation, thus the reason to

optimize fermentation time, enzyme processing, homogenization, and additions of gel/hydrocolloid (Tang *et al*., 2024).

Fermentation time has a profound influence on such physicochemical properties: acidification (pH decrease) occurs slowly during the lag phase until LAB start exponential growth; after pH falls to the plant protein's isoelectric point, gelation and coagulation, as well as gel network formation, intensify (Tachie *et al*., 2024).

Maximum fermentation time provides a solid, homogeneous gel with minimum syneresis; over-fermentation results in excessive acid formation, gel network disruption, excessive whey separation, brittle texture, and potential bitterness (Shah, 2018; Qamar *et al*., 2019). In the case of vegetable soy protein mixtures, the buffering effect of soy proteins and oat β-glucan viscosity slows down the acidification and gelation kinetics, and longer fermentation time than for normal dairy yoghurt mixtures can be required (Bernat *et al*., 2015).

2.7.2. Microbiological characteristics

Viability, metabolic activity, and stability of starter culture and of any added adjunct probiotic strains determine the final product safety, acidity development, texture creation by production of exopolysaccharide (EPS), and shelf-life improvement.

LAB, such as *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus plantarum*, can grow in vegetable substrates, but their growth kinetics, substrate affinity, and acidification rates are quite different with the composition (Olodu and Enabulele, 2025).

For instance, in soy yoghurt, *Bifidobacterium animalis subsp. Lactis* HCS04-001 enriched fermentation enhanced isoflavone-equol content and texture and antioxidant activity (Volokh *et al*., 2019). In a mixed plant matrix, the fermentable carbohydrate content maintains acid

production, EPS production, and antimicrobial metabolite biosynthesis responsible for microbial safety and shelf-life extension (Blaszczak *et al*., 2022; Monteiro *et al*., 2022; Olodu and Enabulele, 2025). Long-term fermentation beyond the window of opportunity can, however, destabilize the viability of LAB by substrate depletion and acid stress of pH, thus probiotic activity and storage stability are lost (Tachie *et al*., 2024).

2.7.3. Nutritional properties

Nutrient content in plant yoghurts is subject to the substrate composition of the initial substrate and the type of biochemical conversion resulting from fermentation (Xu *et al*., 2025). Soy contains a high-quality plant protein, oat contains soluble fibre with known physiological actions, and coconut contains MCTs and trace amounts of micronutrients (iron, magnesium, zinc) (Oluwajuyitan *et al*., 2021; Le *et al*., 2025).

Fermentation enhances nutrient bioavailability through the degradation of anti-nutritional factors such as trypsin inhibitors and macromolecules into more digestible compounds (Sanjukta and Rai, 2016). Microbial proteolysis releases bioactive peptides having potential health effects, i.e., antihypertensive and anti-inflammatory activities (Duong *et al*., 2019). In a recent work on quinoa plant yoghurt, fermentation provided organic acid production (lactic and succinic), reduced total sugar, enhanced essential amino acid content, and antioxidant activity on simulated digestion, showing functional enhancement in plant fermented foods (Benavides-Guevara *et al*., 2023). However, nutrition is also controlled by fermentation time. Maximum time enables the degradation of anti-nutritional compounds and release of bioactive compounds; over-fermentation will lead to the production of unwanted metabolites and nutrient loss via extensive acidification or oxidation, thus reducing acceptability and nutritional value (Patil and Benjakul, 2018).

2.8 Microbial Dynamics and Probiotic Viability in Plant-Based Yoghurts

The microbial yoghurt fermentation consortium forms the foundation for its physicochemical transformation, nutrient enrichment, and probiotics. Traditional yoghurt fermentation relies mainly on symbiotic interaction between *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus,* whereby *S. thermophilus* contributes to quick acidification through lactic acid production and *L. bulgaricus* contributes to proteolysis as well as flavour development (Dan *et al*., 2023; Bai *et al*., 2024). This metabolic symbiosis produces the structural and sensory integrity of yoghurt. In plant systems such as soybean, oat, and coconut mixtures, microbial activity in such systems differs from dairy systems depending on differences in nutrient composition, buffering capacity, and substrate available (Mäkinen *et al*., 2016). Such factors necessitate the overall consideration of microbial activity in attempting to achieve optimum fermentation performance and probiotic viability.

They encounter low protein and high carbohydrate conditions in LAB in soy substrates and adjust their metabolic adaptation and growth pattern (Lavelle *et al*., 2025). *L. plantarum, L. casei,* and *S. thermophilus* have been successful in delivering satisfactory acidifying and proteolytic activities in soy milk, which are beneficial for successful fermentation and generation of bioactive peptides (Hati *et al*., 2018). Yoghurt-probiotic synergy is also complemented by the presence of oligosaccharides such as raffinose and stachyose (Zhu *et al*., 2021). The acid buffering capacity of soy protein can slow down acidification and be extended to attain optimal pH levels as compared to dairy substrates (Wang and Guo, 2021). Thus, such microorganisms with desired acid resistance and protease activity have to be employed for attaining desirable fermentation dynamics for soy-based blends.

For oat matrices, the availability of fermentable carbohydrates like β-glucans and maltose favors high bacterial growth and lactic acid production (Rasane *et al*., 2015). *Lactobacillus acidophilus* and *Lactobacillus fermentum* are the best under these conditions and produce exopolysaccharides (EPS), resulting in improved viscosity and texture (Amiri *et al*., 2019). These EPS subunits strengthen the gel network as well as act as yoghurts, which improve the stability of probiotics during storage. Low acidity during oat fermentation reduces microbial stress and allows more extended bacterial action and the formation of metabolites. Oat and soy co-fermentation enhances the performance of the microbes because the carbohydrate-rich oat supplement the protein-based soy substrate to form an excellent nutrient culture for growth and stabilization of lactic acid bacteria (Modgil *et al*., 2020).

Coconut milk presents a unique microbial challenge and opportunity. It's low-carbohydrate, but high-lipid content will inhibit bacterial growth and has its MCTs and micronutrients alongside to promote favorable niches to lipid-tolerant populations (Chetachukwu *et al*., 2019). Certain Lactobacillus species, like *L. rhamnosus* and *L. plantarum*, have been observed to grow well in lipid niches because of their capacity to use the glycerol and fatty acid terminal oxidation products as an alternative energy source (Pandiselvam *et al*., 2024). Coconut milk also contains antimicrobial fatty acids such as lauric acid, which was able to suppress pathogenic bacteria while being selectively probiotic (Sanches *et al*., 2022). Besides soy-oat blends, coconut lipid matrix stabilizes the bacterial cell membrane and hence maintains microbial viability under cold storage conditions as well as acidic stress conditions, in addition to fermentation (Hati *et al*., 2018).

Time of fermentation directly influences microbial succession as well as probiotic viability in such multi-base yoghurt systems. The initial phases of fermentation are dominated by quick acidifiers like *S. thermophilus* that decrease pH levels to enable the development of acid-resistant strains like

L. bulgaricus and *L. plantarum* (Nguyen *et al*., 2022). Secondary metabolites like diacetyl, acetoin, and volatile fatty acids accumulate in continuous fermentation, which play a role in flavour development and microbiological balance (Krastanov *et al*., 2023). But an overlong fermentation period will lead to over-acidification, which causes cell autolysis and deterioration of probiotic viability. Maximum fermentation time and pH value are required in order to achieve the viability of health microbes (Ray *et al*., 2025).

Fermentation storage is also a danger to probiotic viability and stability. Active concentrations of over 10^7 CFU/mL are required to achieve probiotic health benefits (Wendel, 2022). In plant-based yoghurts, such viability is sustained through synergistic substrate-substrate interactions, soy proteins provide surface adsorptive protection to bacterial cells, oat β-glucans protect as colloids, and coconut lipids form emulsified microenvironments that buffer acidic stress (Mäkinen *et al*., 2016). Supplementation with resistant starches and polysaccharides also enhances the cold storage survival of bacteria by preventing osmotic stress and water stability (Rasane *et al*., 2015).

Symbiotic microbial activity in soybean-oat-coconut yoghurt mixtures reiterates the necessity of starter culture adaptation to plant-based substrates. Blend fermentations with *S. thermophilus, L. bulgaricus*, and probiotic adjuncts like *L. acidophilus* or *Bifidobacterium animalis* have proven to be extremely promising with increased rates of acidification, textural uniformity, and probiotic viability (Mäkinen *et al*., 2016; Hati *et al*., 2018). Metabolic compatibility of such strains ensures consistent organic acid content, intensified antioxidant effect, and stable sensory qualities. Microbalance and optimization of fermentation conditions thus ensure plant-based yoghurt substitutes not only fulfill the nutritional needs but also ensure stable probiotic activity and shelf life.

2.9 Optimization in Fermented Food Production and Its Effect on Yoghurt Quality

Optimization of food fermentation is the process of establishing optimal levels of process parameters that will provide optimal product quality, functionality, and microbiological stability.

optimization in yoghurt, or vegetable yoghurt, or dairy yoghurt involves optimizing features like texture, acidity, taste balance, nutrient retention, and probiotic viability because fermentation is a bio-controlled process, minute variations in time, temperature, inoculum size, and substrate composition will lead to humongous variations in product titer. optimization processes, normally using Response Surface Methodology (RSM), Taguchi design, or Artificial Neural Network (ANN) models, enable one to rigorously screen more than one parameter and their interactions to establish optimum process parameters (Mulatu *et al*., 2021).

2.9.1 Temperature of fermentation

Temperature directly affects microbial metabolism and enzyme activity, thus regulating rate of acidification, texture, and flavour development of yoghurt.

Correct temperature provides efficient conversion of lactose or carbohydrate to lactic acid and prevents thermal shock to the starter culture. For milk systems, the range 40°C to 45°C is best, although this will differ with matrix and strain in plant yoghurts (Vedamuthu, 2013). For example, *Lactobacillus plantarum* and *Streptococcus thermophilus* are perfect at 37°C-42°C in soy-oat-coconut blends (Sukma *et al*., 2021; Moss *et al*., 2023). Acidification is slowed down by temperatures lower than the range and can trigger the creation of weak gel networks, while higher temperatures may trigger denaturation of plant proteins, weakening of viscosity, and increased syneresis. Hence, temperature optimization guarantees microbial balance and texture stability through the preservation of probiotic organism viability (Luo *et al*., 2025).

2.9.2 Fermentation time

Fermentation time controls acidification, gelation, and biochemical conversion in yoghurt texture.

Weak gels and flavour are typically built without acidity by low fermentation times, and over-acidification and whey separation are triggered by high fermentation. Maximum fermentation time varies for varying formulations: milk yoghurt takes 4-6 h while plant yoghurts take 6-8 h since they contain varying buffering capacity and available sugar content (Bernat *et al*., 2015; Tang *et al*., 2024). Fermentation time has been found to be optimized to the ultimate pH of 4.3-4.6 in optimization trials to provide efficient coagulation and excellent sensory quality (Tachie *et al*., 2024).

2.9.3 Inoculum concentration and composition

Inoculum concentration, or quantity of viable starter cells placed in the fermentation medium, affects product quality and final rate of acidification. Low levels of inoculum result in slow acidification, contamination, and prolonged lag phases, while extremely high concentrations of inoculum could result in high-rate acidification and overacidification of products (Patil and Benjakul, 2018). Optimal inoculum levels for yoghurt are 2% to 4% (v/v) and are strain- and substrate-dependent. In addition, Optimization of blend cultures such as *Lactobacillus delbrueckii subsp. Bulgaricus*, *Streptococcus thermophilus*, and selected probiotics such as *Lactobacillus acidophilus* enhance textural quality and functional attributes such as antioxidant functionality (Monteiro *et al*., 2022). optimization in this case means providing synergy among strains with acidification kinetics and exopolysaccharide control.

2.9.4 Formulation ratio and substrate composition

Substrate structure is one property that must be optimized in the case of plant yoghurt because it affects the availability of nutrients, protein gelation properties, and sensorics perception.

Soy brings structure-building proteins, oat brings β-glucans to form build-up viscosity, and coconut brings lipids to add creaminess. The ratio between these fractions must be optimized, though, in order to prevent off-flavour, sediment, or unwanted mouthfeel. For instance, the blend of 7.13 mL peanut, 10 mL oat, and 7.86 mL coconut milk was found to provide a smooth texture, low rate of syneresis, and achieve the desired viscosity with good sensory properties (Nehaa *et al*., 2022). optimization develops nutrient synergy, which enhances microbial metabolism and bioavailability of amino acids, vitamins, and minerals.

2.9.5 pH and titratable acidity

pH and titratable acidity control through monitoring and optimization is crucial in guaranteeing yoghurt quality. Such a value is balanced in terms of protein gelation and microbial acid development. A good, balanced pH value of 4.3-4.6 is usually fixed to provide proper gel firmness and flavour balance without over-acidification. Online analysis and feedback-regulated fermenters-controlled fermentations, online measurement, and pH-stat allow for precise optimization to guarantee reproducibility and stability of plant-based yoghurt products (Huppertz, 2017).

2.9.6 Total solids content

Total solids in the base of yoghurt, such as proteins, carbohydrates, and fats, impact viscosity, firmness, and water-holding. Optimization of total solids does not cause syneresis and improves mouthfeel (Makinen *et al*., 2016). In yoghurts made from plants, control of solids is done with hydrocolloids (pectin, carrageenan) or concentration processes such as evaporation and ultrafiltration. Excessively high solids induce graininess but excessively low solids induce watery texture. Optimization hence preserves product uniformity, especially at bulk scale formulation.

2.9.7 Conditions of homogenization and agitation

Before fermentation, the intensity of mechanical agitation and the pressure of homogenisation affect fat dispersion and the homogeneity of protein networks (Ngapo and Vachon, 2017). Optimized homogenization creates an emulsion that will not phase separate throughout fermentation and storage (Shah, 2018; Qamar *et al*., 2019). Over-homogenization destroys protein structure, reduces viscosity, and inhibits gelation. Thus, the balance of shear stress and efficiency in emulsification through optimization assures the desired textural and appearance quality.

2.9.8 Storage conditions after fermentation

Even under optimal fermentation, storage temperature and time influence the microbiological as well as physicochemical stability of yoghurt. Storage optimization is focused on lower temperatures in an attempt to curtail microbial metabolism but without loss of texture or flavour. Storage under non-optimal conditions resulted in post-acidification, whey separation, and loss of probiotic viability (Duong *et al*., 2019; Vieira, 2019). optimization therefore optimizes shelf life in addition to uniformity of quality on the distribution level.

2.9.9 Optimization tools and methods

High-performance optimization computer software has precise control of yoghurt manufacturing. Response Surface Methodology (RSM) allows multi-variable optimization, interaction amongst factors like temperature, inoculum, and solid content having been demonstrated (Mulatu *et al*., 2021). Artificial neural networks and machine learning algorithms are increasingly utilized to forecast the quality of yoghurt from enormous sets of fermentation conditions and sensory data (Zhang *et al*., 2023). Algorithmic models reduce trial-and-error experimentation and improve process replicability.

2.10. Sensory Acceptability and Consumer Acceptance of Plant-Based Yoghurts

Sensory acceptability is also extremely crucial in the market success and consumer acceptability of yoghurt, especially in developing plant-based alternatives that break away from conventional dairy sensory norms (Hati *et al*., 2018). Consumer perception of yoghurt quality primarily depends on sensory attributes including colour, aroma, taste, texture, and mouthfeel, which are correlated and to a significant degree created and fermented based (Dias *et al*., 2020). Lactic acid fermentation of conventional dairy yoghurt provides the characteristic sourness and creaminess that signify freshness and quality by consumers. But soybean, oat, and coconut yoghurts, which are plant yoghurts, must overcome obstacles of flavour, texture, and lightness of colour sensory masking barriers to compete or surpass the sensory appeal of dairy yoghurts (Mäkinen *et al*., 2016).

Sensory limitations in soy yoghurt are typically caused by the volatile chemicals such as aldehydes, ketones, and alcohols that account for the characteristic "beany" or grassy flavour (Krastanov *et al*., 2023). These are obtained through lipoxygenase-catalyzed oxidation of soybean polyunsaturated fatty acids (Vick *et al*., 2018). Its level can be reduced by appropriate heat treatment, soaking, or blending with other vegetable foods that dilute or mask unpleasant notes (Li *et al*., 2019). In addition, limited fermentation duration counteracts off-flavours because microbial metabolism converts undesired volatiles into harmless or acceptable aromatic compounds (Leonard *et al*., 2023). Oat yoghurt, on the other hand, contains a mild natural sweetness and flavour and smooth texture and therefore is a good candidate to enhance the sensory quality of soy foods (Rasane *et al*., 2015).

β-Glucan, apart from enhanced creaminess, adds a desired viscosity which the consumers have a preference for in high-textured yoghurt (Amiri *et al*., 2019). Oats' mild sweetness can balance out the acidity that develops when it ferments, resulting in a less-dominant and more-balanced taste

experience (Modgil *et al*., 2020). Additionally, oat contributes to the sophistication of the product as well by imparting a lighter color and creamier texture, both of which enhance consumer acceptability over the grainy and transparent nature of soy yoghurts by themselves. Coconut milk adds sensory elements to the blended yoghurt matrix as a result of its lipid nature, adding mouth-coating richness and flavour hold (Chetachukwu *et al*., 2019).

The tropical sweetness, flavour, and silken texture of coconut milk provide a rich sensory experience that is extremely comparable to dairy yoghurt (Sanches *et al*., 2022). Employment of coconut not only improves global acceptance but also contributes to the luxurious nature of goods, a significant driving force for plant-based foods to make inroads in the dairy alternative market (Pandiselvam *et al*., 2024). However, the dominance of coconut flavour must be balanced against the best proportion of blending and control of fermentation so that it will not overpower oat and soy's delicate tastes. Fermentation time has a significant influence on sensory properties in plant-based yoghurt blends.

Breakdown of sugars into lactic acid during fermentation influences pH and taste, whereas protein denaturation and polysaccharide-polysaccharide interactions influence texture and mouthfeel (Nguyen *et al*., 2022). Insufficient fermentation time produces an underdeveloped flavour and a weak gel structure, whereas prolonged fermentation results in excessive acidity and syneresis, both of which reduce consumer acceptability (Rahman *et al*., 2024). Optimization of the fermentation time facilitates production of acceptable appealing acidity, stable viscosity, and aromatic richness contributing to increased consumer rating sensory scores (Ray *et al*., 2025). The interaction between the fermentation time and sensory quality is a call for precise process control during production of acceptable plant-based yoghurt products. Consumer tests have indicated that multi-base plant yoghurts consisting of soy, oat, and coconut would have greater acceptability scores

compared to their single-source equivalents due to their improved flavour harmony and texture balance (Li *et al*., 2019; Mäkinen *et al*., 2016).

The coconut creaminess, oat sweetness, and soy protein richness yield a combination sensory profile that would be appropriate for various consumer taste preferences. Besides, sensory panels are found to capture that such blends show higher flavour complexity and mouthfeel stability upon refrigerated storage, an important parameter for commercial viability (Hati *et al*., 2018). As plant-based yoghurt is developing more and more in the functional food industry, consumers' demand of taste, texture, and nutrition perception is more relevant than ever before. The sensory optimization of a product such as this under fermentation control, ingredient interaction, and texture optimization is the most important key to their long-term viability and prolonged acceptance in the dairy alternative industry (Rasane *et al*., 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Sources of Materials

Matured coconut, soybean, rolled oats, and full cream powdered milk was purchased from local markets in Benin City and Akure. Starter culture and stabilizers were purchased from specialized suppliers to ensure consistent and reliable fermentation results.

3.2. Experimental Design

Custom blend ratios of soy, coconut, and oat milk were formulated using an optimal simplex lattice design (Design Expert 13, Stat-Ease Inc., Minneapolis, USA, trial version), as described by Baskar *et al*. (2022). The variables considered were soy milk (25 - 50%), which is the base constituent, coconut milk (25 - 50%), to improve the middle-chain fatty acids, as well as oat milk (25 - 50%) to improve the beta-glucan in the resultant yoghurts. Fermentation time was used as a discrete factor, with values of 6,8 and 12 hours. Sixteen (16) experimental runs were generated, with two centre points to estimate experimental error and assess model curvature and three lack of fit to evaluate the adequacy of the fitted response surface model. The responses are sensory parameters (appearance, flavour, taste, aroma, consistency, and overall acceptability). The resultant mixture blends are presented in Table 3.1

3.2.1. Study Area

The research was conducted at the Department of Biological Sciences, Benson Idahosa University, Nigeria and Faculty of Agriculture, Federal University of Technology, Akure.

3.3. Sample Preparation

Soy milk preparation: Soybeans were washed thoroughly and soaked in water for 8 hours. The soaked beans were then boiled for 5 minutes for blanching. After blanching, the soybeans were milled using a Sokany Multi-Purpose Blender 6000W, 2.5 L, SK-99. The resulting slurry was filtered through a muslin cloth with an approximate pore size of 100 µm using a water-to-slurry ratio of 7:1. The filtrate was subsequently boiled for 20 minutes to obtain soy milk (Hati *et al.*, 2018). All equipment, including the blender, was washed and sanitized with 70% ethanol prior to use to minimize microbial contamination.

Oat milk preparation: Rolled oats were soaked in water for 30 minutes and then wet-milled in the same sanitized Sokany blender to form a slurry. The slurry was filtered through muslin cloth (100 µm pore size) to obtain oat milk (Le *et al*., 2025).

Coconut milk preparation: Mature coconuts were cracked open, and the nuts were removed, washed, and de-skinned. The coconut nuts were diced and crushed using the sanitized Sokany blender 6000W, 2.5 L, SK-99. The resulting slurry was filtered through a muslin cloth with an approximate pore size of 100 µm to extract coconut milk. The extracted milk was then boiled for 10 minutes to ensure microbial safety (Sanful, 2009).

Dairy yoghurt preparation: Full-cream powdered milk was reconstituted using warm water at a ratio of 3:1 (water to milk) until fully dissolved. The reconstituted milk was pasteurized at 85 °C for 30 minutes to improve protein denaturation and ensure microbial safety, after which it was cooled to the incubation temperature of 42 °C. The pasteurized milk was then inoculated with 0.4% of a store-bought dried yoghurt starter culture, containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, as indicated by the manufacturer. Fermentation was

carried out at 42 °C for 8 hours until coagulation was achieved. The starter culture was used as supplied, and no independent assay was performed to confirm the microbial composition. This method followed standard dairy yoghurt fermentation procedures (Dan *et al.*, 2023).

The three milk extracts (soymilk, oat milk and coconut milk) were mixed in different proportions as predicted by Response Surface Methods (RSM) in Table 3.1. It was cooled to 40 ^{o}C before inoculation with the commercial starter culture. It was incubated for 6, 8 and 12 hours. The fermented yoghurt was kept at 4 ^{o}C to terminate fermentation and for further analysis. The production procedure is shown in Figure 3.1 and the produced milk are shown in Plate 3.1

3.3.1. Preliminary Sensory Evaluation

All sixteen (16) formulations were produced and subjected to sensory evaluation to assess attributes including appearance, flavour, taste, aroma, consistency, and overall acceptability. The sensory scores obtained served as response variables for model development and optimization. Sensory evaluation at this stage allowed for rapid screening of the 16 blends to identify the most promising samples for further detailed analyses, reducing time and resource use.

The evaluation was conducted at the Department of Biological Sciences, Benson Idahosa University, Nigeria, using a panel of 30 untrained participants. The panellists assessed the yoghurt samples using a structured sensory questionnaire based on a 9-point hedonic scale, adapted from Ihekoronye and Ngoddy (1985). Each attribute was rated individually, and the percentage overall acceptability was calculated as the mean of all attribute scores.

3.3.2. Response Surface Optimization (RSM) and Model Validation

The sensory data from the initial sixteen (16) runs were analysed and optimized using Response Surface Methodology (RSM) in Design-Expert® 13 to investigate the effects of mixture proportions and fermentation time on all measured sensory attributes.

The design expert software generated six possible solutions from three (3) combinations of the categoric factor level (soy-coconut-oat). The best 3 combination from the solution were selected joined by the best sample from the experimental run (i.e. the sample with the highest overall acceptability) as well as two (2) other control samples (commercially available store-bought yoghurt and yoghurt produced from reconstituted full cream powdered milk). Consequently, the six (6) samples were analysed in the final stage (Table 3.2)

3.3.3. Selection of Optimized Yoghurt Blends

Each selected blend was assigned a unique sample code for identification. For example, S29_CO indicates the sample containing 29% soy milk; S25_CO indicates a sample containing 25% soy milk; S27_CO indicates a sample containing 27% soy milk; S30_CO indicates a sample containing 30% soy milk along with there respective proportions of coconut and oat milk.

The code (DY) represents reconstituted powered milk yoghurt and (CY) represents store bought dairy yoghurt.

The four selected plant-based blends, along with the store-bought yoghurt (CY) and reconstituted powered milk yoghurt were then subjected to comprehensive evaluation, including: Sensory analysis, Proximate composition, Antioxidant activity, Mineral content, Microbial quality.

Table 3.1. Optimal Mixture Experimental design for Milk blend selection

Run	A: Soy Milk	B: Coconut Milk	C: Oat Milk	Fermentation Time (h)
1	50	25	25	6
2	50	25	25	12
3	25	50	25	6
4	25	50	25	12
5	25	25	50	6
6	25	25	50	12
7	25	37.455	37.545	6
8	25	37.614	37.386	12
9	37.586	25	37.414	6
10	37.483	25	37.517	12
11	37.426	37.435	25.139	12
12	37.426	37.435	25.139	8
13	37.463	37.375	25.163	6
14	27.523	42.440	30.037	8
15	29.692	27.910	42.398	8
16	42.264	29.736	28.000	8

Note: This table summarizes the sixteen (16) experimental runs generated using a simplex lattice mixture design with the software Design-Expert® 13 (Stat-Ease Inc., Minneapolis, USA), following Baskar et al., (2022), showing the proportions of soy, coconut, and oat milk and the applied fermentation time

Table 3.2: Selected Yoghurt Samples for Comprehensive Evaluation

Sample Code	Soy Milk (%)	Coconut Milk (%)	Oat Milk (%)	Fermentation Time (h)	Description
S29_CO	29.147	36.340	34.512	8	Selected optimized formulation
S25_CO	25.000	38.213	36.787	8	Selected optimized formulation
S27_CO	26.775	36.545	36.680	12	Selected optimized formulation
S30_CO	29.6919	27.9098	42.3983	8	High-performing original run
CY	–	–	–	–	Commercially available yoghurt (control)
DY	–	–	–	8	Reconstituted powdered milk yoghurt (control)

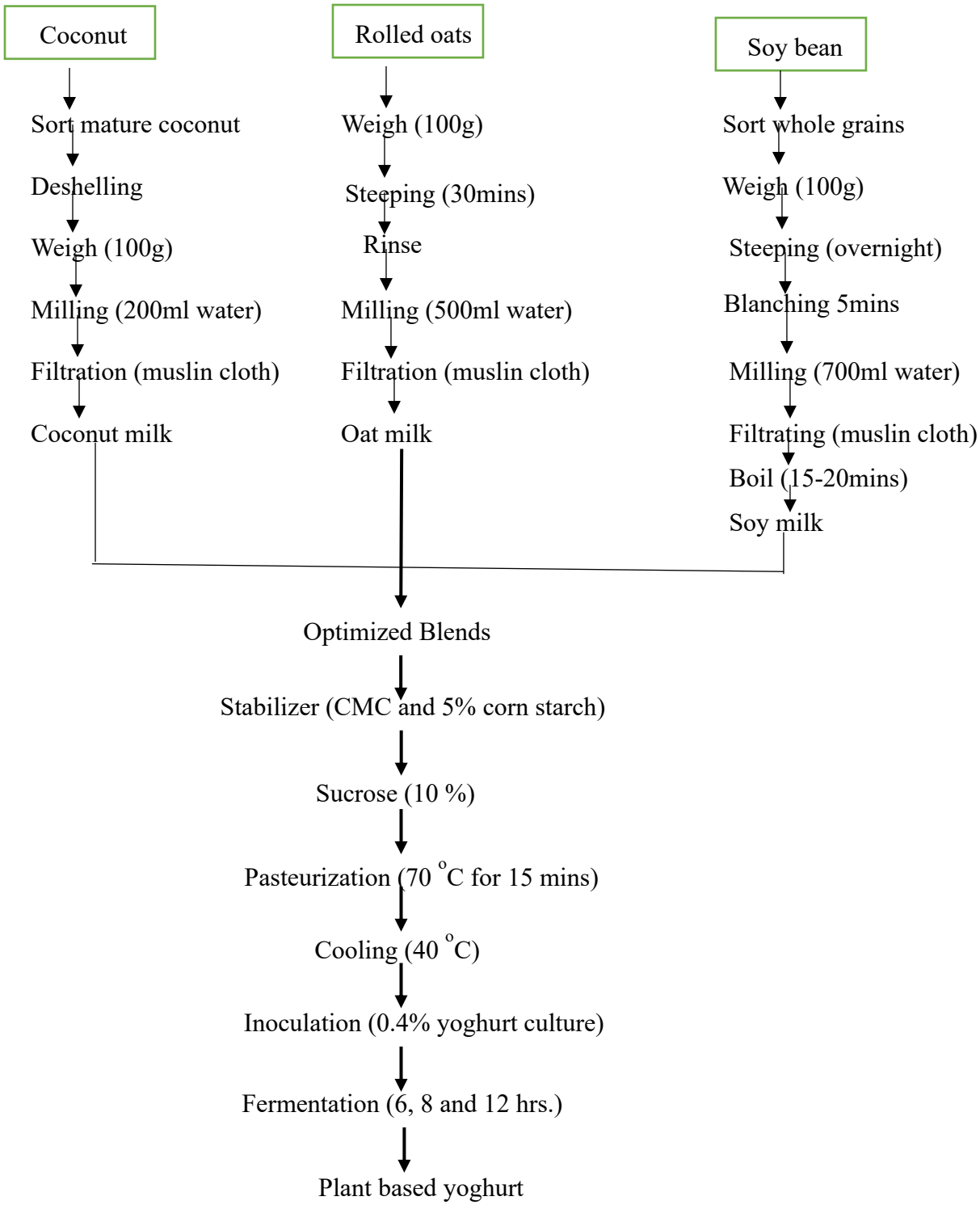


Figure 3.1: Production of Plant-based Yoghurt



Plate 3.1: Optimized Yoghurt Produced.

3.4. Analysis

3.4.1. Proximate

The proximate composition of the yoghurt-based samples was determined using AOAC (2012) methods. The moisture, ash, crude fibre, crude protein, and crude fat was determined, and the carbohydrate was calculated by difference.

3.4.1.1. Determination of moisture content

All samples were weighed in an aluminum dish and allowed to dry in a hot air oven maintained at 110 ^{o}C for 4 hours until constant weight was achieved. It was cooled in a desiccator to room temperature. The difference in percentage weight was reported as moisture according to AOAC (2012).

$$\text{Moisture } (\%) = \frac{W2 - W3}{W1} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ 1$$

Where;

W_1 = Weight of Samples

W_2 = Initial weight of sample + Aluminum dish before drying

W_3 = Final weight of sample + Aluminum dish after drying

3.4.1.2. Determination of fat content

The crude fat content was subjected to the method proposed by AOAC (2012) using a Soxhlet apparatus. 5.0 g of the flour sample was weighed into a thimble and fixed into the Soxhlet extractor, with n-hexane used as the solvent. The hexane was poured into a round-bottom flask and placed on the heating mantle. The extraction was conducted continuously for about 3½ hours, after which the flask was cooled and disconnected. The thimble with the sample was removed and dried to a constant weight in an air oven at 50°C. The difference between the weight of the thimble before

and after extraction was recorded to obtain the crude fat extracted. The percentage of crude fat content was calculated and expressed on a dry weight basis.

$$\%\ crude\ fat\ = \frac{W2-W3}{W1} \times 100 \ldots\ldots\ldots\ldots .2$$

Where:

W_1 = weight of sample;

W_2 = weight of sample + filter paper;

W_3 = weight of sample + filter paper (constant weight after drying)

3.4.1.3. Determination of ash

The crucibles to be used was cleaned and dried in the air oven, and allowed to cool in a desiccator. The crucibles were weighed, and 1g of the sample was weighed into each crucible. The crucibles and their contents were then transferred into a muffle furnace set at 550 °C. The ashing continued until no black speck or white-grey ash was obtained. The crucibles were taken out and immediately covered, and they were placed in a desiccator to cool and later weighed.

Percentage of total ash;

$$\boldsymbol{Ash\ content}\ (\%) =$$

$$\frac{(\boldsymbol{weight\ of\ crucible} + \boldsymbol{weight\ of\ ashed\ sample}) - (\boldsymbol{weight\ of\ crucible})}{(\boldsymbol{weight\ of\ sample} + \boldsymbol{crucible}) - (\boldsymbol{weight\ of\ crucible})} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ 3$$

3.4.1.4. Determination of crude fibre

The crude fibre content of the samples was determined as described by AOAC (2012). 5g of each of the samples was weighed (W_1) and transferred to a fat extraction apparatus for extraction with light petroleum. The sample was transferred to a dry 500 mL conical flask, and about 200 mL of boiling 1.25% sulfuric acid was added and brought to a boil within 1 minute. The content was

allowed to boil gently for 30 minutes, filtered through a muslin cloth, rinsed well with distilled water, and scraped back into the flask using a spatula. A similar procedure was followed using 1.25% sodium hydroxide, after which it was rinsed once with 10% HCl, four times with distilled water, and finally with ethanol. The treated sample was sewaged into a pre-weighed and drained silica dish, oven-dried for 12 hours at 105 °C, cooled, and weighed (W_2). The samples were finally ashed at about 550 °C for 2 hours, cooled in a desiccator, and reweighed (W_3).

$$Crude\ fibre\ (\%) = \frac{W3-W2}{W1} \times 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots 4$$

Where:

W_1 = Initial weight of sample;

W_2 = weight of sample + crucible before ashing;

W_3 = weight of sample + crucible after ashing (constant weight after drying)

3.4.1.5. Determination of crude protein

Content digestion stage:

This was determined by the Kjeldahl method. Each flour sample (1.0 g) was weighed into a Kjeldahl flask, and 3.0 g of hydrated cupric sulphate (catalyst), 20 mL of sodium sulphate solution (Na_2SO_4) and 1.0 mL of concentrated sulphuric acid (H_2SO_4) was added to the sample in the flask. The flask was clamped and heated inside a fume cupboard until the solution became colourless.

Distillation stage:

The clear solution was cooled, diluted with distilled water, and made up to 100mL. 10mL of the resulting solution was mixed with 5 mL of 40 % sodium hydroxide solution in a distillation flask and distilled to release ammonia.

Titration stage:

The resulting solution above was titrated with 0.1 mL of hydrochloric acid (HCl). The titre value or endpoint at which the colour changed from green to pink was noted, and the crude protein was calculated using the expression.

$$\text{Nitrogen}\ (\%) = a\% \times 0.014 \times t\left[\frac{st}{v} \times \frac{100}{1}\right] \ldots\ldots\ldots\ldots\ldots 5$$

Where; a =molarity of HCl used for titration;

0.014 = mole equivalent of nitrogen

t = titre value

st = capacity of standard volumetric flask used

v = volume of pipette used:

100 = conversion to percentage

The percentage Nitrogen (%N) is multiplied by a factor of 6.25 to obtain the crude protein as follows;

$$\%\ Nitrogen\ content\ =$$

$$\frac{(sample\ titre - Blank\ titre)\ X\ Normality\ of\ HCl\ X\ 14\ X\ vol.made\ of\ digest\ X\ 100}{\text{Aliquot of the digest taken X Weight of sample X 1000}} \ldots\ldots\ldots\ldots\ldots..6$$

$$\%\ Protien\ content = \%Nitrogen\ \times 4.25 \ldots\ldots\ldots\ldots\ldots 7$$

3.4.1.6. Determination of carbohydrate content

The carbohydrate content was determined by difference. The percentage total carbohydrate was estimated to be equal to the sum of percentage moisture, protein, ash and fibre subtracted from 100g.

$$\%\,Carbohydrate = 100 - (\%fat + \%fibre + \%protein + \%ash + \%moisture) \ldots\ldots 8$$

3.4.2. Determination of Minerals

The mineral content of the yoghurt samples was determined using the method by Akinyede and Ajewole (2026). Ash samples (0.5 g) were digested using 20mL of nitric acid (HNO_3) and sulfuric acid (H_2SO_4)**.** The mixture was heated until the appearance of white fumes, indicating complete digestion. The resulting clear solution was cooled and diluted with distilled water to a final volume of 50mL, after which it was filtered using Whatman filter paper.

The concentrations of sodium (Na) calcium (Ca), potassium (K), zinc (Zn), magnesium (Mg), iron (Fe), and phosphorus (P)were determined using atomic absorption spectrophotometry (AAS)**.** Both sodium and potassium were determined using flame photometer. Standard working solutions of each element were prepared to generate calibration curves, which were used to quantify the mineral concentrations in the samples. Results were expressed as mg/100g of sample.

3.4.3. Determination of pH and total titratable acidity

Ten grams (10 g) of the yoghurt-based yoghurt sample was mixed with 90 mL of sterile distilled water. The pH of the resulting suspension was measured using a pH meter equipped with a glass electrode. The pH meter was calibrated with buffer solutions of pH 7.0 and 4.0. The total titratable acidity (TTA) was determined by titrating 20mL of the same sample against 0.1 M sodium

hydroxide (Fayemi and Ojokoh, 2012). The process was repeated for all the samples, and the values were recorded. Triplicate determinations were made in all cases.

3.4.4. Determination of phytochemicals

3.4.4.1. Determination of oxalate

Oxalate content was determined according to the method described by Kasimala *et al.* (2018). One gram (1 g) of the sample was soaked in 75mL of 1.5 N sulfuric acid (H_2SO_4) for 1 hour and then filtered using Whatman No.1 filter paper. An aliquot of 25 mL of the filtrate was transferred into a conical flask and titrated while hot (80–90°C) against 0.1 M potassium permanganate **(**$KMnO_4$) until a faint pink colour persisted for 15 seconds.

The oxalate content was calculated using the expression:

$$\text{Oxalate}\left(\frac{\text{mg}}{100\text{ g}}\right) = \frac{\text{V} \times \text{M} \times 45 \times \text{DF}}{W} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots 9$$

Where:

- V = volume (mL) of $KMnO_4$ used
- M = molarity of $KMnO_4$ (0.1 M)
- 45 = molar mass equivalent of oxalate (mg/meq)
- DF = dilution factor
- W = weight of sample (g)

3.4.4.2. Determination of phytate

Phytate content was determined according to the method described by Wheeler and Ferrel (1971). Four grams (4 g) of the sample were soaked in 100 mL of 2% hydrochloric acid (HCl) for 3 hours and subsequently filtered using Whatman No. 1 filter paper. An aliquot of 25 mL of the filtrate was transferred into a conical flask, after which 5 mL of 0.3% ammonium thiocyanate solution was added as an indicator.

Distilled water (53.5 mL) was then added to adjust the acidity of the solution, and the mixture was titrated against a standard iron (III) chloride ($FeCl_3$) solution (0.00566 g/mL $FeCl_3$, equivalent to 0.00195 g Fe/mL) until a brownish-yellow colour persisted for 5 minutes, indicating the endpoint.

Phytate content was calculated using the expression:

$$\text{Phytate } \left(\frac{\text{mg}}{100\text{ g}}\right) = \frac{V \times 0.00195 \times 1.19}{W} \times 100 \ldots\ldots\ldots\ldots 10$$

Where:

- V = volume (mL) of $FeCl_3$ solution used
- 0.00195 = grams of iron per mL of $FeCl_3$
- 1.19 = conversion factor of iron to phytic acid
- W = weight of sample (g)

3.4.4.3. Tannin determination

Tannin content was determined using the Folin–Ciocalteu colorimetric method. About 0.2 g of finely ground sample was weighed into a 50 mL sample bottle, and 10 mL of 70% aqueous acetone was added. The bottle was tightly covered and placed on an ice-bath shaker, where it was shaken for 2 hours at 30 °C to facilitate extraction. The mixture was then centrifuged, and the supernatant was collected and stored on ice until analysis. An aliquot of 0.2 mL of the extract was pipetted into a test tube, and 0.8 mL of distilled water was added.

Standard tannic acid solutions were prepared from a 0.5 mg/mL stock solution and made up to 1 mL with distilled water. To both sample and standard solutions, 0.5 mL of Folin–Ciocalteu reagent was added, followed by 2.5 mL of 20% sodium carbonate (Na_2CO_3) solution. The mixtures were vortexed and allowed to incubate for 40 minutes at room temperature for colour development.

Absorbance was measured at 725 nm using a spectrophotometer against a reagent blank. Tannin concentration was quantified using the tannic acid calibration curve.

Tannin content was calculated using the expression:

$$Tannin\left(\frac{mg\ TAE}{100\ g}\right) = \frac{C \times V \times 100}{W} \ldots\ldots\ldots\ldots\ldots\ldots 11$$

Where:

- C = concentration obtained from the standard curve (mg/mL)
- V = total volume of extract (mL)
- W = weight of sample used (g)
- TAE = Tannic Acid Equivalent

3.4.4.4. Determination of trypsin inhibitor

Trypsin inhibitor was extracted by mixing 1g of the sample with 50ml of 0.01N NaOH at pH of between 8.4-10.00 and allowing the mixture to stand for 3h, while stirring at intervals. 2ml of diluted extract was then dispensed into test tubes to which 2ml of cold trypsin solution (4mg in 200ml of 0.001M HCl) was added, and the tubes were placed in water bath at 370Cfor 15min., 5ml of Benzoyl-DL-arginine-P-nitro anilide hydrochloride (BAPNA) (40mg was dissolved in 1ml of dimethyl sulfoxide and diluted to 100ml with tris buffer 0.05M, pH 8 and diluted to 100ml with tris buffer 0.05M, pH 8.2, pre warmed to 370C) was added as substrate to each tubes. After 10 min the reaction was terminated by adding 30% acetic acid and the content of each tube were thoroughly mixed. Thereafter the content of each tube was centrifuged 3000rpm and the absorbance of the filtrate was measured at 410nm against reagent blank. The reference(std.) was prepared in the same as the sample except that 2ml of that 2ml of distilled water was added in place of extract Smith *et al.* (1980)

3.4.4.5. Saponin determination

The saponin content was quantified via a gravimetric analysis based on the technique adopted by Wirman, *et al*. (2024). The method began by soaking 10 g of the powdered Solanum material in 220 mL of 20% ethanol. This mixture underwent magnetic stirring for 12 hours at 55^{O}C to facilitate extraction. After filtering the solution through Whatman No 1 paper, the resulting extract volume was reduced to 40 mL using a vacuum evaporator. The liquid was then transferred to a separating funnel, where 20 mL of diethyl ether was added and shaken; the ether layer was subsequently discarded. The pH of the remaining aqueous phase was adjusted to 4.5with NaOH. Final extraction was achieved by adding 60 mL of butanol. The *n*-butanol fraction was washed twice with 10 mL

portions of 5% NaCl before being evaporated to complete dryness in a fume hood. The residual crude saponin was then collected and weighed.

3.4.5. Vitamin determination

3.4.5.1. Determination of vitamin C

Vitamin C (ascorbic acid) content was determined using a spectrophotometric method based on the reaction of ascorbic acid with 2,4-dinitrophenylhydrazine (DNPH), as described by Benderitter *et al.* (1998). Ascorbic acid was used as the reference standard. An aliquot of 200µL of the sample extract was pipetted and mixed with 300 µL of 13.3% trichloroacetic acid (TCA) and 75µL of 2,4-dinitrophenylhydrazine (DNPH) reagent. The mixture was incubated at 37 °C for 3 hours to allow color development. Thereafter, 500µL of 65% sulfuric acid (H_2SO_4) was added to form a stable-colored complex, and the absorbance was measured at 520 nm using a Healicom 721S (China) spectrophotometer**.**

Vitamin C concentration was calculated using the equation:

$$\text{Conc. (mg/g)} = \frac{Abs.Of\ sample}{Slope\ of\ the\ standard} \ldots\ldots\ldots\ldots..12$$

3.4.5.2. Determination Vitamin A

A weighed portion of the sample, containing no more than 1g of fat and at least 240 IU of vitamin A, was placed in a round-bottom flask. 30mL of absolute ethanol and 3mL of 5% potassium hydroxide (KOH) was added. The mixture was then boiled gently under reflux for 30 minutes in a stream of oxygen-free nitrogen using a reflux condenser and heating mantle to ensure controlled heating and prevent oxidation of vitamin A during saponification.

After saponification, the mixture was cooled rapidly to room temperature, and 30mL of distilled water was added. The mixture was transferred to a separatory funnel and washed with 3 × 50 mL of diethyl ether. Vitamin A was extracted by shaking for 1 minute during each wash. After complete phase separation, the lower aqueous layer was discarded, and the combined ether extracts were washed with 4 × 50 mL of distilled water, mixing cautiously during the first two washes to avoid emulsion formation. The washed ether solution was then evaporated under reduced pressure to approximately 5 mL, and any residual ether was removed under a gentle stream of nitrogen at room temperature. The resulting residue was dissolved in a sufficient volume of isopropyl alcohol to prepare a final solution (Smith *et al*.,1976)

3.4.5.3. Determination of vitamin D

Vitamin D content was determined using the Carr-Price colorimetric method. The Carr-Price reagent was freshly prepared by dissolving 20% (m/v) antimony trichloride ($SbCl_3$) in chloroform and adding 40% pure acetyl chloride, ensuring the solution was completely alcohol-free. For each assay, 1 mL of chloroform-extracted sample was transferred to a clean test tube, and 9 mL of freshly prepared Carr-Price reagent was added. The mixture was gently stirred to allow immediate development of the blue chromophore. The absorbance (extinction) of the sample solution was measured at 500 nm using a Healicom 721S (China) UV-Visible spectrophotometer against a reagent blank prepared with 1 mL chloroform and 9 mL reagent. The vitamin D concentration was determined by comparing the corrected absorbance to a standard curve prepared from known concentrations of vitamin D

$$Vitamin\ D\ concentration\ \left(\frac{\mu g}{mL}\right) = \frac{Acorrected}{Slope\ of\ standard\ curve} \ldots\ldots\ldots 13$$

Where: $Acorrected = Asample - Ablank$

Asample = absorbance of the sample

Ablank = absorbance of the reagent blank.

3.4.5.4. Determination of vitamin K

Vitamin K content was determined using the ethanol extraction and potassium ferrocyanide colorimetric method. About 0.2 g sample was accurately weighed and transferred into a clean glass vial. 5 mL of ethanol was added to the sample, and the mixture was thoroughly mixed using a vortex mixer for 2–3 minutes to ensure complete extraction of vitamin K. 50 µL of 1% potassium ferrocyanide solution was then added directly to the ethanol extract and mixed gently but thoroughly. The vial was placed in a dark environment, and allowed to incubate for 45 minutes at room temperature to enable complete color development, preventing photodegradation of vitamin K. After incubation, the solution was transferred to a clean cuvette (1cm path length), and the absorbance was measured at 680 nm using a Healicom 721S (China) UV-Visible spectrophotometer. A reagent blank, prepared with 1mL ethanol and 9mL Carr-Price reagent, was measured in parallel. Vitamin K concentration was determined by comparing the corrected absorbance, calculated as

$$Vitamin\ K\ concentration\ \left(\frac{\mu g}{g}\right) = \frac{Acorrected}{Slope\ of\ standard\ curve} \times \frac{Total\ extract\ volume}{Sample\ weight} \ldots\ldots\ldots 14$$

3.4.5.5. Determination of Vitamin E

Vitamin E content of the samples was determined using a classical spectrophotometric method involving saponification, extraction, purification, derivatization, and measurement. About 1.0 g portion of the sample was weighed into a 100 mL round-bottom flask, to which 10 mL of absolute alcohol and 20mL of 1 M alcoholic sulfuric acid were added. The mixture was refluxed for 45 minutes using a reflux condenser to hydrolyze lipids and liberate Vitamin E. After cooling, the saponified mixture was diluted with 50mL + 50mL of water and transferred to a low-actinic separating funnel to prevent photodegradation. The unsaponifiable matter, including Vitamin E, was extracted using five successive 30mL portions of diethyl ether. The combined ether extracts were washed thoroughly to remove residual acid and dried over anhydrous sodium sulfate to remove traces of water. The dried extract was evaporated under low temperature and protected from light, and the residue was dissolved in 10 mL absolute alcohol. Both sample and standard solutions were transferred to 20 mL calibrated volumetric flasks for precise volume measurements. For derivatization, 5mL absolute alcohol and 1 mL concentrated nitric acid were added to each flask. The mixtures were heated at 90°C for 3 minutes in a water bath to produce the Vitamin E-nitric acid colored complex. The solutions were cooled under running water and diluted to 20 mL with absolute alcohol. Absorbance was measured at 470 nm using a Healicom 721S (China) UV-Visible spectrophotometer, with a blank containing only absolute alcohol. Vitamin E concentration was calculated using the corrected absorbance of the sample and the slope of a standard calibration curve prepared from known concentrations of Vitamin E (Pearson 1976)

The corrected absorbance was calculated as:

$$Vitamin\ E\ concentration\ (mg/100g)$$

$$= \frac{Acorrecte}{Slope\ of\ standard\ curve} \times \frac{Total\ extract\ volume}{Sample\ weight} \times 100 \ldots\ldots\ldots\ldots 15$$

3.4.6. Antioxidant compounds determination

3.4.6.1. Determination of Total phenol

Total phenol content was determined using the Folin-Ciocalteu colorimetric method (Singleton *et al*., 1999). 0.2 mL of the sample extract was transferred into a test tube, followed by the addition of 1 mL of Folin-Ciocalteu reagent. The mixture was allowed to react for 3–5 minutes, after which 2 mL of 20% sodium carbonate (Na_2CO_3) solution was added to the reaction mixture. The volume was adjusted with distilled water to ensure proper mixing, and the mixture was incubated at room temperature for 30 minutes to allow complete color development. The absorbance of the developed blue color was measured at 765 nm using a Healicom 721S (China) UV-Visible spectrophotometer against a reagent blank containing all reagents except the sample extract. The total phenol content of the sample was quantified by comparison with a standard calibration curve prepared using gallic acid and expressed as mg gallic acid equivalents per gram of sample (mg GAE/g).

Corrected absorbance was calculated as:

$$Acorrected = Asample - Ablank$$

The total phenol content was calculated using the formula:

$$Total\ phenol\ (mg\ GAE/g)$$

$$= \frac{Acorrected}{Slope\ of\ standard\ curve} \times \frac{Total\ reaction\ volume}{Sample\ volume}$$

$$\times \frac{1}{Sample\ weight} \ldots\ldots\ldots\ldots\ 16$$

3.4.6.2. Determination of Total Flavonoid

Flavonoid content was determined using the colorimetric method described by Bao (2005). 0.2mL of the sample extract was transferred into a test tube, and 0.3mL of 5% sodium nitrite ($NaNO_2$) was added at time zero. The mixture was allowed to react for 5 minutes, after which 0.6 mL of 10% aluminum chloride ($AlCl_3$) was added. Following a further 6-minute interval, 2 mL of 1 M sodium hydroxide (NaOH) was added, and the total reaction volume was adjusted with 2.1 mL of distilled water. The reaction mixture was thoroughly mixed, and the absorbance was measured at 510 nm using a Healicom 721S (China) UV-Visible spectrophotometer against a reagent blank containing all reagents except the sample extract. Flavonoid content was calculated by extrapolation from a standard calibration curve prepared using rutin.

The corrected absorbance was calculated as:

$$Acorrected = Asample - Ablank$$

The flavonoid content of the sample was expressed as mg rutin equivalents per gram of sample (mg RE/g) and calculated using the formula:

$$Flavonoid\ content\ (mg\ GAE/g)$$

$$= \frac{Acorrected}{Slpe\ of\ standard\ curve} \times \frac{Total\ reaction\ volume}{Sample\ volume}$$

$$\times \frac{1}{Sample\ weight} \ldots\ldots\ldots\ldots\ldots\ldots 17$$

3.4.7. Determination of antioxidant properties

3.4.7.1. Extraction of samples

About 5ml of each of the samples was added into a 250mL flask containing distilled water. The mixture was sieved and filtered to obtain a clear solution. The solutions extracted were used for the in-vitro analyses after freeze-drying. The in vitro antioxidant assays of the aqueous extracts of the yoghurt samples were carried out using standard methods.

3.4.7.2. Determination of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability

The DPPH free radical scavenging ability of the yoghurt was determined as previously reported (Agunbiade *et al*., 2022). Briefly, 1 mL of the extract was mixed with 1 mL of the 0.4 mM methanolic solution of the DPPH. The mixture was left in the dark for 30 min before measuring the absorbance at 517 nm with Healicom 721S (China) spectrophotometer. The control consisted of methanol instead of the sample and the radical scavenging ability of the sample was calculated as:

$$\%\ DPPH = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

3.4.7.3. Determination of 2, 2'-azino-bis (3-ethylbenthiazoline-6-sulphonic acid) (ABTS) scavenging ability

The ability of the samples' extract to scavenge ABTS was evaluated using a previously described method (Aderinola *et al*., 2022). ABTS was generated by mixing a 7mM ABTS aqueous solution

with 2.45 mM potassium persulfate and allowing it to stand in darkness for more than 15 h. Before usage, the absorbance was adjusted to 0.7 nm with ethanol. Subsequently, 200µL of the ABTS solution was combined with 20 µL of the extract, and the absorbance was measured at 734 nm using a Healicom 721S (China) spectrophotometer. The percentage of ABTS radical scavenging ability of the extract was determined using the following formula:

$$\% \, ABTS = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

3.4.7.4. Determination of ferric reducing antioxidant power (FRAP)

The extract's reducing capability was evaluated following the method of Aderinola (2018). In short, 0.25 mL of the extract was combined with 0.25 mL of 200 mM sodium phosphate buffer (pH 6.6) and 0.25 mL of 1% potassium ferricyanide. This mixture was then incubated at 50°C for 20 min. Following this, 0.25 mL of 10% trichloroacetic acid was added and centrifuged at 2000 rpm for 10 min. Subsequently, 1 mL of the supernatant was mixed with 1 mL of distilled water and 0.1% $FeCl_3$, and the absorbance was measured at 700 nm using a Healicom 721S (China) spectrophotometer. Ascorbic acid (0.01 mg/mL) served as the standard.

3.4.8. Microbial analysis

Analysis of lactic acid bacteria and of feacal coliform of the samples were carried out. Lactic acid bacteria (LAB) were enumerated using Mann-Rogosa-Sharpe (MRS) agar according to the method described by Alabi *et al.,* (2025). For media preparation, 6.25 g of MRS agar was dissolved in 100mL of distilled water and thoroughly mixed to ensure complete dissolution. The prepared medium was sterilized in an autoclave at 121°C for 15 minutes under 0.15 MPa pressure. The autoclave pressure was monitored using the built-in pressure gauge and digital display, which continuously indicated the internal chamber pressure throughout the sterilization cycle. After cooling, sample aliquots were mixed with the molten agar and poured into sterile Petri dishes using

the pour plate method. Plates were allowed to solidify and then incubated at 37°C for 48 hours under microaerophilic conditions.

Fecal coliforms were enumerated using MacConkey Agar (MCA). Serial dilutions of 10^{-3} and 10^{-6} were prepared in sterile distilled water. Aliquots (0.1 mL) from each dilution were spread evenly on the surface of solidified MCA plates using a sterile glass rod and incubated at 37°C for 24 hours under aerobic conditions. Colonies were counted after incubation, and microbial loads were expressed as CFU/g of sample.

3.4.9. Sensory evaluation

In the sensory evaluation laboratory, the yoghurt samples were positioned randomly on the workbench to minimize bias during assessment. A total of thirty untrained panelists, comprising undergraduate and postgraduate students from the Faculty of Agriculture, Federal University of Technology, Akure, participated in the evaluation. Before tasting, panelists were briefed on the procedure and provided with questionnaires to record their observations. To eliminate any carryover effect between samples, each participant was supplied with water for rinsing the mouth after each tasting. The responses obtained from all panelists were subsequently compiled and analysed .

3.5. Statistical Analysis

The experiment was performed in replicates (n=3). Data obtained were subjected to one-way analysis of variance (ANOVA), and results were presented as mean ± standard deviation (SD) using Statistical Package for Social Sciences (SPSS) version 23. Prism Graph Pad version 8 was used for the graphical illustration of some of the analysed data.

All modelling and optimization were performed using Design Expert version 13 (Stat-Ease Inc., Minneapolis, USA, trial version), and models were checked for significance using the Analysis of Variance (ANOVA)

CHAPTER FOUR

RESULTS

4.1. Response Surface Optimization and Model Transformation for Sensory Quality of Soybean-Oat-Coconut Yoghurt

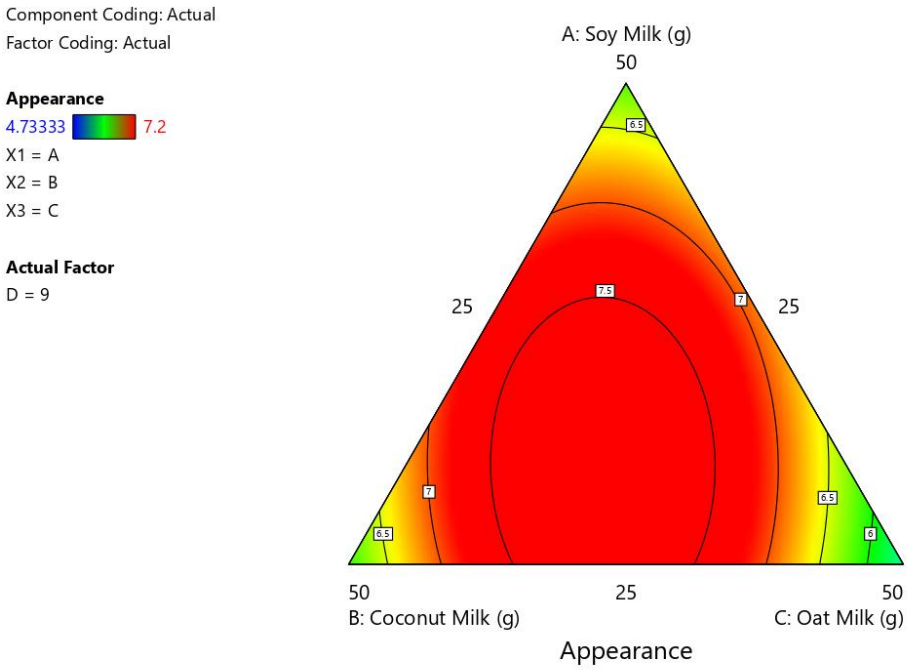
The optimization of soybean-oat-coconut yoghurt using Response Surface Methodology (RSM) revealed that the proportion of the mixture and fermentation time had complex effects on all important sensory characteristics, such as appearance, flavour, taste, aroma, consistency, and overall acceptability (Table 4.1). In some cases, transformation models had to be applied, analysed through ANOVA for better model adequacy and normality of residuals, or to stabilize variance. Models were checked for their respective F-values, R^2, adjusted and predicted R^2, adequate precision, and lack-of-fit tests to see that each response satisfied all the assumptions of RSM validity. ANOVA of the appearance model revealed that this data was heteroscedastic and skewed; thus, it was subjected to an inverse square root transformation to rectify this. The transformation resulted in a highly significant model fit, with F value, p, and R^2 of 22.91, 0.0006, and 0.9717, respectively. The Adjusted R^2 was 0.9293, which is indicative of strong predictive capability. Due to the insignificant lack-of-fit term at $p = 0.8138$, the residual errors were random, while low data variance was accounted for by the transformation. Lastly, the ratio of the adequate precision measure of signal-to-noise ratio was 16.08, way above the desired threshold value of 4.0.

The contour and 3D surface plots (Figure 4.1) of appearance presented a well-pronounced curvature between the coconut milk and oat milk axes. It was during term BC that the highest significance was revealed: $F = 78.87$. Considering soy at an intermediate level, 29-30%, the optimal zone of appearance is at ~36% coconut milk, where a balance between protein and fat enhances its opacity and surface sheen.

Table 4.1: Optimal Mixture Experimental Design for Milk Blend Selection

Run	A: Soy Milk	B: Coconut Milk	C: Oat Milk	D: Fermentation Time	Appearance	Flavour	Taste	Aroma	Consistency	Overall Acceptability	Percentage Acceptability
1	50	25	25	6	5.53333	4.66667	4.8	4.7	4.93333	4.9	54%
2	50	25	25	12	5.23333	4.43333	4.46667	4.46667	4.53333	4.96667	54%
3	25	50	25	6	5.36667	5.16667	5	4.9	5.1	5	55%
4	25	50	25	12	5.23333	5.03333	4.83333	4.9	4.93333	4.96667	54%
5	25	25	50	6	4.73333	4.3	4.43333	4.36667	4.46667	4.6	51%
6	25	25	50	12	5	4.66667	4.86667	4.86667	4.8	4.86667	53%
7	25	37.455	37.545	6	6.26667	5.86667	5.96667	6	5.66667	5.96667	65%
8	25	37.6139	37.3861	12	6.93333	6.56667	6.4	6.63333	6.26667	6.73333	74%
9	37.5861	25	37.4139	6	5.8	5.23333	5.16667	5.26667	5.3	5.16667	56%
10	37.4827	25	37.5173	12	6	5.73333	5.66667	5.7	5.66667	5.83333	64%
11	37.4257	37.435	25.1393	12	6.03333	5.9	5.56667	5.8	5.5	5.86667	64%
12	37.4257	37.435	25.1393	8	6.46667	5.6667	5.7	5.63333	5.3	6.0333	66%
13	37.4629	37.3747	25.1625	6	6.23333	5.96667	5.66667	5.9	5.66667	6	66%
14	27.523	42.4401	30.0369	8	7.2	6.8	6.5	6.7	5.9	6.66667	73%
15	29.6919	27.9098	42.3983	8	7	6.43333	6.36667	6.53333	6.1	6.7	74%
16	42.2642	29.7358	28	8	6.86667	6.36667	6.33333	6.6	6.16667	6.53333	72%

Note: *Values represent the sensory scores for appearance, flavor, taste, aroma, consistency, and overall acceptability of the 16 soybean-oat-coconut yoghurt blends, evaluated by a panel of 30 untrained participants at Benson Idahosa University using a 9-point hedonic scale (Ihekoronye and Ngoddy, 1985). Percentage overall acceptability was calculated as the mean of all attribute scores.*



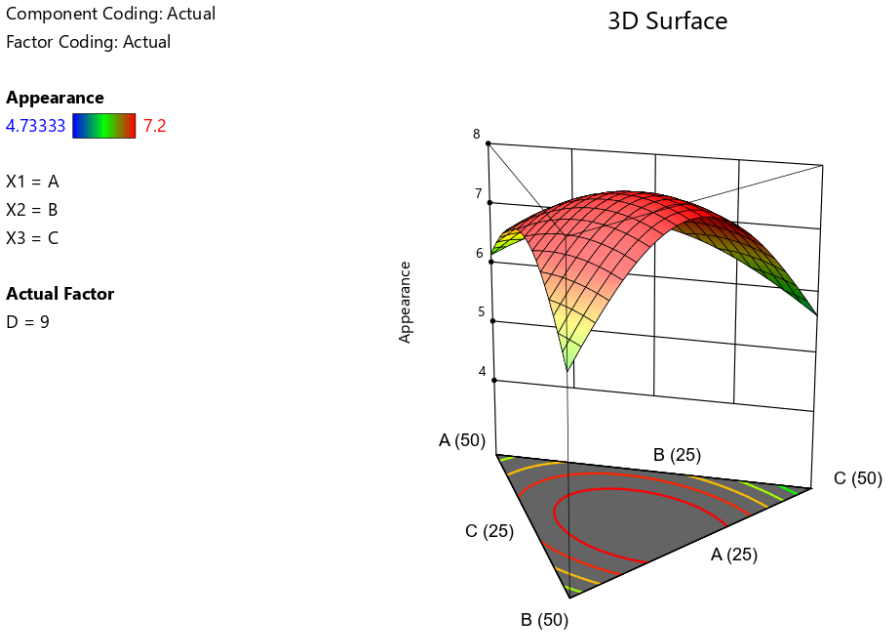


Figure 4.1: Contour and Surface plots for the maximum Appearance of Yoghurt from Milk Mixture

The model then became highly significant after transformation with $F = 37.62$, $p = 0.0001$, $R^2 = 0.9826$, Adjusted $R^2 = 0.9565$, and Adequate Precision = 20.60, indicating the adequacy of the model. This model precisely captured the curvature, as evidenced by the nonsignificant lack-of-fit with $p = 0.6057$.

The 3D and contour plots depicted that flavour intensity increases steeply in the case of balanced levels of soy and coconut milk, 25-35% soy and 35-38% coconut, along with moderate fermentation time at 8 hours. Again, the most significant term was interaction BC, that is, coconut and oat, having an F-value of 99.18, showing that coconut fat and oat β-glucans support flavour-enhancing microbial metabolism.

For the taste model, the response variable was transformed to have a square root in order to stabilize the variance. The model, after transforming, gave rise to a highly significant fit: $F = 38.24$, $p = 0.0001$, $R^2 = 0.9829$, Adjusted $R^2 = 0.9572$, Adequate Precision = 19.75. A non-significant lack-of-fit ($p = 0.4253$) supported the transformation of the variance in this model as it had been stabilized.

The most significant influence of BC, coconut × oat, and D^2, fermentation time, was at $F = 120.98$ and 48.75, respectively.

The quadratic mean model of aroma provided the following results: $F = 47.06$, $p < 0.0001$; $R^2 = 0.9860$, Adjusted $R^2 = 0.9651$, Predicted $R^2 = 0.8502$. The Adequate Precision ratio determined was 21.73, thus confirming the quality of the signal being very good.

The consistency model was significant after inverse transformation: $F = 13.55$, $p = 0.0024$, with $R^2 = 0.9531$, Adjusted $R^2 = 0.8828$, and Adequate Precision = 12.19. Whereas the predicted R^2

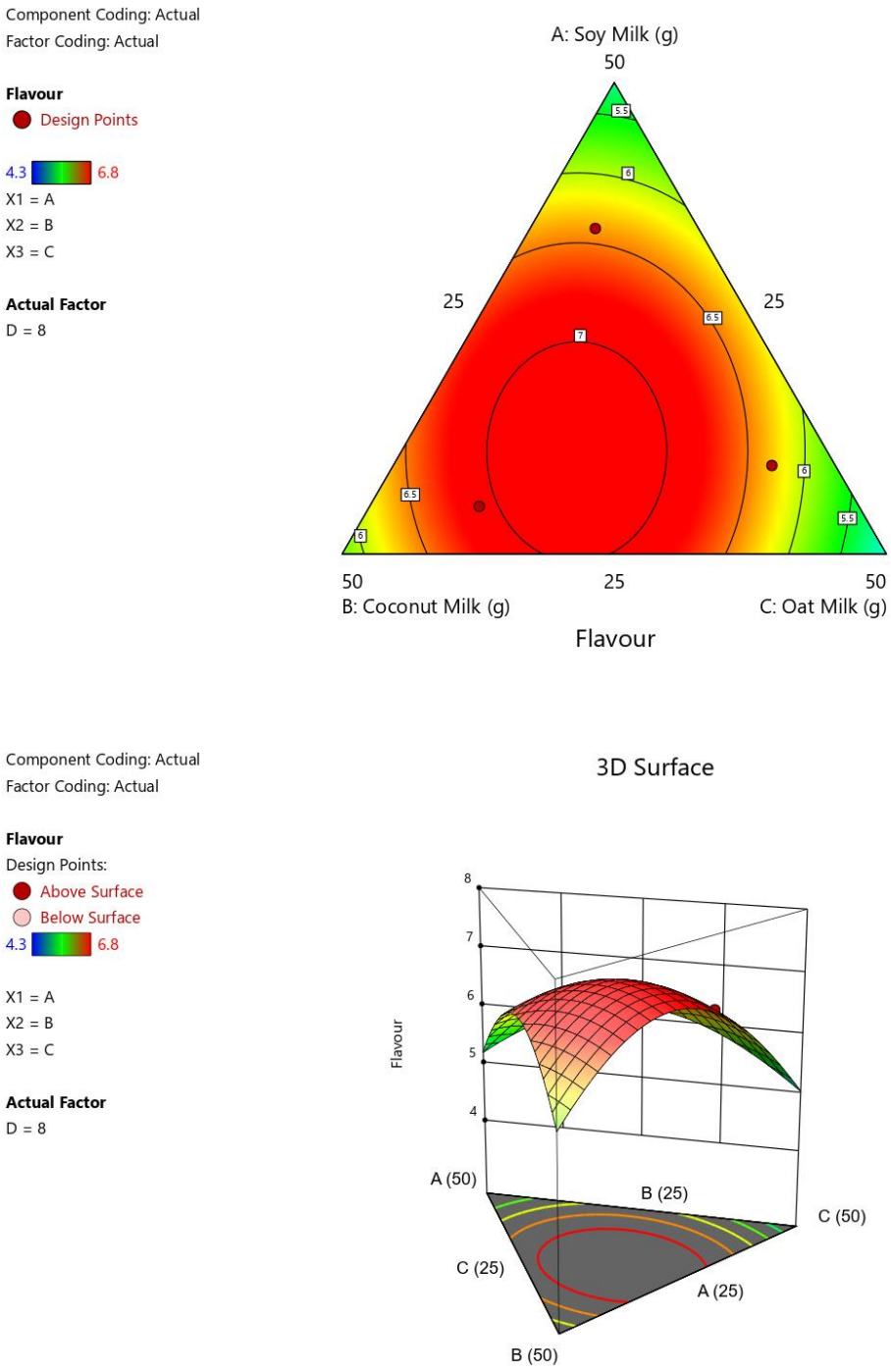


Figure 4.2: Contour and Surface plots for the maximum Flavour of Yoghurt from Milk Mixture

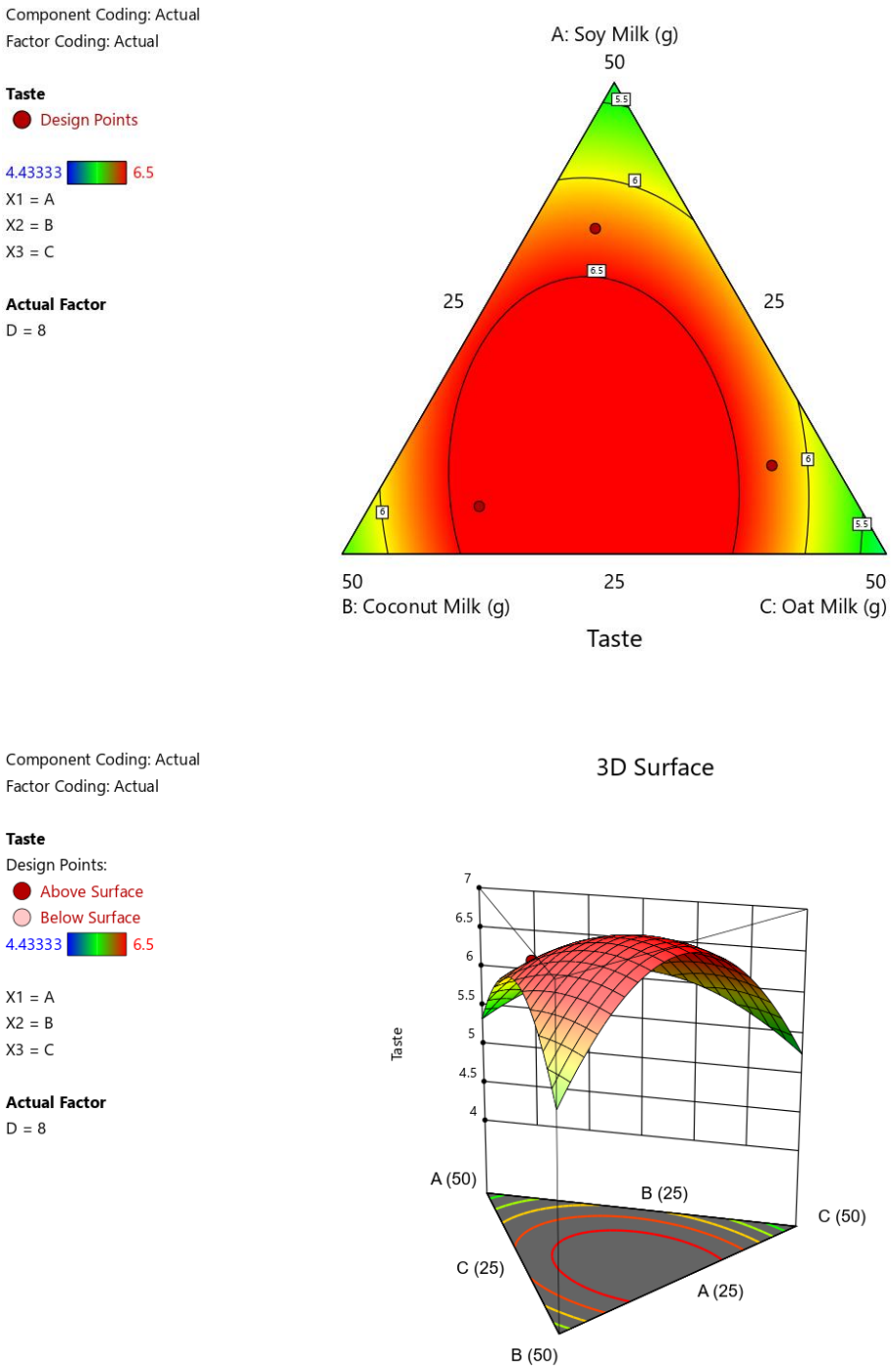


Figure 4.3: Contour and Surface plots for the maximum Taste of Yoghurt from Milk Mixture

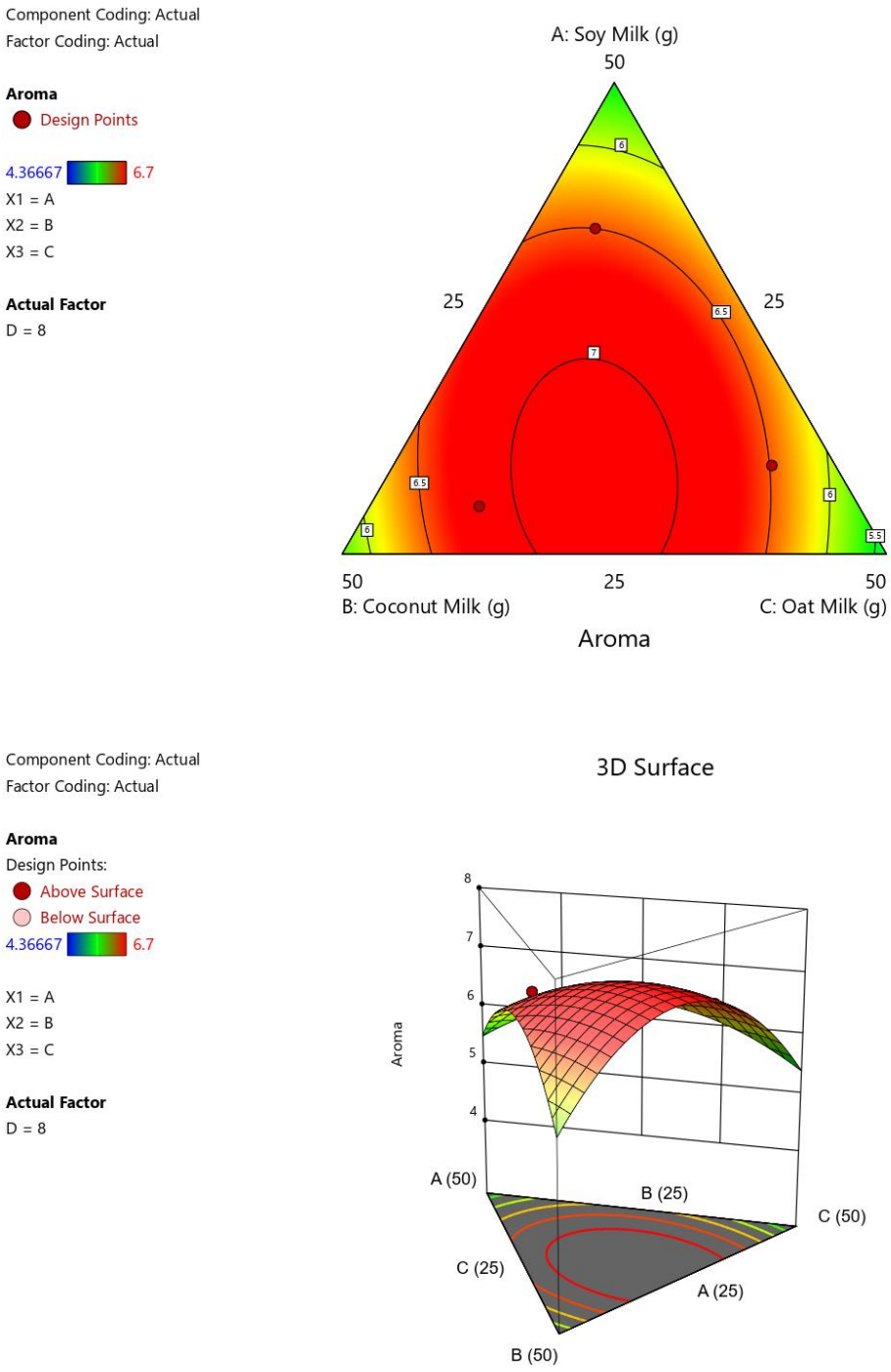


Figure 4.4: Contour and Surface plots for the maximum Aroma of Yoghurt from Milk Mixture

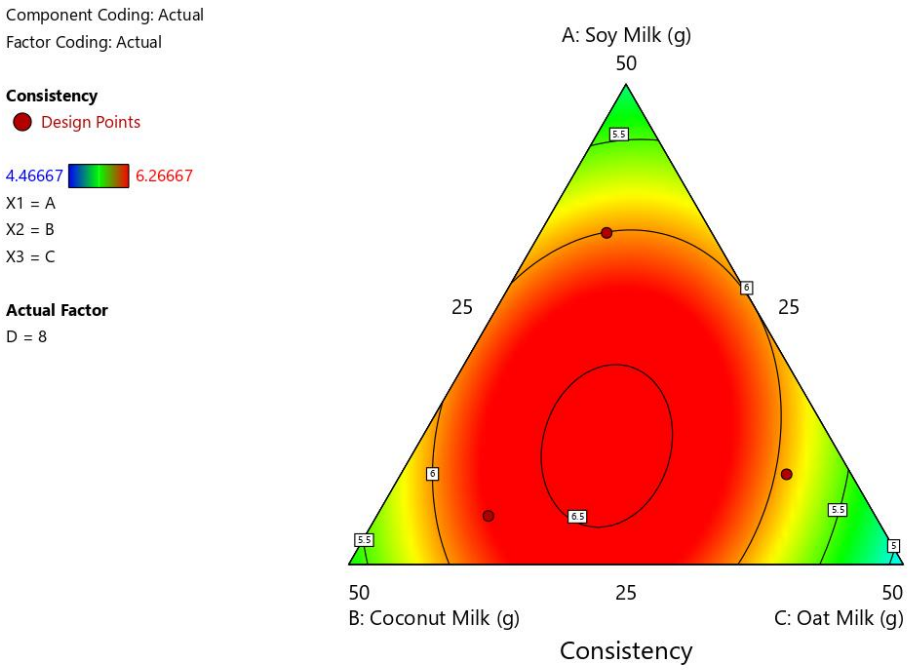
was far from the expected value, 0.5760, a nonsignificant lack-of-fit test, p = 0.4675.

The model for the overall acceptability, which was not transformed, was significant with F = 6.89 and p = 0.0049, while R^2 = 0.7751 and Adjusted R^2 = 0.6626. The predicted R^2 value of 0.5034 was fairly close, and the Adequate Precision of 6.79 also confirmed the satisfactory predictive power. The lack of fit was non-significant (p = 0.1979), so the quadratic model could suitably describe the combined sensory output. The 3D surface plot showed that the highest preference area had a dome shape at approximately 29%, 36%, and 35% for soy, coconut, and oat, respectively. The fermentation time was 8 hours, and it coincided with the desirability function value of 0.768.

4.2 Optimized Solutions from Response Surface Analysis

The response surface methodology (RSM) was used to predict the sensory performance of soybean-oat-coconut yoghurt blends based on the data from the parameter from the preliminary sensory evaluation data. The RSM models incorporated the proportions of soy, coconut, and oat milk, as well as fermentation time, to estimate expected values for appearance, flavour, taste, aroma, consistency, and overall acceptability.

From the model predictions, six optimized solutions were generated (Table 4.2), representing combinations expected to yield the highest sensory quality. Of these, three formulations (Runs 1–3) were predicted to have the highest desirability (i.e. values closer to one). Run 1, consisting of 29.147% soy milk, 36.340% coconut milk, and 34.512% oat milk fermented for 8 hours, was predicted to have the most balanced sensory profile, with the highest predicted scores for appearance, flavour, and aroma among the optimized samples. Run 2, with slightly higher coconut content, was predicted to exhibit improved flavour perception, while Run 3, fermented for 12 hours, was predicted to have marginally lower flavour and aroma scores, likely due to increased acidity from prolonged fermentation.



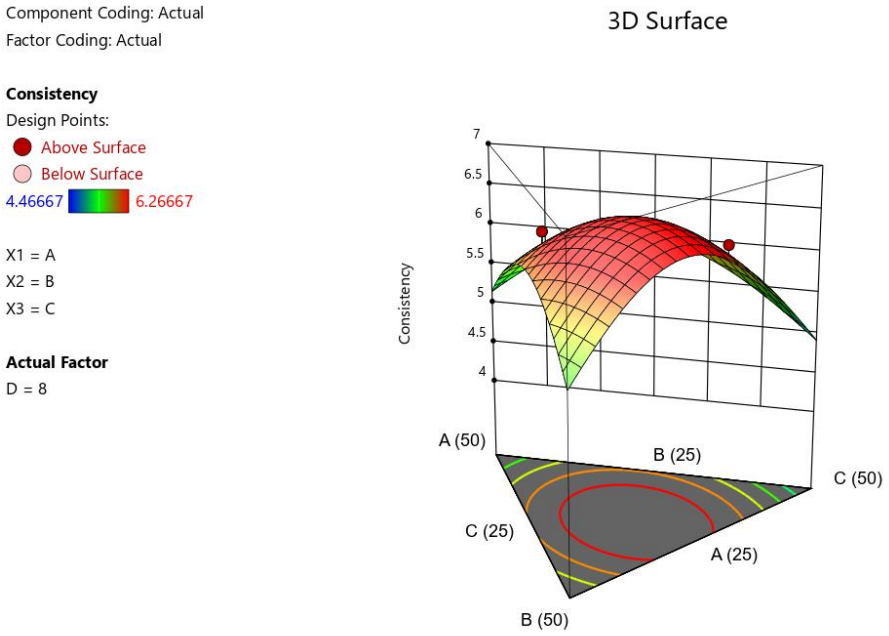
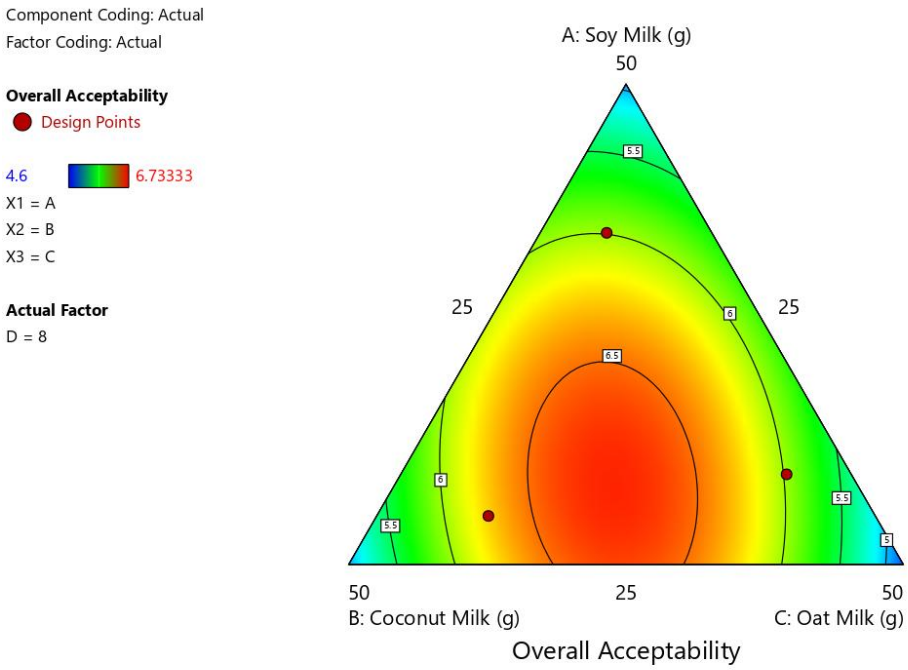


Figure 4.5: Contour and Surface plots for the maximum Consistency of Yoghurt from Milk Mixture



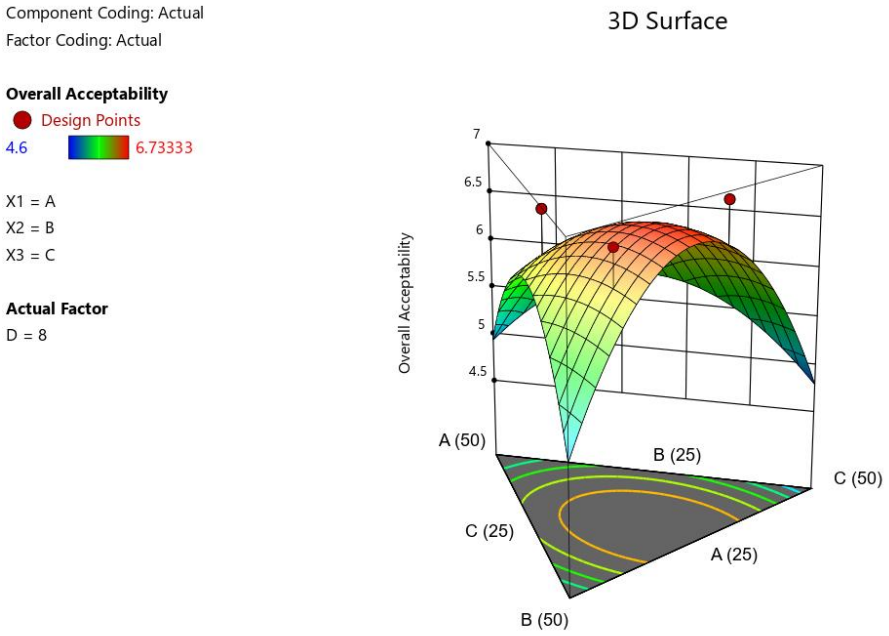


Figure 4.6: Contour and Surface plots for the maximum Overall Acceptability of Yoghurt from Milk Mixture

Table 4.2: Solutions for three Combinations of Categoric Factor Levels

Run	Soy Milk	Coconut Milk	Oat Milk	Fermentation Time	Appearance	Flavour	Taste	Aroma	Consistency	Overall Acceptability	Desirability	
1	29.147	36.340	34.512	8.000	7.686	7.184	6.935	7.144	6.570	6.659	0.768	**Selected**
2	25.000	38.213	36.787	8.000	7.539	7.015	6.882	7.080	6.395	6.594	0.760	**Selected**
3	26.775	36.545	36.680	12.000	6.815	6.449	6.331	6.496	6.129	6.636	0.687	**Selected**
4	25.000	37.353	37.647	12.000	6.774	6.391	6.321	6.484	6.086	6.593	0.686	
5	30.312	36.254	33.433	6.000	6.633	6.252	6.097	6.209	5.972	6.648	0.651	
6	25.000	38.649	36.351	6.000	6.466	6.054	6.017	6.104	5.757	6.588	0.638	

4.3. Proximate Composition of Yoghurt produced from soy, oat, and coconut milk

Six solutions were suggested from the Optimization of the experimental runs. The first three solutions were selected based on the sensory parameters and the desirability level. The best experimental run was also selected as the fourth sample. The other two samples are market yoghurt samples and a reconstituted cow milk, which are both positive and diary reference, respectively.

The proximate parameters of the selected yoghurt samples and the control samples are presented in Table 4.3. According to the results, the moisture content of the yoghurt samples ranged from 76.27% to 89.90%. Significant differences were observed at $p < 0.05$ among all samples. The commercial control, CY, had a significantly higher value than all other samples. Samples S25_CO and S30_CO were statistically similar, having values of 76.27 and 76.76%, respectively.

Ash content varied between 0.10 and 0.61%, with CY having a significantly higher value than other samples. Samples S25_CO and S30_CO gave moderate values of 0.31% and 0.30%, respectively, whereas S27_CO and S29_CO gave the lowest values of 0.15% and 0.10% respectively

Crude protein content was between 2.05% and 6.02% and shows significant differences ($p<0.05$) between samples. The market control, CY, had the lowest protein concentration, but S27_CO and S30_CO were statistically identical.

Fat content varied significantly from 1.52 to 8.14% ($p < 0.05$). The fat value was highest for sample S29_CO at 8.14%, while for CY and DY, it was the lowest at 1.52%, with no significant difference between these two samples. The fiber content varied from 0.27 to 0.33%, presenting significant differences among samples ($p < 0.05$).

Table 4.3: Proximate Composition of Yoghurt Produced from Soy, Oat, and Coconut Milk

	Moisture	Ash	Protein	Fat	Fibre	Carbohydrate	Energy
S29_CO	78.01 ±1.41^{cd}	0.10 ± 0.00^{e}	6.02 ± 0.10^{a}	8.14 ± 0.15^{a}	0.28±0.01^{cd}	7.45±1.66^{d}	127.15±4.91^{a}
S25_CO	76.27 ± 1.37^{d}	0.31 ± 0.01^{c}	4.15 ± 0.07^{b}	4.04 ± 0.07^{b}	0.27±0.00^{d}	14.96±1.39^{ab}	112.82±5.17^{b}
S27_CO	79.78 ± 1.44^{bc}	0.15 ± 0.00^{d}	3.84 ± 0.07^{c}	3.91 ± 0.07^{b}	0.28±0.01^{bc}	12.03±1.58^{c}	98.70±5.43^{c}
S30_CO	76.76 ± 1.38^{d}	0.30 ± 0.01^{c}	3.75 ± 0.06^{c}	3.60 ± 0.06^{c}	0.27±0.00^{d}	15.31±1.39^{a}	108.64±5.25^{b}
CY	89.90 ± 0.15^{a}	0.61 ± 0.01^{a}	2.05 ± 0.03^{e}	1.52 ± 0.03^{d}	0.33±0.01^{a}	5.59±0.23^{d}	44.29±0.52^{e}
DY	81.89 ± 1.48^{b}	0.40 ± 0.01^{b}	3.14 ± 0.05^{d}	1.52 ± 0.03^{d}	0.29±0.01^{b}	12.74±1.46^{bc}	77.28±5.81^{d}

Values represent the means of three replicates ± standard deviation. Mean values followed by different superscripts within columns are significantly different according to Duncan's Multiple Range Test ($p < 0.05$).

Keys:

S29_CO: 29.147 Soy Milk, 36.340 Coconut Milk, 34.512 Oat Milk, for 8 hours fermentation time

S25_CO: 25.000 Soy Milk, 38.213 Coconut Milk, 36.787 Oat Milk, for 8 hours fermentation time

S27_CO: 26.775 Soy Milk, 36.545 Coconut Milk, 36.680 Oat Milk, for 12 hours fermentation time

S30_CO: 29.6919 Soy Milk, 27.9098 Coconut Milk, 42.3983, Oat Milk, for 8 hours fermentation time

DY: Reconstituted Powdered Milk Yoghurt, for 8 hours fermentation time

CY: Commercial Yoghurt

The lowest fiber levels were observed in S25_CO and S30_CO, with 0.27% each, both dominated by soy and coconut bases, while higher levels were observed in the oat-rich formulations.

For carbohydrate, there is no significant difference ($p > 0.05$) between sample S29_CO and CY. This may be a result of high values of other parameters within the samples. The highest carbohydrate values were obtained for S30_CO (15.31%) and S25_CO (14.96%).

Energy values ranged from 44.29 to 127.15 kcal/100 g, corresponding closely with the differences in fat and carbohydrate contents. There was a significant difference, at $p < 0.05$, among samples, where S29_CO had the highest value, while CY had the lowest of 44.29 kcal/100 g.

4.4. Antinutritional Factors of Yoghurt Produced from Soybean-Oat-Coconut Blends

The antinutritional/phytochemical composition of the sample is shown in Table 4.4. The antinutritional profile revealed that the yoghurt blends were quite different ($p < 0.05$). Phytate was measured on the samples, and the results indicated large differences among them. Phytate levels were found to be between 11.92 mg/g and 13.96 mg/g. Sample DY had the highest concentration of phytate, 13.96 mg/g, while S25_CO had the lowest concentration of 11.92 mg/g. The combined samples S27_CO, S29_CO, and S30_CO were found to contain phytate levels of 12.33 to 13.14 mg/g, at medium range.

The oxalate content ranged between 4.58 mg/g for S27_CO and 17.42 mg/g with DY. Low levels, as shown by S27_CO. In other optimized blends such as S25_CO, S29_CO, and S30_CO, the level of oxalate ranged from 8.25 to 11.00 mg/g, in line with the pattern of moderation in antinutrient levels in the optimized formulations.

Trypsin inhibitor activities were within the range of 9.61% and 62.41%. As expected for a higher soy content or minimally processed sample, the highest value of the inhibiting activity was from

the CY sample at 62.41%; the lowest activity, 9.61%, was from DY, showing effective reduction during fermentation. In fact, among the optimized blends, the lowest trypsin inhibition was shown by S27_CO with an activity of 13.13%, while S29_CO and S30_CO exhibited intermediate activities.

The assessment of tannin content showed a range of 1.31 mg QE/g as the lowest to 3.31 mg QE/g as the highest. The sample S30_CO showed the richest content of tannin, while CY showed the poorest. The other samples, S25_CO, S27_CO, S29_CO, and DY, had tannin ranging from 2.70 to 3.11 mg QE/g.

The level of saponins for S25_CO was reported as 82.29 mg/g, the highest concentration, whereas in S27_CO and CY it was 16.50 mg/g. Saponin status in S30_CO and DY was approximately 36.66-48.20 mg/g. There is no significant difference in the saponin level of S27_CO and CY ($p > 0.05$). All other samples show a significant difference at the 95% confidence level.

4.5. Mineral Composition of Yoghurt Produced from Soybean-Oat-Coconut Blends

The analysed elements (sodium (Na), calcium (Ca), potassium (K), zinc (Zn), magnesium (Mg), iron (Fe), and phosphorus (P)) statistical difference was determined to be remarkable at $p < 0.05$: Table 4.5 shows this parameter, and Table 4.6 shows the mineral ratio.

Sodium was found to be in the lowest concentration in the sample CY (0.48 mg/100 g) and in the highest concentration in S30_CO (3.38 mg/100 g). S29_CO, S27_CO, and DY varieties had a range of sodium contents that was similar to the average of 0.85-2.08 mg/100 g; however, S25_CO was more at 2.70 mg/100 g. The differences among them were shown through the corresponding superscripts.

Table 4.4: Phytochemical Composition of Yoghurt Produced from Soybean-Oat-Coconut Blends

Samples	Phytate mg/g	Oxalate mg/g	Trypsin %	Tannin mg (QE)/g	Saponin mg/g
S29_CO	13.14 ± 0.24^{b}	8.25 ± 0.15^{d}	59.59 ± 1.07^{b}	2.84 ± 0.05^{c}	26.94 ± 0.46^{d}
S25_CO	11.92 ± 0.21^{c}	11.00 ± 0.20^{b}	24.39 ± 0.44^{d}	3.11 ± 0.06^{b}	82.29 ± 1.39^{a}
S27_CO	12.33 ± 0.22^{c}	4.58 ± 0.08^{f}	13.13 ± 0.234^{e}	2.70 ± 0.05^{d}	16.50 ± 0.28^{e}
S30_CO	12.84 ± 0.23^{b}	10.09 ± 0.18^{c}	42.70 ± 0.77^{c}	3.31 ± 0.06^{a}	48.20 ± 0.82^{b}
CY	13.23 ± 0.24^{b}	6.42 ± 0.12e	62.41 ± 1.12^{a}	1.31 ± 0.02^{f}	16.50 ± 0.28^{e}
DY	13.96 ± 0.25^{a}	17.42 ± 0.31^{a}	9.61 ± 0.17^{f}	1.62 ± 0.03^{e}	36.66 ± 0.62^{c}

Values represent the means of three replicates ± standard deviation. Mean values followed by different superscripts within columns are significantly different according to Duncan's Multiple Range Test ($p < 0.05$).

S29_CO: 29.147 Soy Milk, 36.340 Coconut Milk, 34.512, Oat Milk, for 8 hours fermentation time

S25_CO: 25.000 Soy Milk, 38.213 Coconut Milk, 36.787 Oat Milk, for 8 hours fermentation time

S27_CO: 26.775 Soy Milk, 36.545 Coconut Milk, 36.680, Oat Milk, for 12 hours fermentation time

S30_CO: 29.6919 Soy Milk, 27.9098 Coconut Milk, 42.3983, Oat Milk, for 8 hours fermentation time

DY: Reconstituted Powdered Milk Yoghurt, for 8 hours of fermentation time

CY: Commercial Yoghurt

From the result, the level of calcium showed a statistically significant difference, $p < 0.05$. The maximum, which was sample S30_CO, was 2.41 mg/100 g, while the minimum was recorded for CY at 0.95 mg/100 g. The concentrations for S25_CO and DY were between 1.54 mg/100 g and 1.61 mg/100 g, respectively. S29_CO and S27_CO were found to have values that were only significantly lower than those of S30_CO, but still higher than those of CY.

The potassium contents in the samples underwent great variation, starting from the lowest value of 0.13 mg/100 g measured in DY, going through the highest value recorded of 2.90 mg/100 g in S25_CO, and ending at S27_CO, which displayed a fairly good amount of potassium at 1.20 mg/100 g. The ranges of potassium content for CY and S29_CO, and S30_CO were moderate, that is, 0.63 to 0.82 mg/100 g. The samples were all found to be different from each other significantly at the $p < 0.05$ level.

The zinc level in S30_CO and DY is significantly the same (0.03 mg/100g), and the value is 50% higher than in other samples. The value of 0.02 mg/100g was observed in S29_CO, S25_CO, S27_CO, and CY. Therefore, the zinc level in S30_CO and DY was 0.03 mg/100 g, which was the highest one with a large difference because S29_CO, S25_CO, S27_CO, and CY had only 0.02 mg/100 g.

The magnesium content was very important and had a p-value < 0.05; S30_CO had the highest value of 1.33 mg/100 g. The mean values for S25_CO and S29_CO were 0.79-0.87 mg/100 g, and thus they were in the mid-range, while CY and S27_CO were in the lower range of 0.51-0.55 mg/100 g. CY had the least amount of magnesium.

Table 4.5: Mineral Composition of Yoghurt Produced from Soybean-Oat-Coconut Blends (mg/100g)

Samples	Na	Ca	K	Zn	Mg	Fe	P
S29_CO	1.19±0.02^{d}	1.34±0.02^{d}	0.63±0.01^{e}	0.02±0.00^{b}	0.79±0.01^{c}	0.02±0.00^{b}	77.28±1.36^{b}
S25_CO	2.70±0.10^{c}	1.61±0.01^{b}	2.90±0.10^{a}	0.02±0.00^{b}	0.87±0.01^{b}	0.02±0.00^{b}	59.66±1.07^{d}
S27_CO	2.08±0.02^{b}	1.05±0.01^{e}	1.20±0.01^{b}	0.02±0.00^{b}	0.55±0.01^{d}	0.03±0.00^{a}	67.21±1.19^{c}
S30_CO	3.38±0.02^{a}	2.41±0.01^{a}	0.72±0.02^{d}	0.03±0.00^{a}	1.33±0.03^{a}	0.02±0.00^{b}	57.14±1.02^{e}
CY	0.48±0.02^{f}	0.95±0.01^{f}	0.82±0.02^{c}	0.02±0.00^{b}	0.51±0.01^{e}	0.03±0.00^{a}	24.40±0.47^{f}
DY	0.85±0.01^{e}	1.54±0.02^{c}	0.13±0.02^{f}	0.03±0.00^{a}	0.77±0.01^{c}	0.03±0.00^{a}	79.80±1.41^{a}

Values represent the means of three replicates ± standard deviation. Mean values followed by different superscripts within columns are significantly different according to Duncan's Multiple Range Test ($p < 0.05$).

Keys:

S29_CO: 29.147 Soy Milk, 36.340 Coconut Milk, 34.512 Oat Milk, for 8 hours fermentation time

S25_CO: 25.000 Soy Milk, 38.213 Coconut Milk, 36.787 Oat Milk, for 8 hours fermentation time

S27_CO: 26.775 Soy Milk, 36.545 Coconut Milk, 36.680 Oat Milk, for 12 hours fermentation time

S30_CO: 29.6919 Soy Milk, 27.9098 Coconut Milk, 42.3983, Oat Milk, for 8 hours fermentation time

DY: Reconstituted Powdered Milk Yoghurt, for 8 hours of fermentation time

CY: Commercial Yoghurt

Table 4.6: Mineral Ratio of Yoghurt Produced from Soybean-Oat-Coconut Blends

Sample	Na/K	Ca/P	Na/Ca	Mg/Ca	Fe/Zn
S29_CO	1.89	0.02	0.89	0.59	1
S25_CO	0.93	0.03	1.68	0.54	1
S27_CO	1.73	0.02	1.98	0.52	1.5
S30_CO	4.69	0.04	1.4	0.55	1
CY	0.59	0.04	0.5	0.54	1.5
DY	6.54	0.02	0.55	0.5	1.5

Keys:

S29_CO: 29.147 Soy Milk, 36.340 Coconut Milk, 34.512 Oat Milk, for 8 hours fermentation time

S25_CO: 25.000 Soy Milk, 38.213 Coconut Milk, 36.787 Oat Milk, for 8 hours fermentation time

S27_CO: 26.775 Soy Milk, 36.545 Coconut Milk, 36.680 Oat Milk, for 12 hours fermentation time

S30_CO: 29.6919 Soy Milk, 27.9098 Coconut Milk, 42.3983, Oat Milk, for 8 hours fermentation time

DY: Reconstituted Powdered Milk Yoghurt, for 8 hours of fermentation time

CY: Commercial Yoghurt

The study infers that the concentration of iron was very strictly controlled, as it fluctuated between 0.02 and 0.03 mg/100 g. S27_CO, CY, and DY showed the highest level of 0.03 mg/100 g, whereas S29_CO, S25_CO, and S30_CO had the lowest of 0.02 mg/100 g; thus, the difference between them was significant.

The phosphorus concentrations were also significantly different in the range of 24.40 mg/100 g (for CY) to 79.80 mg/100 g (DY), the latter being the highest value. S29_CO and S27_CO (67.21-77.28 mg/100 g) also gave relatively high results; concentrations of 57.14-59.66 mg/100 g were attributed to S25_CO and S30_CO. All samples were found to be significantly different from one another according to superscript notations.

The Na/K ratio varied widely in the samples, reflecting the large differences in sodium and potassium contents of the various samples. The maximum Na/K ratio was recorded in S30_CO with a value of 4.69, while those for S25_CO and S27_CO were 0.93 and 1.73, respectively, representing the high increase of sodium levels relative to potassium in these samples.

S29_CO presented a Na/K ratio of 1.89, while DY had the highest Na/K ratio at 6.54, since it consequently had a very low potassium value. CY was intermediate at 0.59 and fell in line with its relatively low sodium content. All the values had marked numerical differences according to their respective Na and K concentrations.

The Ca/P ratio was not the same for all the samples. S30_CO had the highest ratio, 0.04, probably because of the high calcium level in it. S25_CO also showed a fairly high value of the Ca/P ratio, 0.03.

The mean ratio was 0.02 for both S29_CO and S27_CO, while the lowest Ca/P ratio of 0.04 was observed for CY. The determined Ca/P for DY was 0.02, with a high phosphorus concentration

compared to calcium. Variability in these samples shows that there is a measurable difference in the mineral distribution.

Sodium-Calcium Ratio (Na/Ca) The Na/Ca ratio demonstrated clear differences among samples. The highest Na/Ca ratio was observed in S30_CO, reaching a value of 1.40, then followed by S25_CO, reaching 1.68, and finally S27_CO, reaching 1.98. S29_CO and DY had moderate values of Na/Ca, at 0.89 and 0.55, respectively. The least ratio of Na/Ca, 0.50, was given by CY due to its lower concentration of sodium. Obviously, all the samples showed numerical differences. A wide variation in the Mg/Ca ratio was recorded with the highest value of 0.55 in S30_CO, which is directly related to its high magnesium content, and then followed by 0.54 in S25_CO and 0.59 in S29_CO. The ratio of Mg/Ca for S27_CO was even lower at 0.52. The minimum Mg/Ca ratios were for CY and DY with 0.54 and 0.50, respectively. The above-mentioned differences in numbers are indicative of the different measured concentrations of Mg and Ca. On the contrary to these two ratios, the Fe/Zn ratio was in the range of 1.00 to 1.50. The highest Fe/Zn ratio of 1.50 for S27_CO, CY, and DY indeed means that their iron content is very much higher than that of zinc. S29_CO, S25_CO, and S30_CO had lower values which were a Fe/Zn ratio of 1.00, with shared zinc and iron concentrations. All samples indeed manifested obvious numerical variations.

4.6. Antioxidant Properties of Yoghurt Made from Soybean-Oat-Coconut Blends

The antioxidant characteristics of the yoghurt sample made with a mixture of soybean, oat, and coconut were determined by various assays, including Total Phenolic Content, Total Flavonoid Content, DPPH, and ABTS radical scavenging activities. The ferric reducing antioxidant power, FRAP value of the samples, is shown in Figures 4.7 to 4.11.

The amount of total phenolic compounds was established to be in the range of 1.14 to 4.37 mg GAE/g. The S25_CO sample among the different samples indicated the presence of phenolic

compounds the most, with a figure of 4.37 mg GAE/g; on the other hand, commercial yoghurt CY was found to have the least phenolic content of 1.14 mg GAE/g. The result showed a wide range of total flavonoid content, from the lowest, 0.08 mg QE/g, to the highest, 0.55 mg QE/g. Sample S29_CO yielded the highest flavonoid content of 0.55 mg QE/g, followed by S30_CO, with a value of 0.47 mg QE/g. On the other hand, the least value was observed in sample CY.

The values of DPPH radical scavenging activity ranged from 25.16% to 50.64%. The highest value was found in S27_CO (50.64%), which was very close to the commercial yoghurt CY at 50.97%, while the minimum value appeared in S29_CO at 25.16%. ABTS scavenging activity showed wider dispersal, from 5.24% up to 18.29%. The lowest ABTS activity occurred in S27_CO at 5.24%, whereas the highest ABTS activity occurred in CY at 18.29%, followed by DY at 16.02%. For the plant-based samples, ABTS activity clustered mostly between 8.65% and 13.33%, with the high end of this range represented by S25_CO.

The FRAP ranges between 2.66 and 3.09 mg of vitamin C equivalent per gram. Samples S25_CO and S29_CO exhibited the highest antioxidant activities of 3.09 and 3.01 mg VCE/g, respectively. The S27_CO sample was identified as the one with the least reducing power, with a value of 2.66 mg VCE/g.

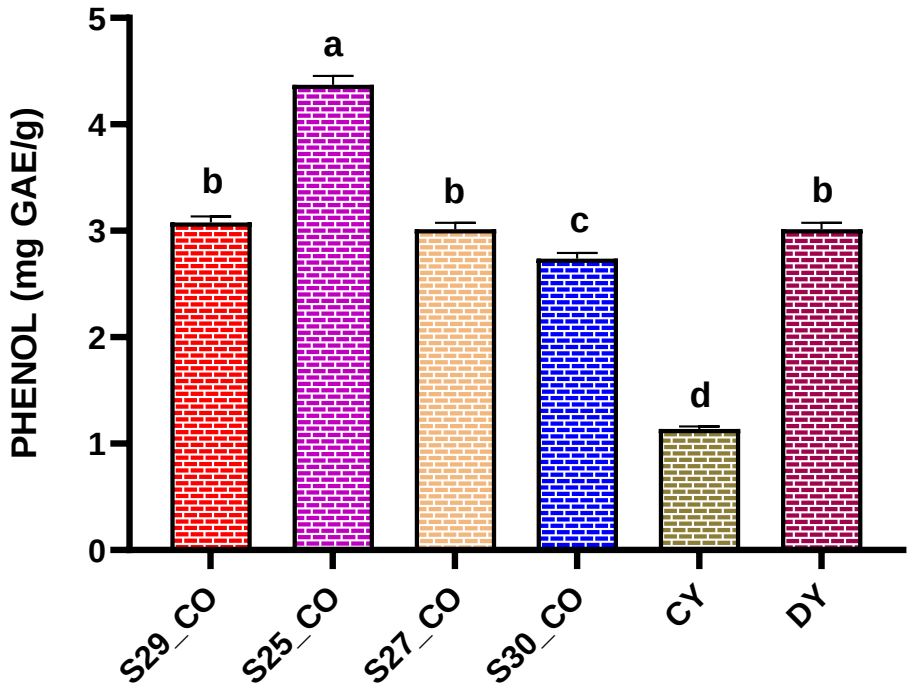


Figure 4.7: Total Phenol Content of Yoghurt Made from Soybean-Oat-Coconut Blends

Values represent the means of three replicates ± standard deviation. Mean values followed by different superscripts within columns are significantly different according to Duncan's Multiple Range Test ($p < 0.05$).

Keys:

S29_CO: 29.147 Soy Milk, 36.340 Coconut Milk, 34.512 Oat Milk, for 8 hours fermentation time

S25_CO: 25.000 Soy Milk, 38.213 Coconut Milk, 36.787 Oat Milk, for 8 hours fermentation time

S27_CO: 26.775 Soy Milk, 36.545 Coconut Milk, 36.680 Oat Milk, for 12 hours fermentation time

S30_CO: 29.6919 Soy Milk, 27.9098 Coconut Milk, 42.3983, Oat Milk, for 8 hours fermentation time

DY: Reconstituted Powdered Milk Yoghurt, for 8 hours fermentation time

CY: Commercial Yoghurt

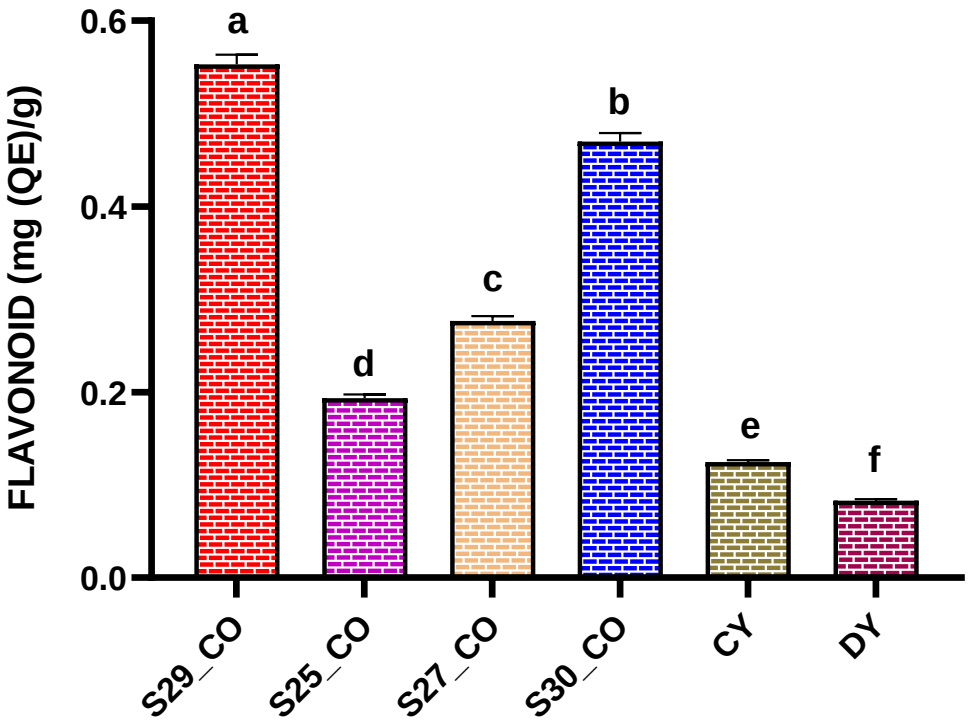


Figure 4.8: Total Flavonoids Content of Yoghurt Made from Soybean-Oat-Coconut Blends

Values represent the means of three replicates ± standard deviation. Mean values followed by different superscripts within columns are significantly different according to Duncan's Multiple Range Test ($p < 0.05$).

Keys:

S29_CO: 29.147 Soy Milk, 36.340 Coconut Milk, 34.512 Oat Milk, for 8 hours fermentation time

S25_CO: 25.000 Soy Milk, 38.213 Coconut Milk, 36.787 Oat Milk, for 8 hours fermentation time

S27_CO: 26.775 Soy Milk, 36.545 Coconut Milk, 36.680 Oat Milk, for 12 hours fermentation time

S30_CO: 29.6919 Soy Milk, 27.9098 Coconut Milk, 42.3983, Oat Milk, for 8 hours fermentation time

DY: Reconstituted Powdered Yoghurt, for 8 hours fermentation time

CY: Commercial Yoghurt

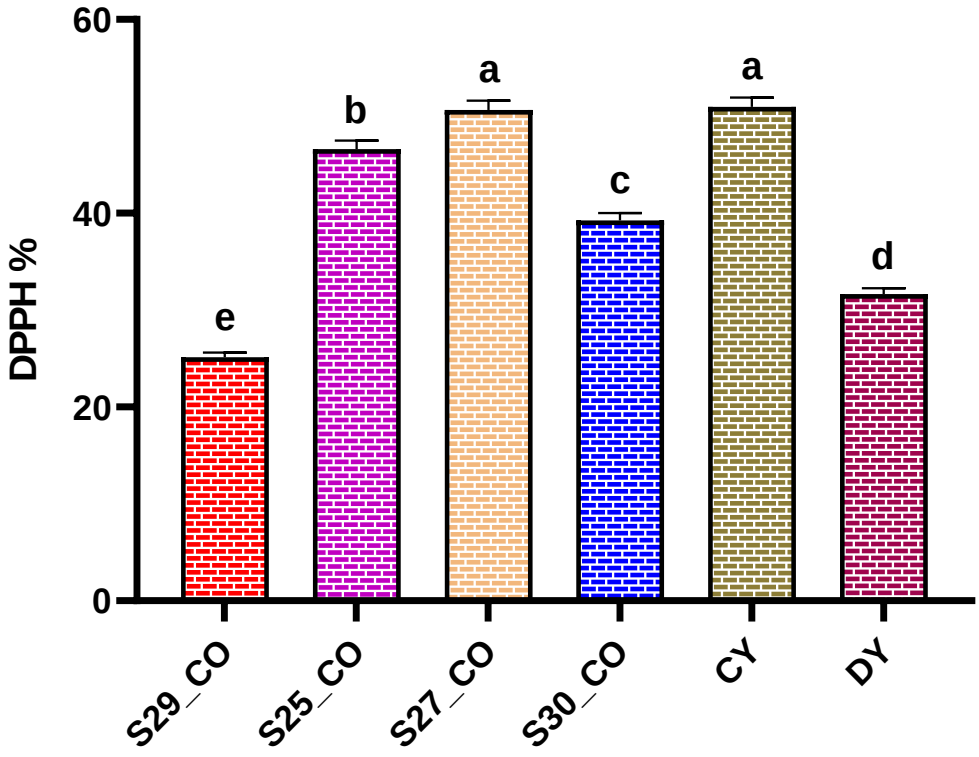


Figure 4.9: DPPH Scavenging Activities of Yoghurt Made from Soybean-Oat-Coconut Blends

Values represent the means of three replicates ± standard deviation. Mean values followed by different superscripts within columns are significantly different according to Duncan's Multiple Range Test ($p < 0.05$).

Keys:

S29_CO: 29.147 Soy Milk, 36.340 Coconut Milk, 34.512 Oat Milk, for 8 hours fermentation time

S25_CO: 25.000 Soy Milk, 38.213 Coconut Milk, 36.787 Oat Milk, for 8 hours fermentation time

S27_CO: 26.775 Soy Milk, 36.545 Coconut Milk, 36.680 Oat Milk, for 12 hours fermentation time

S30_CO: 29.6919 Soy Milk, 27.9098 Coconut Milk, 42.3983, Oat Milk, for 8 hours fermentation time

DY: Reconstituted Powdered Milk Yoghurt, for 8 hours fermentation time

CY: Commercial Yoghurt

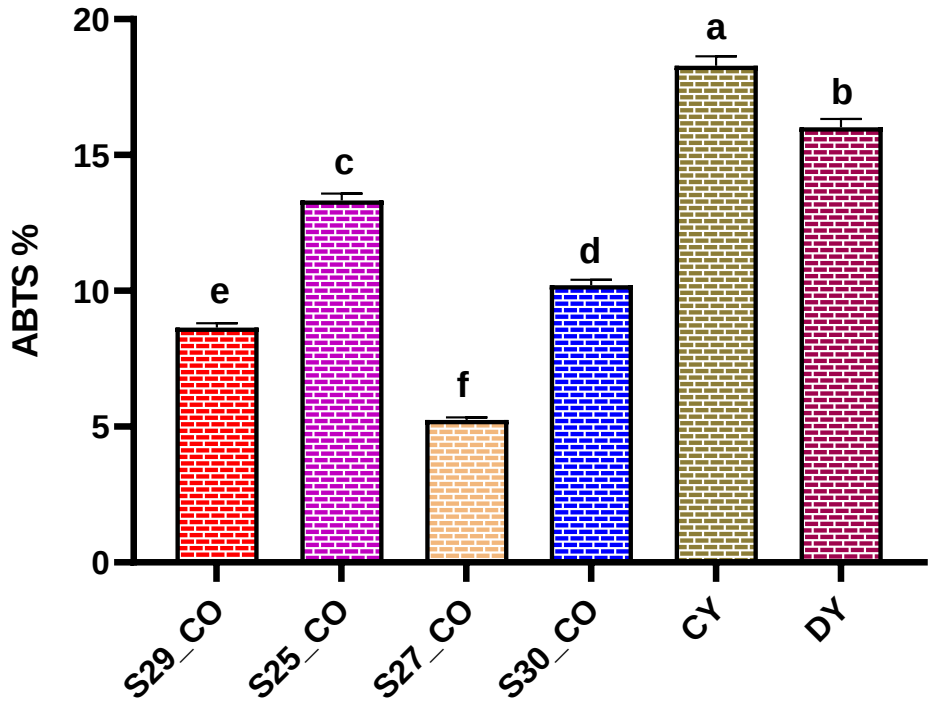


Figure 4.10: ABTS Scavenging Activities of Yoghurt Made from Soybean-Oat-Coconut Blends

Values represent the means of three replicates ± standard deviation. Mean values followed by different superscripts within columns are significantly different according to Duncan's Multiple Range Test ($p < 0.05$).

Keys:

S29_CO: 29.147 Soy Milk, 36.340 Coconut Milk, 34.512 Oat Milk, for 8 hours fermentation time

S25_CO: 25.000 Soy Milk, 38.213 Coconut Milk, 36.787 Oat Milk, for 8 hours fermentation time

S27_CO: 26.775 Soy Milk, 36.545 Coconut Milk, 36.680 Oat Milk, for 12 hours fermentation time

S30_CO: 29.6919 Soy Milk, 27.9098 Coconut Milk, 42.3983, Oat Milk, for 8 hours fermentation time

DY: Reconstituted Powdered Milk Yoghurt, for 8 hours fermentation time

CY: Commercial Yoghurt

4.7. Vitamin Composition of Yoghurt Produced from Soybean-Oat-Coconut Blends

The vitamin content of the soybean-oat-coconut yoghurt samples is shown in Figures 4.11 to 4.16. There were significant differences, as identified at a 95% level of confidence, in the vitamin composition of yoghurt samples between different fermentation times. Vitamin content of the Plant-based vitamin C, A, D, E, and K formulations is very different from commercial controls.

The vitamin C content varied between 1.91 and 3.10 mg/g. The highest value, 3.10 mg/g, was in DY, and the lowest value, 1.91 mg/g, was in S25_CO. The values for plant-based samples were in the middle, with S29_CO at 2.59 mg/g and S27_CO at 2.47 mg/g.

The vitamin A levels in the samples that were tested ranged from 365.17 to 848.28 µg RAE/g. The most were for DY (848.28 µg RAE/g), CY (652.76 µg RAE/g), and S27_CO (641.38 µg RAE/g). Vitamin A was found at the lowest level in the formulated samples, representing 365.17 µg RAE/g in S25_CO.

The Vitamin D contents varied from 0.21 to 0.44 mg/g. The maximum value obtained was for the S30_CO sample, at 0.44 mg/g, whereas a minimum content was obtained for the S27_CO sample, at 0.21 mg/g. The remaining samples have values between 0.23 and 0.40 mg/g.

Vitamin E content ranged from 0.14 to 0.31 mg/g. The highest was for S30_CO, with 0.31 mg/g, while S29_CO closely followed with 0.30 mg/g. The lowest levels measured included samples S25_CO and S27_CO at 0.14 mg/g each. Vitamin K's content ranged from 0.10 to 0.24 mg/g.

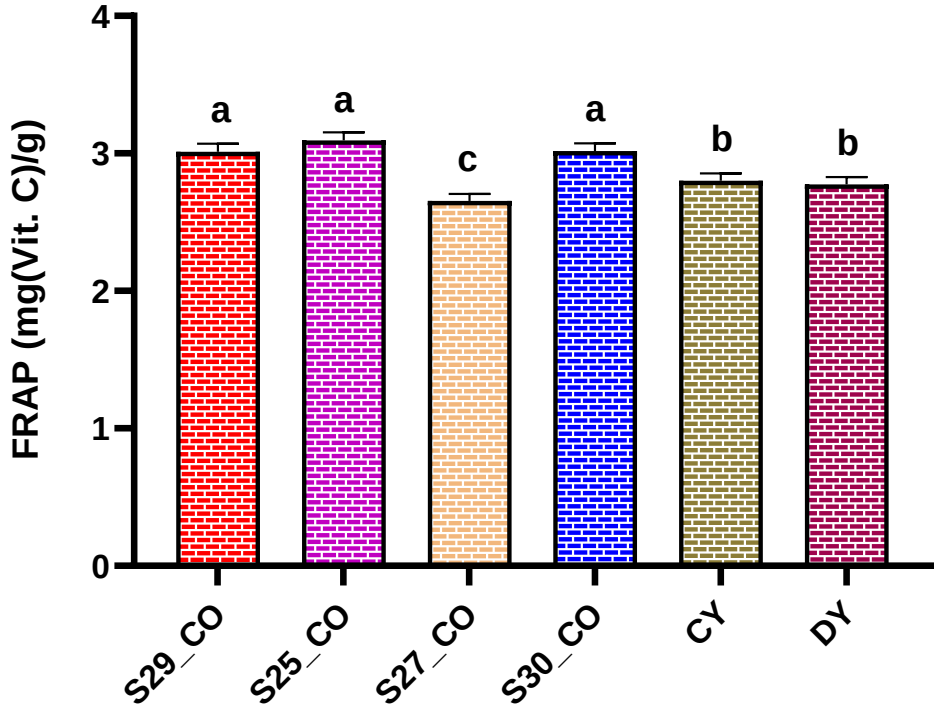


Figure 4.11: Ferric Reducing Power of Yoghurt Made from Soybean-Oat-Coconut Blends

Values represent the means of three replicates ± standard deviation. Mean values followed by different superscripts within columns are significantly different according to Duncan's Multiple Range Test ($p < 0.05$).

Keys:

S29_CO: 29.147 Soy Milk, 36.340 Coconut Milk, 34.512 Oat Milk, for 8 hours fermentation time

S25_CO: 25.000 Soy Milk, 38.213 Coconut Milk, 36.787 Oat Milk, for 8 hours fermentation time

S27_CO: 26.775 Soy Milk, 36.545 Coconut Milk, 36.680 Oat Milk, for 12 hours fermentation time

S30_CO: 29.6919 Soy Milk, 27.9098 Coconut Milk, 42.3983, Oat Milk, for 8 hours fermentation time

DY: Reconstituted Powdered Milk Yoghurt, for 8 hours fermentation time

CY: Commercial Yoghurt

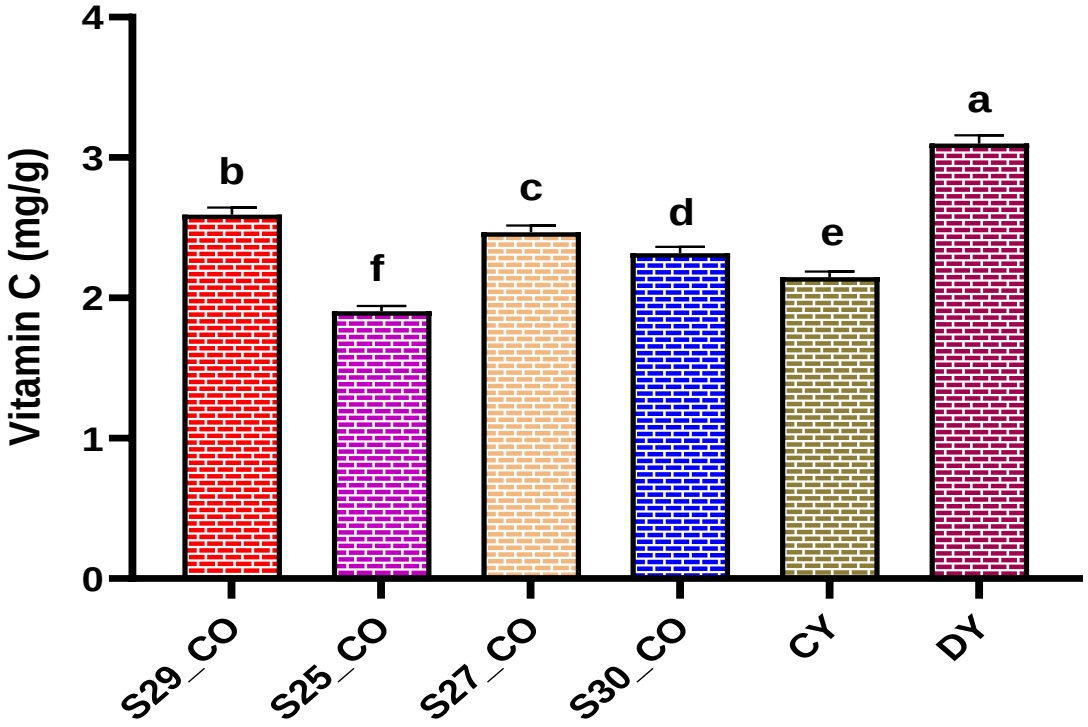


Figure 4.12: Vitamin C Content of Yoghurt Made from Soybean-Oat-Coconut Blends

Values represent the means of three replicates ± standard deviation. Mean values followed by different superscripts within columns are significantly different according to Duncan's Multiple Range Test ($p < 0.05$).

Keys:

S29_CO: 29.147 Soy Milk, 36.340 Coconut Milk, 34.512 Oat Milk, for 8 hours fermentation time

S25_CO: 25.000 Soy Milk, 38.213 Coconut Milk, 36.787 Oat Milk, for 8 hours fermentation time

S27_CO: 26.775 Soy Milk, 36.545 Coconut Milk, 36.680 Oat Milk, for 12 hours fermentation time

S30_CO: 29.6919 Soy Milk, 27.9098 Coconut Milk, 42.3983, Oat Milk, for 8 hours fermentation time

DY: Reconstituted Powdered Milk Yoghurt, for 8 hours fermentation time

CY: Commercial Yoghurt

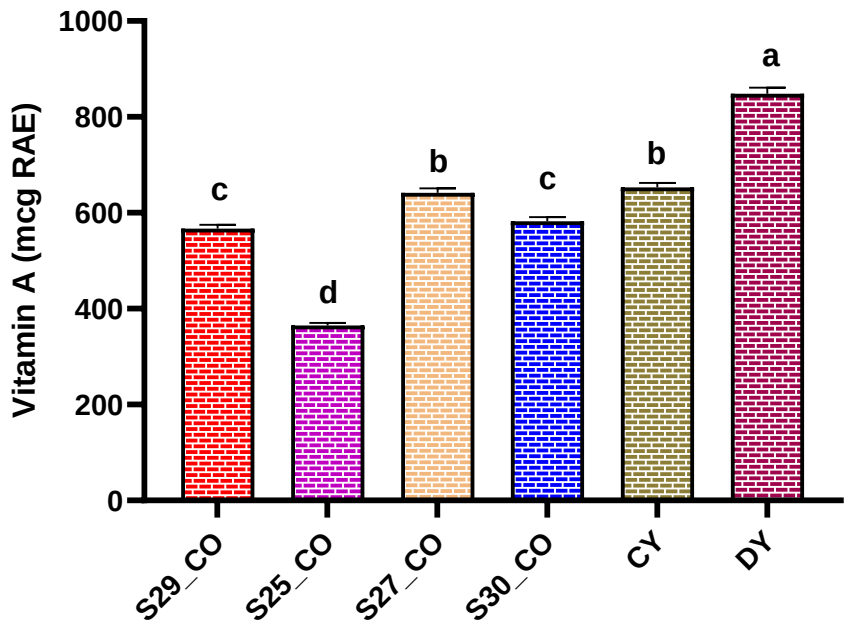


Figure 4.13: Vitamin A Content of Yoghurt Made from Soybean-Oat-Coconut Blends

Values represent the means of three replicates ± standard deviation. Mean values followed by different superscripts within columns are significantly different according to Duncan's Multiple Range Test ($p < 0.05$).

Keys:

S29_CO: 29.147 Soy Milk, 36.340 Coconut Milk, 34.512 Oat Milk, for 8 hours fermentation time

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S30_CO: 29.6919 Soy Milk, 27.9098 Coconut Milk, 42.3983, Oat Milk, for 8 hours fermentation time

DY: Reconstituted Powdered Milk Yoghurt, for 8 hours fermentation time

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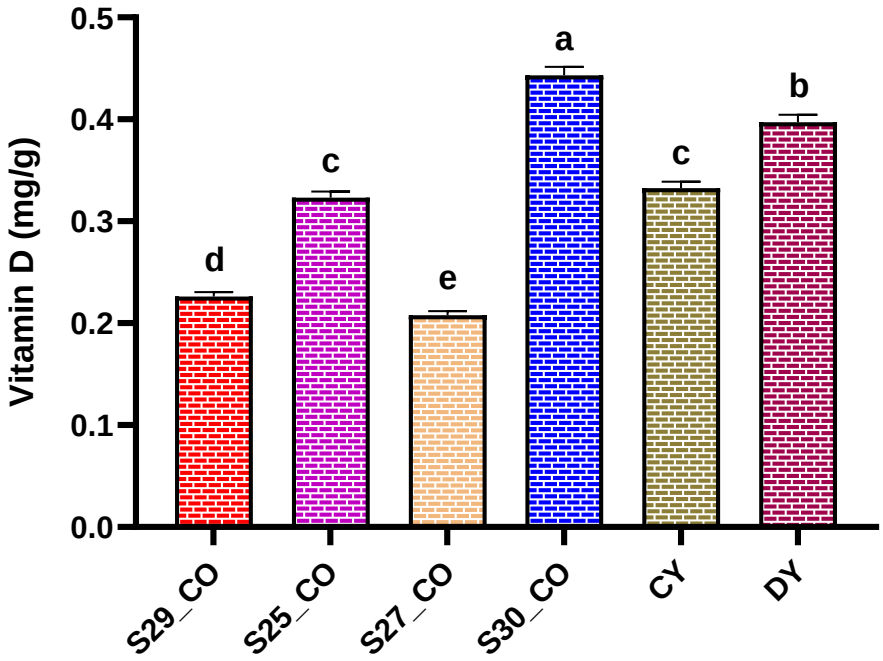


Figure 4.14: Vitamin D Content of Yoghurt Made from Soybean-Oat-Coconut Blends

Values represent the means of three replicates ± standard deviation. Mean values followed by different superscripts within columns are significantly different according to Duncan's Multiple Range Test ($p < 0.05$).

Keys:

S29_CO: 29.147 Soy Milk, 36.340 Coconut Milk, 34.512 Oat Milk, for 8 hours fermentation time

S25_CO: 25.000 Soy Milk, 38.213 Coconut Milk, 36.787 Oat Milk, for 8 hours fermentation time

S27_CO: 26.775 Soy Milk, 36.545 Coconut Milk, 36.680 Oat Milk, for 12 hours fermentation time

S30_CO: 29.6919 Soy Milk, 27.9098 Coconut Milk, 42.3983, Oat Milk, for 8 hours fermentation time

DY: Reconstituted Powdered Milk Yoghurt, for 8 hours fermentation time

CY: Commercial Yoghurt

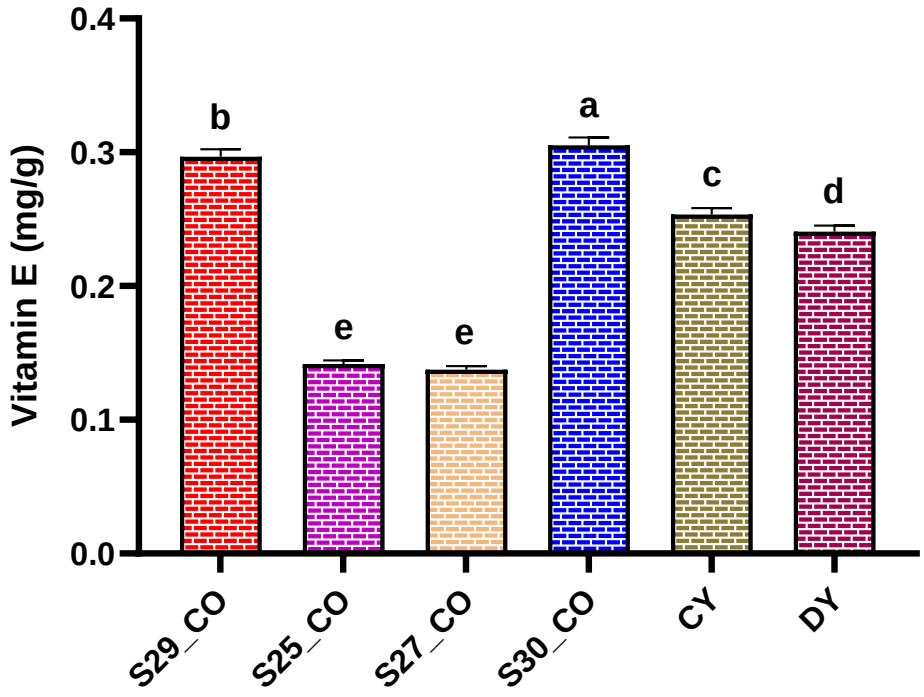


Figure 4.15: Vitamin E Content of Yoghurt Made from Soybean-Oat-Coconut Blends

Values represent the means of three replicates ± standard deviation. Mean values followed by different superscripts within columns are significantly different according to Duncan's Multiple Range Test ($p < 0.05$).

Keys:

S29_CO: 29.147 Soy Milk, 36.340 Coconut Milk, 34.512 Oat Milk, for 8 hours fermentation time

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S30_CO: 29.6919 Soy Milk, 27.9098 Coconut Milk, 42.3983, Oat Milk, for 8 hours fermentation time

DY: Reconstituted Powdered Milk Yoghurt, for 8 hours fermentation time

CY: Commercial Yoghurt

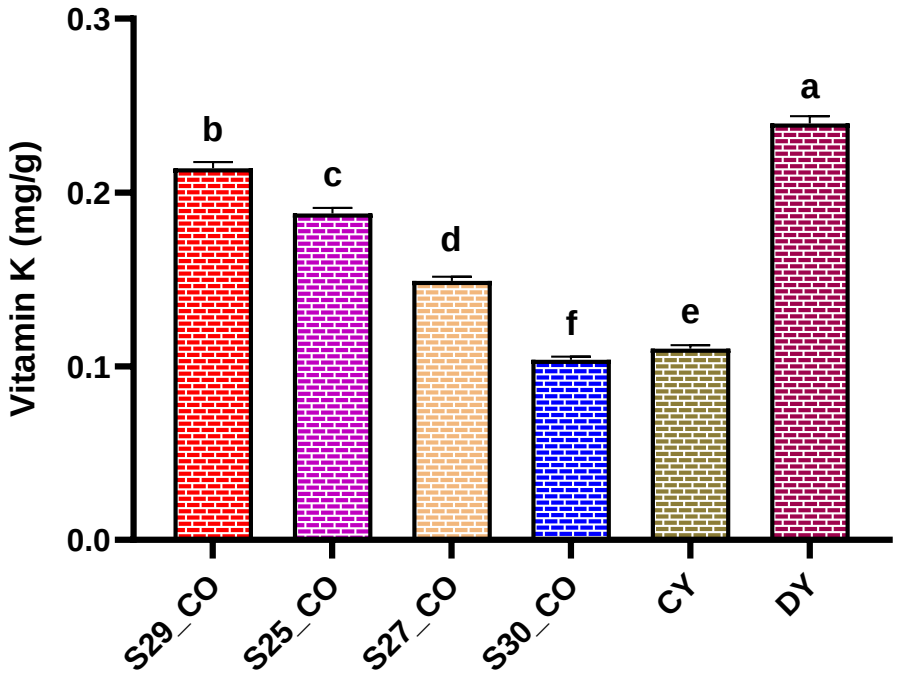


Figure 4.16: Vitamin K Content of Yoghurt Made from Soybean-Oat-Coconut Blends

Values represent the means of three replicates ± standard deviation. Mean values followed by different superscripts within columns are significantly different according to Duncan's Multiple Range Test ($p < 0.05$).

Keys:

S29_CO: 29.147 Soy Milk, 36.340 Coconut Milk, 34.512 Oat Milk, for 8 hours fermentation time

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S27_CO: 26.775 Soy Milk, 36.545 Coconut Milk, 36.680 Oat Milk, for 12 hours fermentation time

S30_CO: 29.6919 Soy Milk, 27.9098 Coconut Milk, 42.3983, Oat Milk, for 8 hours fermentation time

DY: Reconstituted Powdered Milk Yoghurt, for 8 hours of fermentation time

CY: Commercial Yoghurt

Sample DY had the highest value at 0.24 mg/g, while S29_CO and CY had values of 0.21 and 0.11 mg/g, respectively. Sample S30_CO had the least content of 0.10 mg/g.

4.8. Physicochemical Properties of Yoghurt Produced from Soybean-Oat-Coconut Blends

Table 4.9 depicts the physicochemical characteristics of yoghurts made with soybean-oat-coconut, monitored by pH, TTA, and soluble solids content (°Brix).

The pH of the samples was within the range of 3.68 to 4.93. The highest pH values determined were for S25_CO at 4.93, followed by S29_CO at 4.84 and S30_CO at 4.78, while the lowest value of pH belonged to CY, which was 3.68. The pH value of dairy yoghurt DY was relatively lower at 4.34, while among the plant-based samples, the lowest pH value measured was for S27_CO with a pH of 4.65.

Titratable acidity ranged from 0.04 to 0.07 g/100 mL. S27_CO had the highest value with 0.07 g/100 mL; S29_CO and DY showed the minimum with 0.04 g/100 mL, while all the remaining samples presented an acidity value of 0.05 g/100 mL.

Results indicated that the values of °Brix vary between 6.00% and 12.25%. Among all samples analysed, DY presented the highest °Brix value, 12.25%, whereas the remaining plant-based samples presented lower and close values of °Brix between 6.00% and 6.70%. For the formulated samples, the highest values corresponded to S30_CO, with 6.70%, whereas in S29_CO and S27_CO had the minimum values with 6.00%.

The values of viscosity in the samples varied greatly from one another, ranging from 0.6814 to 6.697 kg/m·s. S29_CO was the one with the least viscosity (0.6814 kg/m·s), on the contrary, DY had the highest viscosity (6.697 kg/m·s).

Table 4.7: Physicochemical Properties of Yoghurt Produced from Soybean-Oat-Coconut Blends

Samples	Ph	TTA g/100ml	BRIX%	Viscosity
S29_CO	4.84 ± 0.09^{ab}	0.04 ± 0.00^{d}	6.00 ± 0.00^{c}	0.68
S25_CO	4.93 ± 0.09^{a}	0.05 ± 0.00^{c}	6.25 ± 0.25^{c}	1.60
S27_CO	4.65 ± 0.08^{c}	0.07 ± 0.00^{a}	6.00 ± 0.00^{c}	2.29
S30_CO	4.78 ± 0.09^{bc}	0.05 ± 0.00^{b}	6.70 ± 0.00^{b}	3.72
CY	3.68 ± 0.07^{e}	0.05 ± 0.00^{c}	6.40 ± 0.40^{bc}	1.67
DY	4.34 ± 0.08^{d}	0.04 ± 0.00^{e}	12.25 ± 0.25^{a}	6.70

Values represent the means of three replicates ± standard deviation. Mean values followed by different superscripts within columns are significantly different according to Duncan's Multiple Range Test ($p < 0.05$).

Keys:

S29_CO: 29.147 Soy Milk, 36.340 Coconut Milk, 34.512 Oat Milk, for 8 hours fermentation time

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S27_CO: 26.775 Soy Milk, 36.545 Coconut Milk, 36.680 Oat Milk, for 12 hours fermentation time

S30_CO: 29.6919 Soy Milk, 27.9098 Coconut Milk, 42.3983, Oat Milk, for 8 hours fermentation time

DY: Reconstituted Powdered Milk Yoghurt, for 8 hours fermentation time

CY: Commercial Yoghurt

4.9. Microbial Load of Yoghurt Produced from Soybean-Oat-Coconut Blends

The microbiological evaluation revealed that all samples contained measurable populations of lactic acid bacteria (LAB), while *Escherichia coli* was completely absent across all dilutions tested. For the plant-based yoghurt samples (S25_CO, S27_CO, S29_CO, S30_CO), LAB counts were too numerous to count (TNC) at the 10^{-3} dilution, indicating very high microbial loads. Accurate counts obtained at 10^{-6} dilution ranged from 6.0×10^6 to 8.8×10^6 CFU/mL, with S29_CO (8.4×10^6 CFU/mL) and S30_CO (8.8×10^6 CFU/mL) showing the highest LAB populations.

In contrast, the dairy yoghurt (DY) and commercial yoghurt (CY) recorded lower LAB counts, each with 3.9×10^6 CFU/mL. Although still within a desirable probiotic range, these values were visibly lower compared to the plant-based formulations.

Across all samples and dilutions, *E. coli* was not detected (Nil), suggesting good hygienic practices and the absence of faecal contamination.

4.10. Sensory Properties of Yoghurt Produced from Soybean-Oat-Coconut Blends

Significant variation was clear among sensory attributes in the samples analysed for appearance, aroma, taste, flavour, consistency, and overall acceptability.

Appearance ranged from 5.13 to 8.06, S30_CO having the lowest rating of 5.13 and DY recording the highest of 8.06. S29_CO and S25_CO had similar ratings in appearance of 6.44-6.63, while CY had a rating of appearance of 6.50. Aroma followed a similar pattern, where DY and CY showed higher values

Taste and flavor scores further underlined the difference between the dairy and plant-based matrices. DY scored systematically higher than all other samples.

Table 4.8: Lactic Acid Bacteria and Coliform Load of the Yoghurt.

Sample	LAB $x10^3$	*E. coli* $x10^3$	LAB $x10^6$	*E. coli* $x10^6$
S29_CO	TNC	Nil	8.4	Nil
S25_CO	TNC	Nil	6	Nil
S27_CO	TNC	Nil	6	Nil
S30_CO	TNC	Nil	8.8	Nil
DY	TNC	Nil	3.9	Nil
CY	TNC	Nil	3.9	Nil

Keys:

ssS29_CO: 29.147 Soy Milk, 36.340 Coconut Milk, 34.512 Oat Milk, for 8 hours fermentation time

S25_CO: 25.000 Soy Milk, 38.213 Coconut Milk, 36.787 Oat Milk, for 8 hours fermentation time

S27_CO: 26.775 Soy Milk, 36.545 Coconut Milk, 36.680 Oat Milk, for 12 hours fermentation time

S30_CO: 29.6919 Soy Milk, 27.9098 Coconut Milk, 42.3983, Oat Milk, for 8 hours fermentation time

DY: Reconstituted Powdered Milk Yoghurt, for 8 hours fermentation time

CY: Commercial Yoghurt

TNC: Too Numerous to Count

Nil: Not Detected

Table 4.9: Sensory Properties of Yoghurt Produced from Soybean-Oat-Coconut Blends

Sample	Appearance	Aroma	Taste	Flavour	Consistency	Overall Acceptability
S29_CO	6.63 ±1.31^{b}	5.31 ±1.25^{bc}	5.25± 1.48^{bc}	5.50 ±1.03^{bc}	5.75 ±1.44^{bc}	5.69± 1.54^{bc}
S25_CO	6.44 ±1.46^{b}	5.69 ±1.01^{bc}	5.56 ±1.31^{bc}	5.44 ±1.26^{bc}	5.56 ±1.31^{bc}	5.75± 1.29^{bc}
S27_CO	5.56 ±2.39^{b}	5.36± 1.82^{bc}	4.75± 1.95^{c}	5.00 ±1.93^{c}	5.31± 1.92^{c}	5.12 ±1.75^{c}
S30_CO	5.13 ±2.63^{b}	4.81± 1.91^{c}	4.81± 2.20^{c}	4.81± 2.17^{c}	5.00 ±2.00^{c}	5.31 ±2.21^{c}
CY	6.50 ±2.50^{a}	6.38 ±2.16^{a}	6.44 ±2.22^{a}	6.50± 2.00^{a}	6.69± 2.02^{a}	6.81± 2.04^{a}
DY	8.06 ±1.18^{b}	7.50 ±1.41^{ab}	8.06 ±1.39^{b}	7.94± 1.24^{b}	7.63 ±1.63^{ab}	8.25 ±1.13^{b}

Values represent the means of three replicates ± standard deviation. Mean values followed by different superscripts within columns are significantly different according to Duncan's Multiple PRange Test ($p < 0.05$).

Keys:

S29_CO: 29.147 Soy Milk, 36.340 Coconut Milk, 34.512 Oat Milk, for 8 hours fermentation time

S25_CO: 25.000 Soy Milk, 38.213 Coconut Milk, 36.787 Oat Milk, for 8 hours fermentation time

S27_CO: 26.775 Soy Milk, 36.545 Coconut Milk, 36.680 Oat Milk, for 12 hours fermentation time

S30_CO: 29.6919 Soy Milk, 27.9098 Coconut Milk, 42.3983, Oat Milk, for 8 hours fermentation time

DY: Reconstituted Powdered Milk Yoghurt, for 8 hours fermentation time

CY: Commercial Yoghurt

These values ranged from 4.81 to 7.50. The rating by S30_CO, with an aroma value of 4.81, was the lowest, while that by DY was the highest, with an aroma value of 7.50. For CY, this value was 6.38, while in plant-based samples, S29_CO-S27_CO ranged from 5.31 to 5.69.

Scores for taste ranged from 4.75 to 8.06. S27_CO and S30_CO scored the lowest in taste within the range of 4.75-4.81. The best in taste was DY, scoring 8.06, while CY scored 6.44, and S29_CO and S25_CO scored within the range of 5.25-5.56.

Flavor ranged from 4.81 to 7.94. S30_CO got the lowest rating for flavor, which is 4.81, while DY secured the highest rating of 7.94. For CY, the rating is 6.50, while S29_CO - S27_CO falls in the range of 5.00 to 5.50.

The values of consistency varied within a range from 5.00 to 7.63. Plant-based samples S29_CO-S30_CO ranged from 5.00 to 5.75, and the highest value of consistency was recorded in DY with a value of 7.63. In CY, the value of consistency was moderate, recorded at 6.69.

Overall, the ratings of acceptability ranged between 5.12 and 8.25, S27_CO showing the minimum value at 5.12, while the maximum was for DY with 8.25; for CY, the overall value reached 6.81; the rest were between 5.31 and 5.75 for S29_CO, S25_CO, and S30_CO.

CHAPTER FIVE

DISCUSSION

The development of plant-based yoghurt alternatives has gained increasing attention due to the growing demand for lactose-free, functional, and sustainable food products. In this study, composite yoghurts were produced from blends of soy, coconut, and oat milk using mixture design optimization and controlled fermentation to evaluate their suitability as dairy yoghurt substitutes. This chapter discusses the results obtained from the formulation, optimization, and analytical evaluations, with emphasis on how the blend composition influenced the physicochemical properties, microbial quality, nutritional profile, and overall potential of the products as acceptable plant-based yoghurt alternatives.

5.1. Response Surface Optimization and Model Transformation for Sensory Quality of Optimized Yoghurt

The optimal zone of appearance at ~36% coconut milk showed that a balance between protein and fat enhances its opacity and surface sheen. Besides this, the 3D response surface suggests that with a higher oat content, increased light scattering, and viscosity will lead to a creamier, more stable visual texture. The results agreed with Constantin *et al*. (2025), who reported enhanced glossiness and color homogeneity in plant-based yoghurts using fat-protein synergism.

The residuals of the original flavour model were right-skewed. This is a common feature of sensory responses arising from nonlinear microbial flavour responses. This parameter was natural logarithmically transformed to enhance the significance level of the model.

A slight reduction of flavour scores for excessive oat over 40% may be due to its cereal-like aftertaste and reduced complexity of volatile compounds. From the curvature of the contour plot, one can appreciate that there is nonlinear synergy between the coconut medium-chain

triglycerides, which provide richness, and soy protein hydrolysates which contribute to overall flavour complexity a point also observed by Tangyu *et al*. (2019).

The contour and 3D plots for taste showed a well-pronounced saddle-shaped curvature, evidencing strong two-way interactions among the components. Moderate fermentation, between 8-10 hours, optimized the development of taste, which coincided with maximum proteolytic activity and balanced acid formation. Prolonged fermentation beyond 12 hours increased acidity, thereby decreasing palatability. Maximum desirability for taste was determined to take place with 30% soy, coconut 36%, and oat, 34%, again confirming that the synergy of lipid-protein-carbohydrate dictates the complexity of the taste profile. This finding is in tune with Patel *et al*. (2025), who also emphasized controlled fermentation to ensure a balanced taste sensation in non-dairy matrices.

Unlike for other sensory parameters, the distribution of the aroma response was well-behaved, and no transformation was necessary. Contour and 3D surfaces showed that aroma enhancement was most pronounced when soy and oat concentrations were balanced at about 30-35% each, with coconut at about 36%. Besides, BC interaction dominated with an F-value of 142.14, reflecting that the coconut lipid phase supports volatile retention and ester formation in the process of lactic fermentation. Moderate fermentation for 8-10 h maximized desirable esters and diacetyls, in agreement with Liu *et al*. (2024), who reported an increase in the complexity of aromatic compounds as a result of medium-chain lipid substrates during fermentations using mixed plant-based matrices. Excessive fermentation which reduced the intensity of aroma is probably due to volatilization of the aroma compounds and denaturation of proteins by acid.

The consistency model required an inverse transformation to homogenize variance, which further normalized the residuals. It has a strong precision value which supported adequate model performance. Contour plots indicated that the increase in oat proportions significantly improved

consistency within a range of 35-42%, again supportive of the viscosity-enhancing role played by oat β-glucans and starch fractions. The 3D response surfaces demonstrated dome-shaped curvatures, and the BC interaction was again predominant at F = 41.96, showing that coconut's lipid phase modulated the smoothness and stability of the gel matrix. This is supportive of the rheological trends observed in cereal-legume yoghurts by Chetachukwu *et al*. (2019). The 8-hour fermentation resulted in an optimal consistency that balanced microbial exopolysaccharide formation with protein-polysaccharide interactions.

In terms of the overall acceptability, there was a mixture matched up with S29_CO, which showed the same high uniformity in all sensory parameters.

A longer fermentation period of 12 hours would likely lead to lower overall acceptability because of high acid and volatile compounds; in contrast, a shorter fermentation period would result in less flavour and texture development. These findings corroborate the reports of Okafor and Okoro (2025) and Constantin *et al*. (2025): optimal or moderate fermentation times do ensure a proper balance among acidity, flavour, and texture in plant-based yoghurts. The production of the transformed parameter and the significant fit diagnostics across all sensory responses reinforced the reliability of predictions for the model transformations as well. The log, square root, inverse, and inverse-square root transformations that were applied were able to normalize variance in a way that showed improved model sensitivity, thus confirming their necessity, as validated by the ANOVA metrics. Sound experimental design was ensured by the high R^2 (≥ 0.95), adjusted R^2 (≥ 0.88), and adequate precision for all transformed models (>12).

5.2. Proximate Composition of Yoghurt produced from soy, oat, and coconut milk

The moisture content of all the samples is in tandem with that reported by Ityotagher and Julius (2020), where their value ranged from 78.20 to 86.23%, depending on formulation and processing

parameters. According to Vareltzis (2016), increasing levels of moisture correspond to an increase in water activity that may support microbial spoilage if acidity or refrigeration is not controlled in the product, or if preservatives are not used. CY and DY, therefore, may experience more deterioration and require more stringent hygienic and storage control to maintain shelf stability.

Güzeler *et al*. (2022) explained that ash is the total mineral residue after the complete combustion of organic matter, which expresses the mineral composition in food. Higher ash content in sample CY reflects a probable higher mineral load, such as calcium, magnesium, and phosphorus. Igbabul *et al*. (2014) found the ash content of commercial yoghurts produced within Makurdi, Nigeria, to be between 0.41 and 1.02%.

Generally, Marlapati *et al*. (2024) illustrated that soy-based yoghurts contain 4.0% protein, whereas oat-based yoghurts contain 3.0% protein; both values agree with the result in this study. Yoghurt is a bioavailable source of complete protein, as it provides essential amino acids that enhance muscle maintenance, enzyme synthesis, and metabolic control (Sumi *et al*., 2023). This makes the samples, particularly S25_CO and S25_CO, good choices to combat protein malnutrition. More importantly, the higher protein content in sample S29_CO may be attributed to the higher soymilk of 29.147 in its formulation.

Fat is nutritionally and functionally important since it changes the energy density, mouthfeel, and sensory characteristics of yoghurt (Delikanli and Ozcan, 2017). Also, it modifies the ratio of saturated to unsaturated lipids, affecting sensory perceptions and health. Thus, this higher fat value will likely increase the energy content of S29_CO, since fat contributes more to the caloric value of food (Shakpo *et al*., 2025). Additionally, fat tends to give it a creamier texture and fuller taste, which are among the preferred sensory characteristics of yoghurt among consumers, according to Constantin *et al*. (2025).

Elleuch *et al*. (2011) pointed out that higher levels of fiber in yoghurt preparations are advisable for their role in digestive health promotion, bowel regularity, cholesterol reduction, and control of glycemia. Fiber is also a good satiety promoter, enabling one to reduce his/her intake of calories with possible weight management (Slavin, 2013). Therefore, a high fiber value in CY (0.33%) nutritionally enriched such yoghurt varieties, while the low levels of S25_CO and S30_CO favored the action of getting a smoother consistency, but limited functional health beneficial properties related to crude fiber.

The values observed across all the samples for carbohydrate, are higher than the reported values for yoghurt in the literature. For example, 14.96% observed in sample S25_CO is approximately 147%higher than the highest value of 6.05% reported by Shakpo *et al*. (2025).

Amirdivani and Baba (2011) indicated that the caloric value of yoghurt is determined by its fat, protein, and carbohydrate contents, which together determine its nutritional density. Thus, the high energy content in S29_CO could be attributed to the high fat and carbohydrate concentrations, while the low energy of CY was due to its low level of these macronutrients

5.3. Antinutritional Factors of Yoghurt Produced from Soybean-Oat-Coconut Blends

These changes across the samples S29_CO to S30_CO, as well as the reference yoghurts, CY and DY, were complex interactions involving time, substrate, and microbial metabolism. Additionally, the decrease of bioactive compounds was strongly linked to ratios of ingredients and fermentation time. Generally, phytate, oxalate, trypsin inhibitor, tannin, and saponin were lower in the fermented sample at 12 hours of fermentation (S27_CO), compared to other samples fermented at 8 hours of fermentation, which suggested that longer incubation time improves microbial degradation of anti-nutrients.

The pattern observed in phytate content is because fermentation activates endogenous and microbial phytases, hydrolyzing phytate and consequently releasing the minerals bound during fermentation. This agrees with Gupta *et al*. (2015) and Yang *et al*. (2020). The small reductions across the plant-based formulation indicate that both fermentation time and substrate combination ratio have effects on enzymatic actions. Soybeans and oats are known to have intrinsic phytase. However, their activities are normally poor due to substrate availability and pH issues (Kumar and Sinha, 2018). Thus, the relatively higher phytate amount in S29_CO and S30_CO could be because it takes a longer time for fermentation to degrade it, or probably nutrient metabolisms are competing against the action of lactic acid bacteria. Lower phytate concentration improves mineral bioavailability, such as iron and zinc, hence improving the nutritional quality of food (Adeleye *et al*., 2025). This may position S27_CO well as a good choice in terms of mineral bioavailability.

The lower level of oxalate observed in the sample fermented for twelve hours reveals that long-time fermentation drastically reduces the accumulation of oxalate. In lactic fermentation, microbial metabolism and enzymatic decarboxylation have been shown to degrade oxalates. High values of oxalate in dairy yoghurts at 17.42 mg/g contrast with the low values in most of the plant-based samples and could be an indication that, on a mixed plant substrate, microbes able to utilize oxalate would be favored. High intakes of oxalate may reduce calcium absorption and increase the risk of kidney stones. Thus, this is a nutritionally favorable aspect of controlled fermentation.

Inactivation of trypsin inhibitors in this work corroborates reports that LA fermentation enhances the denaturation of heat-labile protein inhibitors and accelerates proteolytic cleavage (Avilés-Gaxiola *et al*., 2018). The presence of coconut milk in the blend would have increased the action of the protease by supplying medium-chain fatty acids, thus stimulating the growth of microbes (Patil and Benjakul, 2018). Low TI values are indicative of good protein digestibility that ensures

sensory smoothness of fermented products (Manzoor *et al*., 2021). The higher inhibition in S29_CO and CY (59.59 and 62.41%, respectively) reflect the influence of the duration of fermentation and microbial load, being cardinal determinants of enzyme inactivation.

The pattern observed in the tanin level among the samples could be due to the hydrolyzing actions of tannase-producing lactic acid bacteria, which cleave tannin-protein complexes, which may improve flavour perception. According to earlier studies, this could be the case in the works of Adebo *et al*. (2022) and Verni *et al*. (2022). On the other hand, in the case of S30_CO, the tannin concentration was high at 3.31 mg QE/g and may be related to a high inclusion level of oats, since oats contain more polyphenolic compounds. High tannin content has adverse effects on protein digestibility and causes astringency; hence, there is a need to optimize fermentation parameters to improve sensory qualities. This is in contrast to moderate residual tannins, which may contribute to their antioxidant potential, hence desirable in functional plant-based yoghurts.

The higher level of S25_CO compared to the other samples, probably due to the use of a higher proportion of coconut milk, is reported as a good source of steroidal saponins. However, a significant decrease to 16.50 mg/g was observed in S27_CO. Thus, extended fermentation seems to be effective for the cleavage of the glycosidic bonds due to microbial β-glucosidase activity. Saponins improve the stability of foam and mouthfeel at moderate levels but may impart bitterness or even a hemolytic effect at high levels. Thus, lower saponin content in S27_CO might imply a better balance in flavour and consumer acceptance.

5.4. Mineral Composition of Yoghurt Produced from Soybean-Oat-Coconut Blends

The mineral profile of the plant yoghurts reflected strong influences from specific blend ratios of soy milk, coconut milk, and oat milk, together with the conditions applied for fermentation. Since each formulation was made up of different proportions of these plant matrices, the mineral profile

was naturally influenced by the intrinsic mineral densities of legumes (soy), grains (oat), and oil-rich seeds (coconut), all of which have rather different mineral profiles from dairy-based matrices. In contrast, the commercial yoghurt (CY) and reconstituted dairy yoghurt (DY) showed a mineral profile influenced by the naturally lower mineral density of standardized dairy systems and the effects of industrial processing.

Higher sodium in plant-based samples like S30_CO and S25_CO, whose formulations seem to be influenced by sodium-rich soy and oat inputs. The formulations, such as S29_CO and S25_CO, contained 8-hour fermented blends with moderate soy and relatively higher coconut proportions; the latter generally contains lower intrinsic sodium (Alva, 2021), leading to moderate Na levels. CY and DY exhibited much lower sodium, consistent with commercially standardized dairy products, where sodium is not concentrated (Maalouf *et al*., 2015).

The significant variation in calcium level was an outlier due to its ingredients of higher Ca density. The yoghurt sample, which contains a higher level of soy milk (S30_CO), outperformed other samples in terms of calcium content. This may be as a result of a usually higher level of calcium in soybeans compared to both oat and coconut (Walther *et al*., 2022). Interestingly, S27_CO, fermented for 12 hours, did not show significantly higher calcium when compared to its 8-hour fermented counterparts; thus, Ca concentration was not influenced by fermentation time. This showed that the bioavailability of calcium in this study rested mainly on the yoghurt constituents rather than the period of fermentation. However, this negates the claim of Ahmed *et al*. (2020), where their product showed a better level of minerals with an increased fermentation time. Far beyond other samples, S30_CO will help improve bone health.

Soy and oat content strongly influenced potassium, as both ingredients are naturally rich in the mineral (Moisa *et al*., 2024). Thus, formulations such as S25_CO and S29_CO, which contained

balanced soy-oat ratios, exhibited moderate potassium values. S25_CO, the formulation with the highest potassium concentration, seems to reflect even greater contributions from oat-rich matrices. In contrast, DY and CY had lower potassium values consistent with dairy systems where potassium is lower and subject to dilution through reconstitution and processing.

Zinc content was low across all samples, consistent with the naturally low density of zinc in soy, oat, and coconut milk. Samples somewhat higher in zinc-for example, S30_CO and DY-are consistent with minor variations in trace mineral composition. Fermentation time did not significantly affect zinc levels, consistent with minimal microbial breakdown or release of Zn-binding compounds during fermentation.

Magnesium was highest in samples with high oat and soy components, such as in S30_CO and S25_CO. Oat milk is high in magnesium; thus, samples containing higher oat proportions had high levels of Mg. S30_CO exhibited the highest magnesium level due to its high oat proportion. The lowest level of magnesium was in CY and S27_CO, consistent with dairy-based matrices containing rather small quantities of Mg.

Iron values remained low and within a narrow range, a behavior consistent with plant-based milks, which contain trace but not substantial iron levels. Samples with slightly higher iron (S27_CO, CY, DY) reflect minor variations in ingredient composition, while fermentation duration (8 h vs 12 h) did not markedly affect iron. The body uses iron to make certain hormones and neurotransmitters (Gupta, 2014). This positions S27_CO as a good entity in this regard.

Phosphorus varied substantially and was markedly higher in samples with higher soy or oat proportions because both legumes and cereals have higher phosphorus content. DY and S29_CO gave the highest phosphorus, consistent with casein-associated phosphorus in dairy and phytate-

bound phosphorus in plant matrices. CY had the lowest phosphorus, probably a function of commercial dilution and processing.

The Na/K ratio reflected the fundamental electrolyte balance controlled by plant-milk ratios. Samples with higher relative sodium compared to potassium (for example, higher sodium values observed in S30_CO may be related to the combined contribution of soy an oat composition) presented higher ratios. In contrast, CY and S25_CO had more favorable Na/K ratios with their lower sodium or higher potassium. No clear Na/K advantage was observed for the 12-hour sample (S27_CO), suggesting that fermentation time did not greatly change the electrolyte balance. Sample S25_CO and CY had the best ratio. This is because their Na/K value is lower than one. This is a reflection of their suitability for electrolyte balance in the body.

Differences in the Ca/P ratios were strongly related to the inherent distribution of structural minerals in the plant milks. The oat- and soy-enriched formulations, like S29_CO and S30_CO, provided moderate Ca/P ratios, whereas coconut-heavy blends offered a lower ratio due to the limited calcium contribution of coconut. CY and DY showed a lower Ca/P, as is commonly seen in dairy products, where phosphorus tends to be in higher concentration compared with calcium unless fortified. Balancing Ca/P supports efficient bone formation and maintenance (Zhang *et al*., 2024). However, just like majority of African diets, this ratio isn't balanced in the samples.

The Na/Ca ratio varied with the interaction between the monovalent and divalent minerals in each formula. Soy-dominant blends, like S29_CO and S25_CO, showed higher Na/Ca ratios because of the naturally higher sodium in soy. Samples with higher oat and coconut portions, especially S30_CO, showed more moderate ratios.

The ranges of Mg/Ca ratios were narrow, which agreed with the co-distribution of these minerals in the soy and oat matrices. Higher ratios appeared in samples with greater oat influence; the dairy samples showed the lowest ratio because of their lower Mg content and moderate Ca. Fermentation time in S27_CO did not meaningfully shift this ratio.

The Fe/Zn ratio did not change in any of the samples, mostly maintaining within the 1.00-1.50 range, demonstrating the natural dominance of iron over zinc for both plant and dairy matrices. Stability of this ratio suggests a minimum effect of fermentation or blend composition on trace-metal dynamics.

5.5. Antioxidant Properties of Yoghurt Made from Soybean-Oat-Coconut Blends

Fermentation significantly affected the antioxidant properties of soybean-oat-coconut yoghurts, as shown by their DPPH and ABTS radical scavenging activities, total flavonoid and phenolic contents, and ferric reducing antioxidant power. The differences among the experimental samples S29_CO-S30_CO and reference yoghurts CY and DY suggest that fermentation time and compositional balance modulate antioxidant potential through changes in both the bioactive compound matrix and microbial metabolism.

Phenolic enrichment in S25_CO could be due to a synergistic effect of 8-hour fermentation on a mixture rich in coconut milk and oat matrix, which contain phenolic acids and avenanthramides, respectively, as indicated by Santos *et al*. (2018). In any case, microbial fermentation liberates bounded phenols from complex carbohydrates through β-glucosidase and esterase activities, as stated by Adebo *et al*. (2022). Although S27_CO (12-hour) showed a slight decline in TPC, increased DPPH and FRAP suggest that antioxidant derivatives with increased power might have been generated from the phenolic compounds. Therefore, the relationship between the content and

antioxidant activity is not always directly related since compound structure and bioavailability is also critical for the redox efficiency.

The trend observed for TFC indicates that flavonoid retention was higher in the blend with greater proportions of soybean and coconut milk, due to their isoflavones and flavan-3-ols, respectively, as reported by Zeng *et al*. (2020). However, the prolonged fermentation in S27_CO resulted in a net moderate reduction in TFC, at 0.28 mg QE/g, possibly a result of microbial assimilation or oxidation of flavonoid molecules upon extended incubation. Such a tendency follows the observation made by Hur *et al*. (2014), who reported that extended fermentation enhances total antioxidant activity but may reduce measurable flavonoid content since free radicals are utilized during metabolic processes. From a sensory point of view, modest levels of flavonoids contribute to the subtle bitterness and color stability of plant-based yoghurts.

Antioxidant activity could be greatest in S27_CO during 12-hour fermentation because longer microbial activities tend to release bound phenolic compounds and peptides with radical-quenching properties. In general, LABs have been reported for the biotransformation of polyphenols into more small-sized, bioavailable forms by enzymatic hydrolysis. As stated, the strategy of biotransformation has been employed in various foods with potential nutritional benefits (Zhao *et al*., 2021; Kumar *et al*., 2025). The low values obtained for S29_CO and DY could probably be explained by their lower efficiencies in fermentation or fewer substrates for phenolics from the raw blends. Enhanced DPPH activity, hence would mean an improved aptitude against oxidative stress associated with chronic diseases and lipid peroxidation in food systems.

Despite the high values of DPPH shown by S27_CO, its lower ABTS activity underlined the selectivity of antioxidant assays to different radical species (Yang *et al*., 2025). This could be related to the nature, polarity, and solubility of the antioxidant compounds formed during

fermentation. Fortification with an ascorbate-stabilized system may explain the higher ABTS activities shown in commercial yoghurt (CY) and reconstituted dairy yoghurt (DY, 16.02%) reported by Wang *et al*. (2022). However, the moderate ABTS values throughout this plant-based yoghurt have evidenced appreciable antioxidant potential in the plant-based formulations.

The slight S27_CO reduction (2.66 mg/g) in FRAP might show that longer-term fermentation could have shifted the balance toward radical-scavenging activity over ferric ion reduction. This stability of FRAP values has also been observed in the fermented legume-based yoghurts, whereby the equilibrium in the concentration of redox-active compounds maintains itself independently of changes in phenolic concentration (Han *et al*., 2021). The comparative FRAP between the plant-based samples and commercial yoghurts suggests that soy-oat-coconut blends may generate antioxidant capacities comparable to conventional dairy systems and support their functionality as a potential health-promoting food

5.6. Vitamin Composition of Yoghurt Produced from Soybean-Oat-Coconut Blends

The vitamin C content indicates that ascorbic acid retention was significantly influenced by both the duration of fermentation and substrate composition. The high values of vitamin C obtained in dairy yoghurt (DY) and some blends like S29_CO and S27_CO could be indicative that metabolic activity in LAB is probably implicated in the stabilization of ascorbate due to decreased oxidation of the compound during fermentation (Nguyen *et al*., 2022). Alternatively, this may be a result of oxidative losses associated with longer exposure to oxygen or because of lower synergistic protection provided by the phenolics during their protective action in that particular blend (Chen *et al*., 2022). Vitamin C is an important nutrient in immune modulation and antioxidant defense; hence, S29_CO and S27_CO are potential non-dairy yoghurt options with comparable ascorbic acid retention rates to those of dairy yoghurt.

The relatively high vitamin A content found in DY was also seen for the plant-based yoghurts S27_CO, S30_CO, and CY. This suggests that the addition of coconut and soybean could contribute to provitamin A carotenoids that can be transformed into their active forms by enzymatic conversion during fermentation processes (Kaur *et al*., 2019). Some LAB strains were reported to synthesize or conserve carotenoid derivatives during fermentation, improving vitamin A bioavailability, such as *Lactobacillus plantarum* and *Streptococcus thermophilus* (Wang *et al*., 2021). Low levels of vitamin A observed in S25_CO may be related to a low proportion of coconut or soybean or to the oxidative cleavage of β-carotene during long fermentation time, previously reported for cereal-legume yoghurts (El-Said *et al*., 2022).

Vitamin D content varied from 0.21 ± 0.00 mg/g in the case of S27_CO to 0.44 mg/g for S30_CO. The relatively higher value of vitamin D obtained in S30_CO could be because of microbial biotransformation of ergosterol or phytosterols into vitamin D metabolites under conditions of fermentation-promoted oxidative stress (He *et al*., 2025). Though generally, yoghurt is said to have a better natural background for vitamin D, this capability of a nondairy blend such as S30_CO to reach values closer to those obtained in yoghurt establishes the feasibility of fortification through microbial and substrate engineering. This is in tune with very recent trends that have come up, emphasizing plant-based matrices as equally good carriers for vitamin D enrichment (Kumar *et al*., 2023).

Relatively speaking, this higher α-tocopherol content in S30_CO and S29_CO may perhaps be because of the coconut lipid fraction and the unsaturated fatty acids acting as a tocopherol reservoir. Ani *et al*. (2018) explained that partial hydrolysis of lipids during fermentation enhances the release of bound tocopherols, thus boosting their extractable concentration. According to Kumari *et al*. (2017), it could be because of oxidative degradation or a difference in fat composition

among these various blends. Vitamin E plays an antioxidant role, which suggests that such a blend may be capable of supporting the oxidative stability and improving the shelf-life quality of fermented nondairy yoghurts.

LAB, such as *L. lactis* and *Leuconostoc spp*. are known for menaquinone synthesis and thereby enrichment of foods with vitamin K_2. Simes *et al*. (2020) have summarized that fermented foods created by LAB contain high levels of menaquinones. On this basis, the relatively moderate vitamin K contents in yoghurt from plant-based ingredients, in this case S29_CO and S25_CO, would therefore suggest a probable microbial contribution, although substrate differences may influence nutrient availability. Fermentation in a coconut-based matrix favors microbial vitamin K synthesis because of the presence of a rich-lipid medium. According to Liu *et al*. (2024), this increases the microbial vitamin K biosynthesis during fermentation. With respect to calcium metabolism and bone health, these moderate levels of the vitamin may position these yoghurts as complementary dietary sources, particularly in lactose-intolerant individuals.

5.7. Physicochemical Properties of Yoghurt Produced from Soybean-Oat-Coconut Blends

The large differences obtained in the physicochemical properties were not only linked to substrate composition but also to fermentation time. Higher pH values were observed for composite yoghurts (S29_CO-S30_CO) compared to commercial control (CY), indicating moderated acidification. This may be due to the buffering activity of soy proteins and the lipid content of coconut milk, which may neutralize the organic acids that accumulate during the fermentation process (Qadi *et al*., 2023). Such results are in agreement with the expected differences between dairy-based and plant-based formulations, as lactose fermentation in dairy-based yoghurt yields lactic acid as the main end product, while the plant substrates usually present slow acidification rates, partly because of the low fermentable sugars and high protein-to-carbohydrate ratios (Yu *et*

al., 2025). Sample S25_CO presented the highest pH, 4.93, probably because of the balanced blend ratio offering the best microbial substrate availability without fast acid buildup (25% soy, 38% coconut, 37% oat).

The longer time of fermentation of S27_CO resulted in higher acid accumulation due to longer metabolism by lactic acid bacteria, which corroborates the findings of Xu *et al*. (2024), showing that longer incubation supports increased lactic acid yield up to nutrient exhaustion. The moderate acidity of S25_CO and S30_CO (0.05 g/100 mL) confirms the hypothesis that substrate synergy between soy proteins and oat β-glucans could facilitate controlled fermentation, hence maintaining an acceptable acid-base balance that is crucial for flavor and microbial stability. Low acidity in DY, 0.04 g/100 mL, despite its animal-milk base, may be due to reconstitution effects or a lower microbial load compared to fresh fermentations. In general, the inverse trends of pH and TTA, as observed, agree with the lactic fermentation pattern as expected and again establish acid production as a prime driver of pH reduction, as reported by Hidalgo-Fuentes *et al*. (2024).

The highest value obtained in sample DY reflects a higher carbohydrate concentration or added sweeteners common in commercial dairy formulations. Among plant-based blends, S30_CO gave relatively higher soluble solids at 6.70 °Brix, probably due to its increased proportion of oat milk (42.4%), supplying maltose and other soluble carbohydrates that add to sweetness and viscosity. Yuan *et al*. (2022) say that fairly stable values of °Brix, within the range 6.00-6.70%, across S29_CO-S30_CO, reflect good consistency in sugar utilization efficiency by the fermenting microbes throughout the blends and thus infer proper substrate availability for metabolic conversion without excessive saccharide degradation. These variations in physicochemical parameters underline how fermentation time and compositional matrix may modulate microbial metabolism, acid production, and the dynamics of soluble solids. Results have shown that

improved blending of soy, oat, and coconut milks can produce acidity and sweetness comparable to yoghurt from cow milk while sustaining moderate pH and well-balanced sensory acceptability. Such improvements bear the potential for formulating plant-based yoghurts that have desirable profiles of acidity and stability for lactose-free or vegan consumers (Constantin *et al*. 2025; Okafor and Okoro, 2025)

Since viscosity is an intensive property, the pattern of viscosity across the samples is indicative of the combined influence of the blend composition, matrix structure, and intrinsic rheological behaviors of plant-based versus dairy-based systems. In the case of the plant-based yoghurts, represented by S29_CO-S30_CO, a definite gradation in viscosity was observed. This suggests that with increasing proportions of soy, coconut, or oat milk, changes had been induced in the structural density and water-binding capacity of the resulting gels. The lowest viscosity observed in S29_CO probably represents a formulation with either reduced total solids or weakened gel-forming components, while the higher viscosities obtained in S25_CO and S27_CO correspond to progressively stronger or more cohesive networks of the plant milk. Among plant-based samples, S30_CO was one of the most viscous. This may be due to the higher oat level in the formulation. This high viscosity in DY reflects the interaction between casein micelles and milk fat globules during fermentation, which builds a strong three-dimensional network typical of traditional dairy yoghurts. This may also reflect the probable addition of a thickening agent by the producers.

5.8. Microbial Load of Yoghurt Produced from Soybean-Oat-Coconut Blends

Microbiological outcomes showed distinct trends across the samples, with clear differences in lactic acid bacteria and S. thermophilus levels between the plant-based formulations and the dairy-based yoghurts. Higher LAB counts were found in the plant-based samples (S29_CO–S30_CO) than DY and CY, suggesting that the soy–oat–coconut matrix favored more extensive microbial

proliferation during fermentation. This agrees with the availability of fermentable substrates in plant-based milks, including especially oligosaccharides, soluble fibres, and other non-dairy sugars, which enhance LAB growth kinetics compared to dairy substrates.

On the contrary, DY and CY showed lower counts for both LAB in response to dairy matrices' characteristics. Usually, commercial yoghurts are subjected to homogenization, addition of stabilizers, or thermal treatments that can reduce microbial availability of substrates or impact

culture viability. Similarly, reconstituted DY normally has lower solid content and reduced microbial-supporting nutrients compared to fresh dairy milk, explaining why it recorded the lowest values of LAB. The absence of *E. coli* in all samples shows conformity to microbiological safety standards for fermented foods. Such an outcome reflects appropriate fermentation conditions, hygienic handling, and natural inhibitory properties of LAB, which through lower pH and generated metabolites suppress the growth of pathogens.

5.9. Sensory Properties of Yoghurt Produced from Soybean-Oat-Coconut Blends

The clear differences of the sensory profiles, particularly between the plant-based yoghurts S29_CO-S30_CO and the commercial CY and DY, paralleled the trends in the compositional and structural properties of the formulations. Higher scores for appearance in the CY and DY than for the plant-based yoghurts suggest that commercial dairy systems have perhaps benefited from standardized processes that optimize visual homogeneity, whereas the plant-based systems, and particularly S27_CO and S30_CO, showed higher appearance variability, likely owing to the varying optical properties of the soy, oat, and coconut matrices.

The higher aroma values in DY and CY point out their more marked volatile profiles shaped by dairy fermentation pathways. On the contrary, the plant-based samples showed relatively lower

aroma scores, consistent with the milder aromatic release of soy-oat-coconut systems. S30_CO, featuring the poorest sensory balance, presented the lowest rating in terms of aroma, indicating that its specific blend did not produce marked aromatic notes. The higher aroma observed in S25_CO may be attributed to a much higher coconut milk in the sample. This claim is supported by Xing *et al*. (2025), who reported high volatile compounds in coconut milk. From this study, it was observed that the higher the coconut milk, the better the aroma.

Lactose fermentation products, fat content, and protein structure are responsible for the richness of flavor of dairy yoghurts (Lesme *et al*., 2020). CY scored high in taste and flavour, aligning with commercially optimized formulations. The plant-based samples showed low taste and flavour scores, with S27_CO and S30_CO presenting the least preferred taste/flavor profiles. These findings correspond to the intrinsic taste/flavor profiles of soy, oat, and coconut milks, which do not develop lactic notes during fermentation comparable to those resulting from dairy fermentation.

Consistency scores showed the highest degree of structural differences. DY presented the highest viscosity and also the highest rating in consistency, which directly related the textural rheology with sensory perception. The casein network in dairy yoghurt forms a more integrated gel structure (Li *et al*., 2025) compared to plant-based matrices. CY followed closely, reflecting industrial stabilization. This evidence shows that plant-based samples proved to have a good consistency; this attests to proper performance from S29_CO and S25_CO when compared to S27_CO and S30_CO. These results are a testament to the fact that the blend composition impacts the gel strength inside the plant-based matrix (Nasrollahzadeh *et al*., 2023).

Overall acceptability reflected trends in the individual attributes. The panelists ranked DY, followed by CY highest in terms of overall acceptability, thus evidencing that dairy-based yoghurts

are generally more desirable to the consumer. This may be attributed to their familiarity with such products. For example, Ojiaku *et al*. (2024) stated that familiarity with a product affects/influences consumer perception of such products. On the contrary, the plant-based yoghurts reached a more moderate score of acceptability, while S27_CO and S30_CO got the minimum scores due to their combined lower ratings in taste, flavor, and consistency. S29_CO and S25_CO presented an intermediate overall acceptability, attesting that some of these soy-oat-coconut combinations are closer to the sensory expectations than others.

CONCLUSION AND RECOMMENDATION

6.1. Conclusion

Optimizations and developments made on soybean-oat-coconut yoghurts showed that nutritionally sound and sensorially acceptable plant yoghurts can be successfully made from raw materials available locally. These optimizations have, therefore, allowed the identification of mixing ratios with better sensory properties, and such optimized formulations performed well in nutritional, antioxidant, and physicochemical characterizations. Yoghurt samples prepared in this work exhibited high contents of phenolics and flavonoids, which translated into significant antioxidant activities; some of them matched the radical scavenging ability of commercial dairy yoghurt. Vitamin profiles were similarly impressive, with vitamins A, C, and E reflecting well on the density of these micronutrients in the base plant materials. Fermentation proceeded admirably, as reflected in the appropriate pH, titratable acidity, and soluble solids measures, while the microbiological findings confirmed the safety and functionality of the products. Sensorially acceptable samples were obtained for all optimized yoghurt samples, with some of the blends receiving very high ratings to questions on the aroma, mouthfeel, and overall acceptability. In general, these findings support the idea that if soybean-oat-coconut mixes are perfectly optimized, then they can be used as high-quality, functional, and consumer-friendly alternatives to dairy yoghurt.

6.2. Recommendation

It is proposed that further research be carried out to apply the findings of this study to large-scale production. The process should be evaluated for its consistency, and fermentation conditions determined for each batch during pilot-scale trials. The different storage scenarios should be put through shelf-life testing to check stability, microbial count, and quality of the yoghurt over time.

More formulation work is required to try the mixing of natural flavor enhancers or fruit-based ingredients that could make the sensory experience more inviting, especially for larger market adoption.

The functional food characteristics of these products would be more evident through an in-depth assessment of probiotic fortification, nutrient bio-accessibility, and glycemic response.

Consumer research considering various demographic categories and economic evaluations will be necessary for the products to be marketed successfully.

Furthermore, appropriate packaging, cold-chain handling, and natural preservatives will make sure that the product is still good for consumption over a long period of time.

6.3. Contribution to Knowledge

1. This research adds to the body of knowledge on nutritional important approaches in plant-based yoghurt showing how the combination of legumes, cereal and coconut materials improve the nutritional competitiveness of dairy alternatives
2. Production of empirical knowledge on the influence of fermentation time on physicochemical properties, microbial growth kinetics and product shelf life in composite plant base yoghurt systems
3. Scientific confirmation of the acceptability of optimized plant-based yoghurt formulations by consumers through comprehensive sensory analysis
4. The study also makes further contributions to the new knowledge of the functional and antioxidant abilities of composite plant yoghurt by identifying correlations between raw material ratios, fermentation, and the retention of bioactive compounds.

5. The study also makes further contributions to the existing body of knowledge on the viability of probiotics in non-dairy materials by confirming the suitability of plant-based milk systems as probiotic carriers during fermentation and storage.

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APPENDIX I

Sensory Data for RSM optimization

Sample	Appearance	Taste	Aroma	Flavour	Consistency	General Acceptability
Run1	7	7	7	6	6	7
Run1	7	7	7	8	7	7
Run1	8	7	5	7	7	7
Run1	7	7	6	6	6	6
Run1	7	6	7	7	6	6
Run1	8	7	8	7	5	8
Run1	8	8	8	8	5	8
Run1	7	7	7	7	7	7
Run1	7	7	6	7	7	7
Run1	7	6	6	6	6	6
Run1	8	7	7	7	8	8
Run1	7	7	7	7	7	7
Run1	8	7	7	7	8	8
Run1	7	6	6	7	7	7
Run1	6	6	6	6	6	6
Run1	6	6	6	6	6	6
Run1	6	6	7	6	7	7
Run1	8	7	6	7	8	8
Run1	7	6	7	7	7	7
Run1	6	5	4	4	4	4
Run1	7	5	5	5	5	5
Run1	7	6	7	7	5	7
Run1	7	6	6	7	6	7
Run1	7	7	6	7	5	7
Run1	7	6	5	5	4	6
Run1	6	6	6	6	6	6
Run1	7	6	7	7	6	7
Run1	8	7	6	7	5	7
Run1	6	6	7	6	5	6
Run1	6	6	6	6	6	6
Run2	6	5	6	6	5	6
Run2	6	5	5	5	6	5
Run2	8	6	6	6	6	6
Run2	7	4	4	4	4	4
Run2	7	6	6	6	6	6
Run2	6	6	6	6	6	6

Run2	6	6	6	6	6	6
Run2	6	6	5	5	5	5
Run2	5	4	5	4	5	4
Run2	7	5	5	5	7	6
Run2	5	4	5	4	5	5
Run2	3	3	3	3	3	3
Run2	6	5	5	4	5	5
Run2	6	4	5	5	5	5
Run2	4	3	3	3	3	3
Run2	3	3	3	3	3	3
Run2	6	4	5	5	5	5
Run2	5	5	5	5	5	5
Run2	6	5	5	5	5	5
Run2	4	2	3	3	3	3
Run2	7	6	6	6	6	6
Run2	6	5	5	5	5	6
Run2	6	5	4	5	5	5
Run2	5	5	4	4	5	5
Run2	5	5	4	4	4	5
Run2	5	5	5	5	5	5
Run2	6	4	5	5	5	5
Run2	4	4	5	4	5	4
Run2	5	5	5	5	5	5
Run2	5	5	5	5	5	5
Run3	6	7	6	6	5	5
Run3	7	6	6	6	6	7
Run3	8	5	5	4	4	4
Run3	8	8	7	8	5	8
Run3	6	8	8	8	5	8
Run3	8	8	8	8	5	8
Run3	8	8	7	8	6	8
Run3	4	4	4	4	4	4
Run3	7	7	7	7	7	7
Run3	8	6	7	6	7	6
Run3	7	6	6	7	6	7
Run3	8	8	8	8	8	8
Run3	7	7	6	7	6	7
Run3	7	7	6	7	6	6
Run3	7	7	7	7	7	7
Run3	8	8	8	8	8	8
Run3	8	7	7	7	6	8
Run3	7	6	7	7	6	7
Run3	8	8	7	7	8	6

Run3	7	6	6	6	6	6
Run3	8	8	8	8	8	8
Run3	7	7	6	7	5	6
Run3	7	7	6	7	6	7
Run3	8	7	7	7	5	8
Run3	8	5	4	4	4	4
Run3	5	5	5	5	5	5
Run3	8	7	6	7	6	7
Run3	8	8	7	8	6	7
Run3	6	6	6	5	5	6
Run3	7	7	7	7	6	7
Run4	6	6	6	5	5	6
Run4	7	5	6	5	6	6
Run4	7	5	4	5	5	5
Run4	7	6	6	6	6	6
Run4	6	6	6	6	6	6
Run4	6	5	5	5	5	5
Run4	5	4	5	4	6	4
Run4	7	7	7	7	7	7
Run4	6	6	6	6	6	6
Run4	7	5	5	5	6	5
Run4	6	5	5	5	6	5
Run4	4	4	4	4	4	4
Run4	6	5	6	6	6	6
Run4	6	5	5	6	5	5
Run4	5	4	5	4	5	4
Run4	5	5	5	5	5	5
Run4	6	6	6	6	5	6
Run4	6	6	5	6	5	6
Run4	6	6	5	6	6	4
Run4	5	4	4	4	4	4
Run4	6	6	6	6	6	6
Run4	6	5	6	6	5	6
Run4	6	5	6	6	6	5
Run4	6	5	5	5	6	5
Run4	6	6	4	4	4	4
Run4	4	4	4	4	4	4
Run4	6	6	5	6	5	6
Run4	6	6	5	6	4	6
Run4	5	5	4	5	6	5
Run4	4	4	4	4	4	3
Run5	7	6	4	4	5	7
Run5	7	5	5	5	5	5

Run5	7	5	5	5	6	6
Run5	7	4	4	4	4	4
Run5	6	5	5	5	5	5
Run5	8	8	8	8	5	8
Run5	3	3	3	3	5	3
Run5	8	8	8	8	8	8
Run5	4	4	4	4	4	4
Run5	7	6	5	6	7	6
Run5	2	2	2	2	2	2
Run5	6	5	5	5	5	5
Run5	5	4	5	5	4	6
Run5	3	3	3	3	3	3
Run5	2	2	2	2	2	2
Run5	5	5	5	5	5	5
Run5	5	5	5	4	5	6
Run5	4	4	5	4	4	7
Run5	5	2	3	3	3	3
Run5	6	2	3	3	3	3
Run5	5	6	6	6	5	6
Run5	6	5	5	5	5	5
Run5	6	4	5	5	5	6
Run5	5	4	4	4	5	4
Run5	5	5	5	4	4	5
Run5	5	5	5	5	5	5
Run5	5	4	5	5	4	5
Run5	5	4	4	5	6	5
Run5	4	5	4	5	4	5
Run5	3	3	3	3	3	6
Run6	7	5	6	6	6	7
Run6	6	6	7	6	5	6
Run6	7	6	5	5	5	5
Run6	7	6	6	6	6	6
Run6	7	6	6	6	6	6
Run6	7	8	7	7	5	7
Run6	7	7	7	7	5	7
Run6	5	5	5	5	5	5
Run6	5	5	5	5	5	5
Run6	8	5	6	5	7	6
Run6	6	6	6	6	6	6
Run6	8	7	8	7	8	7
Run6	5	6	6	6	5	6
Run6	5	6	6	6	6	5
Run6	7	6	6	6	6	6

Run6	8	7	8	7	8	8
Run6	5	6	6	6	6	6
Run6	6	5	5	6	6	6
Run6	7	6	6	7	6	5
Run6	6	7	6	6	6	6
Run6	6	6	6	6	6	6
Run6	5	6	6	6	5	6
Run6	5	6	6	6	6	6
Run6	6	4	4	6	4	6
Run6	7	4	5	5	4	5
Run6	5	5	5	5	5	5
Run6	5	5	6	6	5	5
Run6	7	6	6	7	6	7
Run6	7	7	6	6	5	7
Run6	6	6	6	6	6	5
Run7	7	7	7	7	7	7
Run7	6	6	5	5	5	5
Run7	7	6	6	6	6	6
Run7	6	6	6	6	6	6
Run7	6	5	5	5	5	5
Run7	5	5	5	5	5	5
Run7	4	3	4	3	5	5
Run7	1	1	1	1	1	1
Run7	5	5	5	5	5	5
Run7	8	6	7	6	8	7
Run7	5	6	5	5	5	4
Run7	5	5	5	5	5	5
Run7	5	6	6	5	5	5
Run7	5	6	5	5	5	3
Run7	5	5	5	5	5	5
Run7	5	4	4	4	5	5
Run7	5	6	4	5	5	5
Run7	5	6	5	5	5	5
Run7	5	5	5	5	5	5
Run7	7	3	3	3	3	3
Run7	8	8	8	8	8	8
Run7	5	5	5	5	5	5
Run7	5	5	5	5	5	5
Run7	5	5	5	5	5	5
Run7	6	5	5	4	4	5
Run7	4	4	4	4	4	4
Run7	5	5	5	5	5	5
Run7	6	6	5	5	6	6

Run7	5	5	5	5	5	5
Run7	7	8	7	7	7	7
Run8	7	5	5	5	5	6
Run8	6	5	5	5	4	5
Run8	6	5	5	6	5	6
Run8	6	4	5	5	5	5
Run8	6	5	5	5	5	5
Run8	7	7	7	7	6	7
Run8	1	1	2	1	6	3
Run8	3	3	3	3	3	3
Run8	6	6	6	6	6	6
Run8	7	5	5	5	6	5
Run8	4	4	4	4	4	4
Run8	1	1	1	1	1	1
Run8	5	4	5	5	4	5
Run8	5	4	4	3	3	5
Run8	4	3	3	3	3	3
Run8	1	2	1	2	1	2
Run8	5	5	5	5	4	5
Run8	4	4	4	4	4	4
Run8	4	3	3	3	4	6
Run8	6	6	6	6	6	6
Run8	7	7	7	7	7	7
Run8	5	6	5	5	5	5
Run8	5	5	5	5	4	5
Run8	4	4	4	4	6	4
Run8	4	5	6	5	4	5
Run8	3	3	3	3	3	3
Run8	5	4	5	4	4	4
Run8	4	3	4	4	5	3
Run8	6	5	5	5	6	5
Run8	5	5	5	5	5	5
Run9	7	5	5	6	5	6
Run9	6	6	5	6	6	6
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Run9	6	5	6	6	5	5
Run9	6	5	5	5	5	5
Run9	6	6	6	6	6	6
Run9	4	4	4	4	6	4
Run9	5	5	5	5	5	5
Run9	6	6	6	6	6	6
Run9	8	6	7	5	7	7
Run9	5	5	5	5	5	5

Run9	4	4	4	4	4	4
Run9	5	6	5	5	5	5
Run9	5	5	5	5	5	5
Run9	3	4	3	4	3	3
Run9	4	4	4	4	4	4
Run9	5	6	5	5	5	5
Run9	5	6	5	5	5	6
Run9	5	5	5	5	5	5
Run9	6	5	5	5	5	5
Run9	5	5	5	5	5	5
Run9	5	6	5	5	5	5
Run9	5	6	5	5	5	5
Run9	5	5	5	5	6	5
Run9	5	3	3	4	4	3
Run9	3	3	3	3	3	3
Run9	5	5	4	3	4	5
Run9	5	5	5	5	5	5
Run9	7	5	5	5	4	5
Run9	5	5	5	5	5	5
Run10	6	6	5	6	6	6
Run10	7	6	6	6	6	6
Run10	7	7	4	5	6	7
Run10	7	6	6	7	5	6
Run10	7	6	7	7	5	7
Run10	8	8	8	8	6	7
Run10	7	6	6	6	5	7
Run10	6	6	6	6	6	6
Run10	4	4	4	4	4	4
Run10	8	6	6	5	7	5
Run10	7	7	7	7	7	7
Run10	6	6	6	6	6	6
Run10	6	6	5	6	6	6
Run10	6	6	5	5	6	6
Run10	6	7	7	7	7	7
Run10	6	6	6	6	6	6
Run10	5	6	5	5	6	6
Run10	7	6	7	7	6	7
Run10	7	6	6	7	7	7
Run10	7	6	6	6	6	6
Run10	5	5	5	5	5	5
Run10	6	6	5	6	5	6
Run10	6	6	5	5	4	6
Run10	6	6	5	6	6	4

Run10	5	5	5	4	4	5
Run10	6	4	6	6	6	6
Run10	5	6	5	6	5	6
Run10	7	7	6	6	6	6
Run10	6	6	5	6	5	6
Run10	6	6	7	6	5	7
Run11	7	6	7	7	6	6
Run11	6	6	6	6	6	6
Run11	7	7	4	6	6	7
Run11	7	5	6	5	5	6
Run11	7	7	7	7	6	7
Run11	7	7	7	7	6	7
Run11	6	6	6	6	5	6
Run11	6	6	6	6	6	6
Run11	4	4	4	4	4	4
Run11	8	7	7	7	8	7
Run11	6	6	6	6	5	6
Run11	5	5	5	5	5	5
Run11	6	6	5	6	6	6
Run11	6	6	5	5	6	7
Run11	7	7	7	7	7	7
Run11	5	5	5	5	5	5
Run11	7	7	6	6	6	6
Run11	6	6	6	6	6	6
Run11	6	6	6	6	6	6
Run11	5	6	5	6	5	5
Run11	6	5	6	5	6	6
Run11	6	6	5	6	5	6
Run11	6	6	5	6	5	6
Run11	6	6	5	6	6	5
Run11	7	6	5	5	4	5
Run11	7	7	5	7	3	7
Run11	6	6	5	6	5	6
Run11	6	6	5	6	6	6
Run11	5	6	6	6	4	6
Run11	5	5	5	5	5	5
Run12	7	7	7	7	7	7
Run12	7	6	6	7	7	7
Run12	7	3	3	4	6	4
Run12	8	7	8	8	8	8
Run12	7	8	7	7	6	7
Run12	6	6	7	7	5	7
Run12	9	9	9	9	5	9

Run12	5	5	5	5	5	5
Run12	5	5	5	5	5	5
Run12	8	6	6	6	8	6
Run12	8	5	7	7	7	7
Run12	7	7	7	7	7	7
Run12	7	7	6	7	6	7
Run12	7	6	6	7	6	4
Run12	8	8	8	8	8	8
Run12	9	9	9	9	9	9
Run12	6	6	6	6	7	7
Run12	7	7	7	7	6	7
Run12	7	7	7	7	7	7
Run12	7	7	7	7	7	7
Run12	7	7	7	7	7	7
Run12	7	6	6	7	5	6
Run12	7	6	5	6	6	6
Run12	7	6	6	7	6	6
Run12	6	6	5	5	4	6
Run12	4	4	4	4	4	4
Run12	8	7	7	8	5	7
Run12	7	7	7	7	8	7
Run12	7	7	6	5	5	7
Run12	5	5	5	5	5	5
Run13	7	6	6	6	6	6
Run13	7	7	7	6	6	7
Run13	7	5	5	5	5	5
Run13	7	6	5	5	5	5
Run13	7	5	6	6	6	6
Run13	8	7	7	7	5	7
Run13	7	6	6	6	5	7
Run13	6	5	5	5	6	6
Run13	5	4	5	5	5	5
Run13	7	6	6	5	5	7
Run13	6	5	6	6	5	6
Run13	6	6	6	6	6	6
Run13	6	7	7	7	6	6
Run13	5	5	5	5	5	8
Run13	5	5	6	6	6	6
Run13	5	4	4	4	4	4
Run13	7	5	5	5	5	6
Run13	6	6	5	5	5	5
Run13	6	5	6	6	5	6
Run13	7	6	7	6	6	6

Run13	7	7	7	7	7	7
Run13	7	6	6	6	5	6
Run13	7	7	5	7	6	7
Run13	7	6	6	6	6	7
Run13	6	5	5	5	4	5
Run13	5	3	3	3	3	4
Run13	6	6	6	7	6	7
Run13	8	7	7	7	5	7
Run13	7	7	6	5	6	7
Run13	6	5	5	5	5	5
Run14	7	6	8	8	5	7
Run14	6	5	5	5	5	5
Run14	7	4	4	6	5	5
Run14	6	6	6	6	6	6
Run14	7	5	7	6	6	6
Run14	8	8	8	8	5	8
Run14	2	1	2	2	5	1
Run14	6	6	6	5	6	6
Run14	6	6	6	6	6	6
Run14	7	5	5	6	6	5
Run14	4	5	4	4	4	4
Run14	6	6	6	6	6	6
Run14	5	5	5	5	4	5
Run14	4	3	6	4	4	6
Run14	2	2	2	2	2	2
Run14	2	2	2	2	2	2
Run14	5	4	5	5	4	5
Run14	5	5	5	5	4	5
Run14	4	4	4	4	5	4
Run14	5	4	4	4	4	4
Run14	6	6	6	6	6	6
Run14	5	5	5	5	5	5
Run14	5	5	4	4	6	5
Run14	4	4	4	4	6	4
Run14	6	7	5	5	4	7
Run14	4	4	4	4	4	4
Run14	3	3	3	3	5	3
Run14	5	6	6	7	5	6
Run14	4	5	6	6	6	5
Run14	3	3	3	3	3	3
Run15	7	5	6	6	7	6
Run15	7	7	7	7	7	7
Run15	7	6	6	7	6	7

Run15	7	7	7	7	5	7
Run15	7	8	8	8	6	8
Run15	7	7	7	7	5	7
Run15	9	8	8	8	5	9
Run15	7	7	7	7	7	7
Run15	6	6	6	6	6	6
Run15	8	6	6	6	7	6
Run15	8	7	8	8	8	8
Run15	6	6	6	6	6	6
Run15	7	8	6	7	7	7
Run15	7	6	6	8	7	6
Run15	5	5	5	5	5	5
Run15	9	8	8	8	9	9
Run15	8	7	6	7	7	8
Run15	8	8	8	8	8	8
Run15	7	8	8	8	8	8
Run15	6	6	6	6	6	6
Run15	6	4	4	4	4	4
Run15	7	7	6	7	5	7
Run15	7	7	6	7	7	7
Run15	8	7	7	6	6	7
Run15	5	7	5	5	4	7
Run15	5	5	5	5	5	5
Run15	8	7	6	6	7	7
Run15	7	6	6	7	6	6
Run15	6	5	6	6	6	5
Run15	6	6	6	6	6	6
Run16	7	6	6	6	5	6
Run16	6	6	6	6	6	6
Run16	7	5	6	7	6	6
Run16	5	5	5	5	5	5
Run16	7	7	5	5	6	6
Run16	7	7	7	7	5	7
Run16	3	5	5	5	5	5
Run16	7	7	7	7	7	7
Run16	6	6	6	6	6	6
Run16	8	6	6	6	8	7
Run16	6	5	6	6	6	6
Run16	6	6	6	6	6	6
Run16	6	6	6	6	6	6
Run16	6	6	6	6	6	6
Run16	6	6	6	6	6	6
Run16	6	5	6	6	6	6

Run16	6	5	4	4	6	5
Run16	6	6	6	6	6	6
Run16	5	5	5	5	5	5
Run16	7	5	5	5	5	5
Run16	6	6	6	6	5	6
Run16	6	6	6	6	5	6
Run16	6	6	5	5	6	6
Run16	5	7	5	5	4	7
Run16	5	5	5	5	5	5
Run16	6	5	5	5	5	5
Run16	7	6	6	7	6	6
Run16	5	5	6	5	6	5
Run16	7	5	5	5	6	5
Run16	5	5	5	5	5	5

APPENDIX II

RSM Optimization

ANOVA for KCV Model

Response 1: Appearance
Transform: Inverse Sqrt
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0104	9	0.0012	22.91	0.0006	significant
[(1)]Linear Mixture	0.0006	2	0.0003	5.59	0.0426	
AB	0.0017	1	0.0017	34.29	0.0011	
AC	0.0013	1	0.0013	26.31	0.0022	
AD	0.0000	1	0.0000	0.8703	0.3869	
BC	0.0040	1	0.0040	78.87	0.0001	
BD	4.305E-06	1	4.305E-06	0.0856	0.7796	
CD	0.0002	1	0.0002	4.23	0.0855	
D^2	0.0014	1	0.0014	28.27	0.0018	
Residual	0.0003	6	0.0001			
Lack of Fit	0.0002	5	0.0000	0.4264	0.8138	not significant
Pure Error	0.0001	1	0.0001			
Cor Total	0.0107	15				

(1) Inference for linear mixtures uses Type I sums of squares.

Mixture Component coding is L Pseudo.
Sum of squares is Type III - Partial

The Model F-value of 22.91 implies the model is significant. There is only a 0.06% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The Lack of Fit F-value of 0.43 implies the Lack of Fit is not significant relative to the pure error. There is a 81.38% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Fit Statistics

Std. Dev.	0.0071	R^2	0.9717
Mean	0.4109	Adjusted R^2	0.9293
C.V. %	1.73	Predicted R^2	0.7491
		Adeq Precision	16.0753

ANOVA for KCV Model

Response 2: Flavour
Transform: Natural Log
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.3105	9	0.0345	37.62	0.0001	significant
[(1)]Linear Mixture	0.0354	2	0.0177	19.29	0.0024	
AB	0.0629	1	0.0629	68.59	0.0002	
AC	0.0519	1	0.0519	56.54	0.0003	
AD	0.0009	1	0.0009	1.02	0.3521	
BC	0.0910	1	0.0910	99.18	< 0.0001	
BD	0.0000	1	0.0000	0.0156	0.9047	
CD	0.0115	1	0.0115	12.58	0.0121	
D^2	0.0354	1	0.0354	38.64	0.0008	
Residual	0.0055	6	0.0009			
Lack of Fit	0.0047	5	0.0009	1.15	0.6057	not significant
Pure Error	0.0008	1	0.0008			
Cor Total	0.3161	15				

(1) Inference for linear mixtures uses Type I sums of squares.

Mixture Component coding is L_Pseudo.
Sum of squares is Type III - Partial

The Model F-value of 37.62 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The Lack of Fit F-value of 1.15 implies the Lack of Fit is not significant relative to the pure error. There is a 60.57% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

The Predicted R^2 of 0.7491 is in reasonable agreement with the Adjusted R^2 of 0.9293; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 16.075 indicates an adequate signal. This model can be used to navigate the design space.

Fit Statistics

Std. Dev.	0.0303	R^2	0.9826
Mean	1.70	Adjusted R^2	0.9565
C.V. %	1.78	Predicted R^2	0.7797
		Adeq Precision	20.6011

The Predicted R^2 of 0.7797 is in reasonable agreement with the Adjusted R^2 of 0.9565; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 20.601 indicates an adequate signal. This model can be used to navigate the design space.

ANOVA for KCV model

Response 3: Taste
Transform: Square Root
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.3369	9	0.0374	38.24	0.0001	significant
[(1)]Linear Mixture	0.0180	2	0.0090	9.20	0.0149	
AB	0.0626	1	0.0626	63.95	0.0002	
AC	0.0415	1	0.0415	42.34	0.0006	
AD	0.0012	1	0.0012	1.21	0.3141	
BC	0.1184	1	0.1184	120.98	< 0.0001	
BD	0.0001	1	0.0001	0.0691	0.8015	
CD	0.0138	1	0.0138	14.11	0.0094	
D^2	0.0477	1	0.0477	48.75	0.0004	
Residual	0.0059	6	0.0010			
Lack of Fit	0.0055	5	0.0011	2.78	0.4253	not significant
Pure Error	0.0004	1	0.0004			
Cor Total	0.3428	15				

(1) Inference for linear mixtures uses Type I sums of squares.

Mixture Component coding is L_Pseudo.
Sum of squares is Type III - Partial

The Model F-value of 38.24 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The Lack of Fit F-value of 2.78 implies the Lack of Fit is not significant relative to the pure error. There is a 42.53% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Fit Statistics

Std. Dev.	0.0313	R^2	0.9829
Mean	2.34	Adjusted R^2	0.9572
C.V. %	1.34	Predicted R^2	0.7631
		Adeq Precision	19.7498

The Predicted R^2 of 0.7631 is in reasonable agreement with the Adjusted R^2 of 0.9572; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 19.750 indicates an adequate signal. This model can be used to navigate the design

ANOVA for KCV model

Response 4: Aroma

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	9.53	9	1.06	47.06	< 0.0001	significant
[(1)]Linear Mixture	0.4495	2	0.2247	9.98	0.0123	
AB	1.82	1	1.82	80.84	0.0001	
AC	1.07	1	1.07	47.71	0.0005	
AD	0.0400	1	0.0400	1.78	0.2307	
BC	3.20	1	3.20	142.14	< 0.0001	
BD	0.0041	1	0.0041	0.1819	0.6846	
CD	0.3642	1	0.3642	16.18	0.0069	
D^2	1.52	1	1.52	67.52	0.0002	
Residual	0.1351	6	0.0225			
Lack of Fit	0.1212	5	0.0242	1.74	0.5168	not significant
Pure Error	0.0139	1	0.0139			
Cor Total	9.67	15				

(1) Inference for linear mixtures uses Type I sums of squares.

Mixture Component coding is L_Pseudo.
Sum of squares is Type III - Partial

The Model F-value of 47.06 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The Lack of Fit F-value of 1.74 implies the Lack of Fit is not significant relative to the pure error. There is a 51.68% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Fit Statistics

Std. Dev.	0.1500	R^2	0.9860
Mean	5.56	Adjusted R^2	0.9651
C.V. %	2.70	Predicted R^2	0.8502
		Adeq Precision	21.7349

The Predicted R^2 of 0.8502 is in reasonable agreement with the Adjusted R^2 of 0.9651; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 21.735 indicates an adequate signal. This model can be used to navigate the design space.

ANOVA for KCV model

Response 5: Consistency
Transform: Inverse

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0058	9	0.0006	13.55	0.0024	significant
(1)Linear Mixture	0.0003	2	0.0001	2.94	0.1290	
AB	0.0010	1	0.0010	21.41	0.0036	
AC	0.0014	1	0.0014	29.16	0.0017	
AD	0.0002	1	0.0002	3.55	0.1086	
BC	0.0020	1	0.0020	41.96	0.0006	
BD	2.616E-06	1	2.616E-06	0.0550	0.8224	
CD	0.0004	1	0.0004	7.60	0.0330	
D^2	0.0004	1	0.0004	9.06	0.0237	
Residual	0.0003	6	0.0000			
Lack of Fit	0.0003	5	0.0001	2.23	0.4675	not significant
Pure Error	0.0000	1	0.0000			
Cor Total	0.0061	15				

(1) Inference for linear mixtures uses Type I sums of squares.

Mixture Component coding is L_Pseudo.
Sum of squares is Type III - Partial

The Model F-value of 13.55 implies the model is significant. There is only a 0.24% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The Lack of Fit F-value of 2.23 implies the Lack of Fit is not significant relative to the pure error. There is a 46.75% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Fit Statistics

Std. Dev.	0.0069	R^2	0.9531
Mean	0.1874	Adjusted R^2	0.8828
C.V. %	3.68	Predicted R^2	0.5760
		Adeq Precision	12.1920

The Predicted R^2 of 0.5760 is not as close to the Adjusted R^2 of 0.8828 as one might normally expect; i.e. the difference is more than 0.2. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response transformation, outliers, etc. All empirical models should be tested by doing confirmation runs.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 12.192 indicates an adequate signal. This model can be used to navigate the design space.

ANOVA for Quadratic x Mean model

Response 6: Overall Acceptability

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	6.51	5	1.30	6.89	0.0049	significant
(1)Linear Mixture	0.2622	2	0.1311	0.6939	0.5221	
AB	2.30	1	2.30	12.18	0.0058	
AC	1.22	1	1.22	6.48	0.0291	
BC	4.25	1	4.25	22.51	0.0008	

Residual	1.89	10	0.1889			
Lack of Fit	1.88	9	0.2084	15.00	0.1979	not significant
Pure Error	0.0139	1	0.0139			
Cor Total	8.40	15				

(1) Inference for linear mixtures uses Type I sums of squares.

Mixture Component coding is L_Pseudo.
Sum of squares is Type III - Partial

The Model F-value of 6.89 implies the model is significant. There is only a 0.49% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The Lack of Fit F-value of 15.00 implies the Lack of Fit is not significant relative to the pure error. There is a 19.79% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Fit Statistics

Std. Dev.	0.4346	R^2	0.7751
Mean	5.68	Adjusted R^2	0.6626
C.V. %	7.66	Predicted R^2	0.5034
		Adeq Precision	6.7938

The Predicted R^2 of 0.5034 is in reasonable agreement with the Adjusted R^2 of 0.6626; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 6.794 indicates an adequate signal. This model can be used to navigate the design space.

APPENDIX III

Proximate Raw Data

		Moisture	Ash	Protein	Fat	Fibre	Carbohydrate	Energy
		%	%	%	%	%	%	%
S29_CO	a	76.6	0.1	5.922747	7.990196	0.273125	9.11393218	132.0584806
	b	79.41022	0.103669	6.126817	8.283916	0.283145	5.792228928	122.2314264
		78.00511	0.101834	6.024782	8.137056	0.278135	7.453080554	127.1449535
S25_CO	a	77.64786	0.311006	4.077253	4.117277	0.276861	13.56974663	107.6434884
	b	74.9	0.3	4.217737	3.971292	0.267063	16.34390808	117.9882056
		76.27393	0.305503	4.147495	4.044284	0.271962	14.95682736	112.815847
S27_CO	a	78.34331	0.149701	3.776824	3.843284	0.279341	13.60753749	104.1269983
	b	81.21749	0.155193	3.906956	3.984563	0.289589	10.44620502	93.27370834
		79.7804	0.152447	3.84189	3.913923	0.284465	12.02687126	98.70035334
S30_CO	a	78.13912	0.310076	3.690987	3.66142	0.278613	13.91978424	103.3958661
	b	75.37388	0.299103	3.818162	3.531599	0.268753	16.70850577	113.8910559
		76.7565	0.304589	3.754574	3.596509	0.273683	15.314145	108.643461
CY	a	89.75124	0.597015	2.017167	1.496063	0.320017	5.818493885	44.807212
	b	90.04395	0.618918	2.08667	1.551058	0.331757	5.367649268	43.77680024
		89.8976	0.607966	2.051919	1.523561	0.325887	5.593071576	44.29200612
DY	a	83.36777	0.412202	3.090129	1.55514	0.297256	11.27750186	71.46678245
	b	80.4175	0.397614	3.1966	1.5	0.286737	14.20155366	83.09261614
		81.89263	0.404908	3.143365	1.52757	0.291996	12.73952776	77.2796993

APPENDIX IV

Phytochemicals/Antinutrients Raw Data

	Phytate	Oxalate	Trypsin	Tannin	Saponin
	mg/g	mg/g	%	mg (QE)/g	mg/g
S29_CO	12.9057	8.1036	58.51942	2.793371844	26.48649
	13.37883	8.400678	60.66474	2.895776856	27.39909
	13.14226	8.252139	59.59208	2.84457435	26.94279
S25_CO	12.13431	11.2009	24.83036	3.162863071	80.9009
	11.7052	10.8048	23.95227	3.051012936	83.68837
	11.91976	11.00285	24.39131	3.106938003	82.29464
S27_CO	12.10439	4.502	12.89078	2.644211211	16.21622
	12.54814	4.667043	13.36336	2.741147994	16.77495
	12.32627	4.584522	13.12707	2.692679603	16.49558
S30_CO	13.06772	10.2675	43.46424	3.373720609	47.38739
	12.6056	9.9044	41.92719	3.254413799	49.02014
	12.83666	10.08595	42.69571	3.314067204	48.20376
CY	12.98737	6.3028	61.28479	1.288205462	16.21622
	13.46349	6.533861	63.53149	1.335431074	16.77495
	13.22543	6.41833	62.40814	1.311818268	16.49558
DY	14.20975	17.73476	9.77992	1.644688797	36.03604
	13.70724	17.1076	9.434067	1.586526727	37.27767
	13.95849	17.42118	9.606993	1.615607762	36.65685

APPENDIX V

Antioxidant Raw Data

	DPPH	ABTS	Flavonoid	Phenol	FRAP
	%	%	mg (QE)/g	mg GAE/g	Mg (Vit. C)/g
S29_CO	25.638139	8.4840056	0.5424023	3.0193237	2.95549768
	24.680851	8.813072	0.56344028	3.1364332	3.07013162
	25.159495	8.6485388	0.55292129	3.0778784	3.01281465
S25_CO	47.518965	13.580799	0.1972041	4.4537352	3.15247232
	45.744681	13.073713	0.1898408	4.2874396	3.0347639
	46.631823	13.327256	0.19352245	4.3705874	3.09361811
S27_CO	49.680851	5.1460362	0.27120115	2.9589372	2.60446156
	51.607806	5.3456338	0.28172014	3.0737045	2.70547997
	50.644329	5.245835	0.27646065	3.0163209	2.65497076
S30_CO	40.004338	10.402314	0.47892424	2.7914256	3.07405261
	38.510638	10.013908	0.46104195	2.6871981	2.95927226
	39.257488	10.208111	0.4699831	2.7393118	3.01666243
CY	50	17.941586	0.12204052	1.1171498	2.74789567
	51.939334	18.63748	0.12677406	1.1604803	2.85447742
	50.969667	18.289533	0.12440729	1.138815	2.80118655
DY	32.268693	16.325855	0.08451604	3.0737045	2.82703052
	31.06383	15.716273	0.08136034	2.9589372	2.7214736
	31.666261	16.021064	0.08293819	3.0163209	2.77425206

APPENDIX VI

Vitamin Raw Data

	Vit. C	Vit. A	Vit. D	Vit. E	Vit. K
	mg/g	mcg (RAE)	mg/g	mg/g	mg/g
S29_CO	2.545045	558.5216	0.221921	0.290904	0.21021
	2.6437589	575.277248	0.230529	0.3021872	0.217453
	2.594402	566.899424	0.226225	0.2965456	0.213831
S25_CO	1.941876	370.570928	0.329327	0.1445243	0.18473
	1.8693694	359.7776	0.31703	0.139128	0.191095
	1.9056227	365.174264	0.323178	0.1418262	0.187912
S27_CO	2.4211712	631.904	0.203805	0.134912	0.14651
	2.5150804	650.86112	0.21171	0.1401448	0.151558
	2.4681258	641.38256	0.207757	0.1375284	0.149034
S30_CO	2.3630057	591.023888	0.451648	0.3109462	0.10192
	2.2747748	573.8096	0.434784	0.299336	0.105432
	2.3188903	582.416744	0.443216	0.3051411	0.103676
CY	2.1058559	643.1152	0.326088	0.248744	0.10829
	2.187535	662.408656	0.338736	0.258392	0.112021
	2.1466954	652.761928	0.332412	0.253568	0.110156
DY	3.158473	860.81632	0.404601	0.2452534	0.23569
	3.0405405	835.744	0.389494	0.236096	0.243811
	3.0995068	848.28016	0.397048	0.2406747	0.23975

APPENDIX VII

Minerals Raw Data

	Na	Ca	K	Zn	Mg	Fe	P
	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g
S29_CO	1.2	1.36	0.62	0.0198	0.78	0.0215	75.91584
	1.17	1.32	0.63	0.02	0.79	0.0213	78.64498
	1.185	1.34	0.625	0.0199	0.785	0.0214	77.28041
S25_CO	2.8	1.62	3	0.0214	0.86	0.0199	58.58911
	2.6	1.6	2.8	0.0216	0.88	0.0197	60.72125
	2.7	1.61	2.9	0.0215	0.87	0.0198	59.65518
S27_CO	2.1	1.06	1.2	0.0169	0.54	0.0314	66.01485
	2.06	1.04	1.19	0.0166	0.56	0.0316	68.40285
	2.08	1.05	1.195	0.01675	0.55	0.0315	67.20885
S30_CO	3.4	2.42	0.7	0.0314	1.36	0.0217	56.11386
	3.36	2.4	0.73	0.0316	1.3	0.0215	58.16072
	3.38	2.41	0.715	0.0315	1.33	0.0216	57.13729
CY	0.46	0.96	0.8	0.0242	0.5	0.0325	23.93564
	0.49	0.94	0.83	0.024	0.52	0.0323	24.87379
	0.475	0.95	0.815	0.0241	0.51	0.0324	24.40472
DY	0.85	1.52	0.11	0.0261	0.78	0.031	78.39109
	0.84	1.56	0.14	0.026	0.76	0.0314	81.20552
	0.845	1.54	0.125	0.02605	0.77	0.0312	79.79831

APPENDIX VII

Physicochemical Properties Raw Data

	PH	TTA	BRIX
		g/100ml	%
S29_CO	4.75	0.0432	6
	4.92	0.044784	6
	4.835	0.043992	6
S25_CO	5.01	0.047272	6
	4.84	0.0456	6.5
	4.925	0.046436	6.25
S27_CO	4.57	0.064	6
	4.73	0.066346	6
	4.65	0.065173	6
S30_CO	4.86	0.053077	6.7
	4.69	0.0512	6.7
	4.775	0.052138	6.7
CY	3.61	0.0458	6
	3.74	0.047479	6.8
	3.675	0.04664	6.4
DY	4.41	0.042296	12
	4.26	0.0408	12.5
	4.335	0.041548	12.25

APPENDIX IX

Sensory Raw Data II

Samples	Appearances	Aroma	Taste	Flavour	Consistency	Overall Acceptabilty
S29_CO	7	7	8	7	8	8
S29_CO	4	4	4	4	4	4
S29_CO	7	6	6	5	5	6
S29_CO	6	5	4	4	4	4
S29_CO	5	5	5	7	7	6
S29_CO	7	6	6	6	7	6
S29_CO	9	6	6	6	7	7
S29_CO	9	7	6	7	6	7
S29_CO	6	6	5	5	6	5
S29_CO	7	5	6	6	7	7
S29_CO	6	4	3	5	5	6
S29_CO	7	6	7	6	7	7
S29_CO	7	6	6	5	6	6
S29_CO	5	5	5	5	4	4
S29_CO	7	2	2	4	3	2
S29_CO	7	5	5	6	6	6
S25_CO	6	7	6	6	7	7
S25_CO	4	4	4	4	4	4
S25_CO	7	5	5	4	5	5
S25_CO	5	5	5	5	4	6
S25_CO	6	6	6	6	6	6
S25_CO	7	7	7	7	7	7
S25_CO	9	8	8	8	8	8
S25_CO	9	6	7	7	7	7
S25_CO	7	5	4	4	5	4
S25_CO	7	5	5	6	6	6
S25_CO	5	6	6	4	5	6
S25_CO	7	6	7	6	7	7
S25_CO	5	5	5	5	5	5
S25_CO	5	5	5	5	4	4
S25_CO	8	5	3	4	4	4
S25_CO	6	6	6	6	5	6
S27_CO	8	5	6	6	7	6
S27_CO	6	7	4	3	4	4

S27_CO	1	1	1	1	1	1
S27_CO	6	6	5	6	5	5
S27_CO	6	5	6	5	4	5
S27_CO	4	5	6	6	6	6
S27_CO	7	5	4	5	7	7
S27_CO	9	6	7	7	7	6
S27_CO	6	8	4	4	7	5
S27_CO	6	7	4	5	5	5
S27_CO	2	5	2	4	4	5
S27_CO	7	7	5	8	7	5
S27_CO	4	5	7	7	7	7
S27_CO	2	2	2	2	2	2
S27_CO	9	5	5	4	5	5
S27_CO	6	7	8	7	7	8
S30_CO	7	7	9	9	7	9
S30_CO	9	6	6	6	6	6
S30_CO	4	4	4	4	4	4
S30_CO	2	2	2	2	2	2
S30_CO	5	6	5	3	6	7
S30_CO	2	2	2	2	2	2
S30_CO	8	6	7	7	8	8
S30_CO	9	5	5	5	5	6
S30_CO	5	6	6	6	7	6
S30_CO	5	5	6	5	5	5
S30_CO	2	3	2	3	4	4
S30_CO	7	5	6	5	5	6
S30_CO	4	7	4	7	5	6
S30_CO	1	1	1	1	1	1
S30_CO	4	5	5	6	6	6
S30_CO	8	7	7	6	7	7
DY	9	9	6	6	8	7
DY	9	9	9	9	9	9
DY	5	5	5	6	5	5
DY	7	8	8	6	8	7
DY	5	6	5	7	6	7
DY	5	6	5	7	6	7
DY	6	5	6	5	8	7
DY	9	5	8	8	8	9
DY	9	9	9	9	8	9
DY	7	6	6	7	8	8
DY	9	6	9	8	7	7

DY	7	8	7	7	6	7
DY	3	7	6	6	7	7
DY	4	4	4	4	4	4
DY	1	1	1	1	1	1
DY	9	8	9	8	8	8
CY	8	8	9	9	8	9
CY	9	7	9	9	9	9
CY	9	9	9	9	9	9
CY	9	9	9	9	9	9
CY	6	6	5	7	3	6
CY	9	9	9	9	9	9
CY	8	7	9	7	8	9
CY	9	5	8	8	8	9
CY	8	7	8	7	6	7
CY	6	5	7	6	6	7
CY	6	6	7	6	7	8
CY	8	8	8	8	8	8
CY	9	8	9	9	7	9
CY	9	9	9	9	9	9
CY	9	9	9	9	9	9
CY	7	8	5	6	7	6

APPENDIX X



Sensory Panel