EFFECT OF EXTENDED FERMENTATION TIME ON THE MICROBIAL, NUTRITIONAL AND SENSORY PROPERTIES OF OAT-BASED YOGURT

BY

EZEABATA EMMANUEL CHUKWUEMEKA

GOU/U22/BTG/167

BIOTECHNOLOGY

DEPARTMENT OF BIOLOGICAL SCIENCES

FACULTY OF NATURAL SCIENCES AND ENVIRONMENTAL STUDIES

GODFREY OKOYE UNIVERSITY, ENUGU NIGERIA.

JUNE, 2026

TITTLE PAGE

EFFECT OF EXTENDED FERMENTATION TIME ON THE MICROBIAL, NUTRITIONAL AND SENSORY PROPERTIES OF OAT-BASED YOGURT

BY

EZEABATA EMMANUEL CHUKWUEMEKA
GOU/U22/BTG/167

BIOTECHNOLOGY
DEPARTMENT OF BIOLOGICAL SCIENCES
FACULTY OF NATURAL SCIENCES AND ENVIRONMENTAL STUDIES

GODFREY OKOYE UNIVERSITY, ENUGU NIGERIA.

A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) DEGREE IN BIOTECHNOLOGY.

SUPERVISOR: Dr. I. NWAJOBI
CO-SUPERIOR: Mrs Ruth Ugwu

JUNE, 2026

APPROVAL

This research titled "Effect of extended fermentation time on the microbial, nutritional and sensory properties of oat-based yogurt" has been assessed and approved by the Committee of Department of Biological Sciences and the Faculty of Natural Sciences and Environmental Studies, Godfrey Okoye University Enugu, Nigeria.

Dr. I. NWAJOBI	____________________	________________
Supervisor	**Signature**	**Date**
Prof. Florence I. Akaneme	____________________	________________
Supervisor	**Signature**	**Date**
Dr. Frances N. Olisaka	____________________	________________
HOD, Department of Biological Sciences	**Signature**	**Date**
Prof. Chidi C. Uhuegbu	____________________	________________
Supervisor	**Signature**	**Date**

CERTIFICATION

This is to certify that this research titled (Effect of extended fermentation time on the microbial, nutritional and sensory properties of oat-based yogurt) was carried out by **EZEABATA EMMANUEL CHUKWUEMEKA** under the supervision of Dr. I. NWAJOBI in the Department of Biological Sciences, Godfrey Okoye University Enugu, Nigeria.

EZEABATA EMMANUEL CHUKWUEMEKA ________________ ________________

Signature **Date**

DEDICATION

I dedicate this work to our Lord for all His mercy and strength in all of my studies. I would like to dedicate this work to my family, who have been stepping stones in my life, helping me become successful through their love, sacrifice, and prayers. Finally, to Godfrey Okoye University, for the academic environment and challenges that have shaped my mind and spirit.

ACKNOWLEDGEMENT

My sincere gratitude goes to my supervisor, Dr. I. Nwajiobi, who made sure that this research work was a great success. In a special way I thank my Head of Department, Dr. Frances N. Olisaka for her timeliness and word of advice on the preparations to embark on the laboratory activities and most importantly, the Dean of the Faculty of Natural Sciences and Environmental Studies, Prof. Chidi Uchegbu for his seamless effort. I would like to thank the entire staff of the Department of Biological Sciences, Godfrey Okoye University, for their massive support during the project, especially Dr. I. Nwajiobi and Mrs Ruth Ugwu. My fellow supervisees, thank you for your time, patience, and hardworking spirit in the project of this course.

Thanks a lot to Mr Patrick Ezeabata and Mrs Fransisca Ezeabata, Mrs Prisca Ezeabata and Miss Maryjane Ezeabata for their financial support, unconditional love and words of wisdom throughout my studies; thanks to my siblings for their support throughout my studies; and thanks to my friends Gideon, Samuel, Daniel, Princess, Fechukwu, Paul, thanks to all of you.

Last but not least, I would like to thank all those whom I didn't include but are also deserving of praise; may God bless you all.

ABSTRACT

The growing demand for plant-based dairy alternatives driven by lactose intolerance, dairy allergies, and health-conscious consumers has positioned oat-based yogurt as a promising functional food. Oats are rich in β-glucan, offering cholesterol-lowering and gut health benefits. This study aimed to investigate the effects of extended fermentation durations on the nutritional, microbial, and sensory characteristics of oat-based yogurt. Oat milk was prepared from whole-grain oat flour and dates, then fermented with commercial lactic acid bacteria starters at 42°C using a Completely Randomized Design with four treatments: 0 h (control), 12 h, 24 h, and 48 h fermentation periods. Microbial viability was determined using deMann, Rogosa and Sharpe (MRS), Nutrient, and Potato Dextrose Agars; pH and proximate composition (moisture, ash, crude protein, fat, fibre, carbohydrate) were analyzed following AOAC methods; sensory evaluation was conducted by 52 panelists using a 9-point hedonic scale. Data were analyzed using one-way ANOVA and expressed as mean ± standard deviation. Results revealed that viable lactic acid bacteria counts increased with fermentation time, reaching up to 1.09×10^7 CFU/mL on MRS and 1.72×10^7 CFU/mL on Nutrient Agar at 48 h. Yeast and mould counts were moderate but declined over time. The 12-hour fermented sample showed the best nutritional balance with favourable moisture, protein, fat, and fibre contents among fermented treatments, while sensory scores (taste, aroma, colour, texture) were highest in the control and declined progressively with longer fermentation, with the 12-hour sample being the most acceptable among fermented yogurts. The study concludes that 12-hour fermentation offers the optimal balance of probiotic viability, nutritional quality, and sensory acceptability, making oat-based yogurt a suitable nutritious alternative for lactose-intolerant individuals and consumers of plant-based diets.

TABLE OF CONTENTS

CHAPTER ONE

INTRODUCTION

1.1 Background of study

The growing popularity of health, sustainability, and ethical food options among consumers has been a driving force behind the rise of plant-based dairy alternatives worldwide. Among them, the oat-based products are distinguished by their unique nutritional value and functionality. Oats are also well-known due to their high contents of soluble dietary fibre, especially β-glucan, which is linked to such benefits as the cholesterol-reducing effect, the positive effect on glycaemic control, and the health of the gut. These properties render oat milk and its fermented analogs appealing choices among people with lactose intolerance, allergy to dairy products, or people on plant-based diets (Alemayehu *et al*., 2023; Yu*et al*., 2023).

Traditionally made by fermenting dairy milk using lactic acid, yogurt is known to possess sensory properties and health-promoting properties that come as a result of the metabolic processes of lactic acid bacteria (LAB). The universal starter cultures, *Streptococcus thermophilus*and *Lactobacillus delbrueckii*subsp. *Bulgaricus*, is the most important in that they convert the available sugars into lactic acid. The process leads to acidification, coagulation of proteins, and the acquisition of the typical gel texture, aroma, and flavour profile of yogurt. In systems based on oats, though, the natural lack of lactose necessitates prior enzymatic degradation of oat starch to release fermentable sugars, with a subsequent inoculation of LAB to produce a similar fermented product (Xu *et al*., 2024; Pua*et al*., 2022; Kut*et al*., 2023).

Studies on fermented oat drinks and yogurts have always emphasized the decisive importance of processing parameters on the quality of the final product. Enzymatic liquefaction and saccharification with the help of alpha-amylase and glucoamylase are actively used to reduce the viscosity of oat slurries and increase substrate availability to fermentation. The following fermentation of lactic acid under controlled temperatures (usually 37-42 o C) and different inoculum concentrations results in quantifiable modifications in pH, titratable acidity, rheological behavior, and microbial viability (Xu *et al*., 2024; Silventoinen-Vejalainen*et al*., 2023; Benmezaine and Belleili, 2023). The steps would be necessary in breaking the technological barriers posed by plant-based matrices, including poor gel formation and phase separation.

Research comparing dairy oat hybrid and pure oats systems has indicated that the length of fermentation has a direct impact on the major quality characteristics. Common standard fermentation times of between 5 and 6 hours are typically employed to achieve a desired pH range of 4.5, although it has been indicated that longer fermentation periods can enhance exopolysaccharide (EPS) production by some LAB strains, thus increasing gel strength and water retention. Extended fermentation in oat matrices has also been associated with increased degradation of complex carbohydrates, possible increases in bioactive compound bioavailability, and changes in sensory properties, such as decreased cereal-like flavors and increased creaminess (Ziarno*et al*., 2025; Hoxha *et al*., 2023; Kuehn*et al*., 2023). Stability of storage also proves to be a crucial factor in this and the majority of studies involve monitoring the physicochemical, microbial, and sensory variations over days of storage at refrigeration (4°C). It is commonly accepted that to maintain the probiotic functionality, LAB viability must be maintained at above 8 log CFU/g throughout this time (Hoxha *et al*., 2023; Ziarno*et al*., 2025; Parvarei*et al*., 2021).

Consumer research supports the market potential of oat-based yogurts, with the perception of health benefits, environmental friendliness, and clean-label acceptance. Nevertheless, the recurring issues are to attain the same texture and flavour characteristics as conventional dairy yogurt, especially in 100% oat formulations, as opposed to hybrid blends. Oat cultivar selection, flour properties, and processing have been demonstrated to impact particle size, viscosity, whitening index, and general sensory acceptability (McCarron*et al*., 2024; Zhou *et al*., 2023; Halme*et al*., 2023; Rovai*et al*., 2025). All these insights foster the significance of methodical optimisation of fermentation conditions to liberate the entire functional capabilities of oat-based yogurt.

1.2 Statement of the Problem

Despite the growing interest in oat-based yogurt as a functional plant-based alternative, there is still much to learn about the particular effects of long fermentation duration on its nutritional, microbial, and sensory characteristics. Literature is generally based on brief and standardised fermentation times (usually 5-6 hours) to achieve a desired pH of around 4.5, and the presence of intentionally extended fermentation times in pure oat systems has not been investigated extensively (Hoxha *et al*., 2023; Xu *et al*., 2024; Kuett*et al*., 2023). This is very limited in grasping the potential changes that could be caused by extended fermentation to the product results which could either be positive or detrimental to the overall quality.

Nutritionally, short durations of fermentation might not effectively harness the release of bioactive constituents or the degradation of anti-nutritional constituents within oat starch. Long-term use of LAB activity has the potential to enhance β-glucan solubility, increase free phenolic content, and protein digestibility, but empirical data specific to oat yogurt is limited (Decker *et al*., 2014; Leszczyńska*et al*., 2023; Alemayehu *et al*., 2023; Xu *et al*., 2024).

Microbially, although LAB viability is typically maintained when stored at refrigeration conditions, the impact of prolonged fermentation on strain dominance, EPS production, and long-term stability in oat matrices has not been thoroughly studied (Hoxha *et al*., 2023; Ziarno*et al*., 2025; Parvarei*et al*., 2021).

Sensory and rheological-wise, oat yogurts are often adversely characterized by poor gel properties, high syneresis, and strong cereal out-of-place off-flavours, which do not go well with consumer acceptance. Despite the studies on enzymatic pre-treatment and starter culture optimisation, the effects of fermentation duration on apparent viscosity, water-holding capacity, syneresis, colour, aroma, taste, and overall hedonic scores have not been sufficiently studied over a variety of prolonged periods (McCarron*et al*., 2024; Zhou *et al*., 2023;

Ziarno*etal*., 2025). Moreover, most research is focused on dairy-oats blends, and there is a significant knowledge gap regarding purely plant-based oat yogurts (Ziarno*et al*., 2025; Pua*et al*., 2022).

The absence of specific studies on long-term fermentation consequently hinders the development of uniform, optimised practices of production of commercial oat yogurt. In the absence of this data, manufacturers are likely to create products that are not up to the expectations of consumers in terms of texture, flavour, nutritional value, and probiotic efficacy. This research directly fulfills these gaps by methodically investigating the impact of a longer fermentation period on nutritional, microbial, and sensory characteristics of oat-based yogurt.

1.3 Aim and Objectives

This study was aimed at how long a fermentation period would affect nutritional, microbial, and sensory characteristics of oat-based yogurt.

The specific objectives were to:

i. Prepare oat milk from whole-grain oat flour and ferment it into yogurt using both standard and extended fermentation durations.

ii. Evaluate the microbial viability and growth dynamics of lactic acid bacteria throughout 21, 24, and 48 hours of fermentation.

iii. Determine the changes in nutritional composition, including moisture, protein, fat, fibre, carbohydrate content, resulting from different fermentation times.

iv. Assess the sensory characteristics (colour, texture, aroma, taste, and overall acceptability) of the yogurts produced at each fermentation interval.

v. Identify the optimal fermentation duration that best balances nutritional enhancement, microbial stability, and sensory quality in oat-based yogurt.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Avena sativa* (oats)

2.1.1 Nutritional Composition of Oats

Due to the high protein and lipid content compared with other cereals, oats are very rich in nutrients,including starch, protein, fibre (beta-glucans), antioxidants, vitamins, and healthy fats (Kütt*et al*., 2023). Oats are a rich source of quality protein with balanced amino acids, fibre (β – glucan), carbohydrates, and minerals they also possess a well-balanced nutritional composition (Gupta and Bisla, 2019). The oat grains are rich in protein, fibre, minerals, and many other antioxidants (vitamins, avenanthramides, phenolic acids, sterols, phytic acid, flavonoids) (Gupta and Bisla, 2019).

Oats are a source of many health benefits, such as lowering the risk of cardiovascular diseases, type 2 diabetes, gastrointestinal disorders, and cancer (Djorgbenoo*et al*., 2023), and are regarded as one of the healthiest foods around the globe. The oats are rich in bioactive compounds, particularly natural antioxidant phenolic compounds and β-glucan (Chen *et al*., 2020). Oats have a high content of dietary fibre, antioxidants, and a well-balanced protein fraction (Angelov*et al*., 2018). The lipid content of oats is higher than that of all other cereals. Lipids present in oats are of great importance in human nutrition due to their high content of unsaturated fatty acids (linoleic acid and oleic acid) and essential fatty acids (myristic acid, palmitic acid, stearic acid) (Das and Maria, 2017).

βeta-Glucans are generally found in cereals and fungi and are polysaccharides with a bioactive effect. Cereal, a good source of β-glucan, is mainly composed of barley and oats. Oat β-glucan has beneficial physiological activities such as lowering cholesterol, reducing postprandial blood glucose response, preventing diabetes, and regulating immunity (Zhao *et al*., 2023). The β-glucan content in oat ranges from 1.8 to 8.1% (dry matter) (Angelov*et al*., 2018). Oat products are a primary source of available carbohydrates as starch. It is approximately 82%

(dry basis) of the endosperm of the oat kernel (Angelov*et al*., 2018). The protein yield of whole oatis 13 to 20%. The proteins are found mostly in the embryo ~30% (Paudel*et al*., 2021). Oats also contain a wealth of micronutrients such as thiamine, folate, biotin, riboflavin, niacin, iron, potassium, copper, magnesium, zinc, and phosphorus (Das and Maria, 2017).

2.2 Health Challenges Associated with Dairy Yogurt

2.2.1 Lactose Intolerance

One of the common fermented dairy products that is commonly consumed all over the world is yogurt. It is achieved by lactic acid fermentation of milk by the action of a starter culture containing *Lactobacillus delbrueckii*ssp. *Bulgaricus* and *Streptococcus thermophilus* (Gupta and Bisla, 2019). Conventional fermented dairy products are widely used for delivering probiotics. However, as lactose intolerance and milk allergy become more prevalent, and due to the world's interest in low-cholesterol products, there is growing demand for non-dairy probiotics.

2.2.2 Non-Palatability of Traditional Plant Yogurts

While plant-based yogurts, such as oat, are generally popular, they are often less palatable than dairy yogurt and pose a considerable challenge for consumer acceptance. Multiple studies have noted that plant-based beverages are often inadequate in the sensory attributes that are desirable in dairy products and thus need to be overly fortified or processed to achieve the desired level of acceptance.

Plant-based drinks are typically not suitable for consumption in place of dairy products and have to be overly fortified (Kütt*et al*., 2023). Although fermentation is an easy and natural method for enhancing the sensory, food, and nutritional quality of plant-based dairy alternatives, there are sensory constraints. The sensory evaluation indicated that all fermented oat beverages were sour-smelling and tasting. The volatile compounds isolated were of different classes such as ketones, alcohols, aldehydes, acids, and furans. In the sensory evaluation, however, all samples were connected to cereals instead of dairy regarding their taste and odor (Kütt*et al*., 2023).

This is a sensory characteristic commonly found in oat-based and other plant yogurts that is similar to a cereal. Unfortunately, plant-based drinks are rarely nutritionally suited to conventional dairy products and need more fortification (Kütt*et al*., 2023). The variants of oat milk and cow milk were prepared and five variants of oat milk based yogurt were developed in oat milk-based yogurt development. It was accepted as 60:40 for oat and cow's milk-based yogurt and was found to be less acceptable with a higher proportion of oat than a higher proportion of cow's milk (Gupta and Bisla, 2019). Using oat milk to substitute cow milk in yogurt reduces fat content, as pure oat products have sensory problems.

The lack of palatability is also attributed to texture and mouthfeel. Many plant-based yogurts suffer from weak gel quality, high syneresis, and chalky or sandy mouthfeel, stemming from the differences in protein structure and starch gelatinization in such systems. When fermented oat drinks were analysedrheologically, they formed weak gel-like structures (Kütt*et al*., 2023). The quality characteristics of low-fat pea protein yogurt were specifically compared with a control sample having higher oil content and which was formulated with oat β-glucan, showing prominent pea protein-based fruity, sweet, and creamy flavors that addressed the issues mentioned earlier (Zhao *et al*., 2023).

Usually, enzymatic hydrolysis and fermentation optimization are necessary to reduce beany/ cereal off-notes and enhance viscosity, respectively. In addition to the fermentative process, enzymatic hydrolysis processing was needed to optimize the acidity and coagulated texture of set-type oat yogurts, traditional plant yogurts have poor texture and flavors (Xu *et al*., 2024). In the same way, the characteristics of oat flour (particle size, viscosity, dispersion stability, and colour) were found to affect the physicochemical properties of the oat-based milk substitutes, which in turn affect the sensorial properties (Silventoinen-Vejalainen*et al*., 2023).

Home-made and commercial plant-based yogurts (almond, oat, soy) made with yogurt starter culture (standard culture) differed in their sensory profiles; oat-based yogurts often had different sensory attributes compared to the creamy, dairy-like properties that consumers expect (Łopusiewicz, 2024). The development of a plant-based yogurt using peanut, oat, and coconut matrices by means of simplex lattice design to optimize the yogurt's rheological and sensory properties highlighted the challenges in making a plant-based yogurt with a dairy-like taste and texture attributes (Baskar*et al*., 2022).

The non-palatability of traditional plant yogurts can be attributed to the cereal-related flavor and odor, poor texture, high syneresis, and chalky mouthfeel overall. In addition to the limitations, advanced processing methods like enzymatic hydrolysis, incorporation of β-glucan, use of specific starter cultures, and blending techniques have been explored to improve consumer acceptance of oat-based yogurt (Kütt*et al*., 2023; Zhao *et al*., 2023; Gupta and Bisla, 2019; Silventoinen-Vejalainen*et al*., 2023; Xu *et al*., 2024).

2.3 Advantages of Oat yogurt

2.3.1 Nutritional Advantages

The nutritional benefits of oat yogurts compared to conventional dairy yogurts and many other plant-based yogurts are significant, largely because of the special properties of oats and the added benefits from fermentation. Oats are an excellent source of starch, protein, fibre (beta-glucans), antioxidants, vitamins, and healthy fats, containing more protein and lipids than other cereals (Kütt*et al*., 2023). The nutritional profile of oats is well balanced, as it contains a good amount of quality protein, which is rich in balanced amino acids, fibre (β-glucan), carbohydrates, and minerals (Gupta and Bisla, 2019). The protein, fibre, minerals, and various other compounds that are present in oat grains have been found to exhibit antioxidant activity due to their Vitamin, Avenanthramides, Phenolic acids, Sterols, Phytic acid, and Flavonoids (Gupta and Bisla, 2019).

The most important nutritional advantage is the presence of a high level of bioactive polysaccharides β-glucans, which are mainly present in oats. Oat β-glucan has beneficial physiological activities such as lowering cholesterol, reducing postprandial blood glucose response, preventing diabetes, and regulating immunity (Zhao *et al.*, 2023). The amount of β-glucan in oat is in the range of 1.8–8.1% (dm) (Angelov*et al.*, 2018) and thus, oat yogurt can provide the recommended daily value for cholesterol-lowering (Angelov*et al.*, 2018). Fermentation further benefits these with better nutrient bioavailability and bioactive compounds. Under the influence of fermentation, the nutritional value, sensory properties, and shelf-life of the product are improved (Kütt*et al.*, 2023).

Another attribute of oat yogurts is the protein quality. The protein content in oat groats is 13-20%, and proteins are largely located in the embryo. Oat proteins have a higher lysine content than wheat, which is the main limiting amino acid in cereals (Paudel*et al.*, 2021). Results indicate that the amino acid content of oats (lysine and methionine + tryptophan) improved by fermentation which led to an increase in the protein content and digestibility of cereals (Das and Maria, 2017). This makes oat yogurt a unique source of high-quality plant protein, especially for populations that are looking for this type of protein.

The lipid profile of oat yogurt offers other benefits. The lipids of oats are rich in unsaturated fatty acids (linoleic acid and oleic acid) and essential fatty acids and are unique among cereals. These lipids are important for human nutrition (Das and Maria, 2017). Whereas, oat-based yogurt is naturally cholesterol-free and has less saturated fat than its dairy counterpart, which can be important for cardiovascular health (Djorgbenoo*et al.*, 2023).

Another advantage is the micronutrient density. Oats are rich in micronutrients like thiamine, folate, biotin, riboflavin, niacin, iron, potassium, copper, magnesium, zinc, and phosphorus (Das and Maria, 2017). Other B vitamins can be further improved by lactic acid bacteria fermentation. During the fermentation of cereal, Lactic acid bacteria affect the production of Vitamin B2, Vitamin B9, Vitamin B11, and Vitamin B12, (Das and Maria, 2017). Further, the fermentation process yields optimum pH for enzymatic breakdown of phytate, hence making minerals like Fe, Zn, and Ca, soluble and bioavailable (Das and Maria, 2017).

Oat Yogurt is a lactose-free, cholesterol-free, and allergen-free option rich in functional benefits, similar to or better than those of dairy yogurt. The use of oat milk to replace cow milk in yogurt decreases fat content and the major problems associated with cow milk fat, cholesterol, and lactose contents (Gupta and Bisla, 2019). Oat yogurt is a better option for lactose-intolerant people, people with dairy allergies, and health-conscious people who are looking for sustainable nutrition, as the result of fermentation, the antinutrients are reduced and the bioavailability of nutrients is increased (Kütt*et al.*, 2023; Silventoinen-Vejalainen*et al.*, 2023).

Fortified versions may further increase the nutritional benefits with the inclusion of β-glucan or other prebiotics. In addition to providing a positive impact on texture, the addition of oat β-glucan also provides validated physiological benefits in the areas of cholesterol and glucose management (Zhao *et al.*, 2023). In general, the oat yogurts possess a functional food quality in addition to the natural nutritional richness of oats, which is enhanced by the fermentation process, thereby providing greater protein quality, fiber, antioxidant activity, mineral bioavailability, and probiotic delivery.

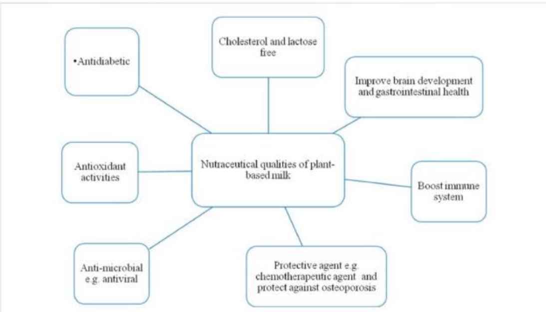


Figure 1. Some nutraceutical qualities of plant based milk.

source : Irondi*et al.*, 2023

2.3.2 Environmental Advantages

Oat yogurts and other oat-based fermented products offer substantial environmental benefits over conventional dairy products, mainly due to the lower use of resources, lower greenhouse gas emissions, sustainable sourcing of raw materials, and efficient valorization of by-products. The benefits will reflect the trend of plant-based diets and the circular economy in food production, which is gaining popularity in the world.

As consumers seek healthy alternatives to dairy products, the production of oat milk is also rising. Oat milk production leaves oat pulp as the major by-product, which is insoluble. Some 0.2–0.45 kg of oat pulp is generated for each 1 kg of oat milk; globally, some 228 kilotons are produced annually, and this is estimated to rise to 500 kilotons by 2030 (Le *et al.*, 2025). Oat pulp contains a lot of proteins, dietary fibre, β-glucan, lipids, and bioactive compounds, but it is very easy to break down by microorganisms, and this by-product is being wasted today in landfills and has a negative effect on the environment. The understanding of the nutritional profile, the oat milk processing, and the existing value of oat milk is crucial for the valorization of oat pulp. The efficient use of oat pulp and other by-products is crucial for addressing environmental concerns and the growing demand for sustainable food production solutions (Le *et al.*, 2025).

The eco-credentials of oat yogurts are largely due to the reduced overall ecological impact of growing and processing oats compared to dairy systems. Oats require cool, moist conditions and sandy loam to heavy clay soils with good drainage to grow oats, which makes it possible to grow oats in areas where soy or nut production may not be possible because of weather-and agronomic conditions (Kütt*et al.*, 2023). This flexibility means that the land requirement for the dairy farm does not need to be converted for feeds, nor does the farm need a high level of inputs to grow feeds. Oat yogurt production uses relatively low resource inputs, helps to use land resources more sustainably, and reduces water and energy usage in the supply chain.

In addition, the transition from dairy to oat yogurts helps to solve the environmental issues associated with the consumption of animal products. In conventional dairy yogurt manufacturing, the input of milk, which includes high GHG emissions from livestock, land use for grazing and feed, and water use, is a major input. Oat yogurts, on the other hand, contribute to the larger push for plant-based eating which minimizes impacts. It also offers a healthy and sustainable diet for mankind from plant-based foods. In order to address these needs, innovative plant-based dairy products that closely replicate animal-based dairy products need to be developed (Kütt*et al*., 2023). The production of fermented oat beverages began in Europe, when the functional food market began to grow, encouraged by a growing consumer interest in functional foods as a product which can carry with it a nutritional and health value and promotes environmental actions (Angelov*et al*., 2018).

The by-product valorization contributes to an environmental benefit. Oat pulp, an insoluble by-product of the process used to make oat milk, when added to yogurts and other formulated products, can reduce waste sent to landfills and act as a useful ingredient in a product with nutritional benefits, such as fiber, protein, and β-glucan. The circular model ensures that waste is minimized, methane emissions from landfills are lower, and the efficiency of resource use in oat processing is enhanced (Le *et al*., 2025). Those are examples of sustainable food production, closing the loop in the value chain for oats.

Fermentation technologies for extending the shelf life and minimizing food waste are also available for oat yogurts. This fermentation has the effect of extending the shelf life of the product, enriching its nutritional value, enhancing its sensory characteristics, reducing the risk of spoilage, and minimizing environmental waste due to the disposal of food waste (Kütt*et al*., 2023; Bernat*et al*., 2015). Product stability of 21–28 days in refrigerated storage also helps to minimize losses in the supply chain.

Oat-based systems show benefits in sustainability metrics when compared with other systems. Oats are an alternative functional protein source that provides separation technologies and functionality, which are useful in the context of lower-impact food systems (Holopainen-Mantila*et al*., 2024). The use of oat-based milk alternatives has also significantly grown, and optimizing resource use (Silventoinen-Vejalainen*et al*., 2023) by identifying factors that affect the quality of the product has a significant economic and environmental impact.

In general, the environmental benefits of oat yogurt are the reduction of greenhouse gas emissions, the reduction of water and land consumption, the versatility of the use of the product in different climatic conditions, the valorization of by-products through fermentation, and the possibility of reducing food waste during fermentation. Overall, the properties of oat yogurt make it a promising alternative to conventional yogurt, offering a range of nutritional benefits and sustainable options to help reduce environmental impact and promote a healthier future (Le *et al*., 2025; Kütt*et al*., 2023; Angelov*et al*., 2018).

2.3.3 Economic Advantages

A significant economic benefit of the oat fermented products and oat yogurts is their lower raw material expenses, potential market expansion, by-product valorization, and production cost savings compared to dairy yogurts. The benefits of oat yogurt make it an interesting product for manufacturers and consumers interested in the low-cost, value-added functional food.

Oats have a low taste and are a healthy raw material. In recent times, the use of oat-based milk substitutes (OBMS) has significantly risen and consequently, understanding the factors affecting the quality of OBMS is of the utmost importance and economic value (Silventoinen-Vejalainen*et al*., 2023). Oat yogurt producers have great potential in the market as consumers are increasingly seeking plant-based alternatives. As value-added and functional foods with high nutrient value of oat and the demand of health-conscious consumers, fermented oat beverages have excellent market potential (Yu*et al*., 2023). This market expansion contributes to the economic benefits by means of an increase in sales volume, product diversification, and premiums for functional claims.

Oats' economic advantages include their low cost as a raw material. Oats tend to be cheaper and easier to grow than dairy production systems, which are highly capital-intensive with the needs of animal husbandry, feed, and processing. The use of oat milk instead of cow milk in yogurt also reduces the fat content, and the key issues of cow milk fat (CMF) such as cholesterol content and lactose content are reduced which makes the product more economical and acceptable with optimized blends (Gupta and Bisla, 2019). This alternative cuts the reliance on costly dairy ingredients and related supply chain expenses.

The rising functional food market due to health-conscious consumers offers significant economic incentives. Plant-based fermented drinks represent the new reality for a sustainable lifestyle… new plant-based dairy alternatives, that are similar to animal-based foods, have to be developed (Küttet *al*., 2023). Oat yogurts offer an interesting positioning in the functional foods market due to their health-promoting properties including β-glucan as a cholesterol-lowering agent, probiotics, and antioxidants, which help maintain a higher profit margin (Zhao *et al*., 2023; Djorgbenoo*et al*., 2023; Angelov*et al*., 2018).

Economic efficiency is further improved by optimizing processing methods including the use of enzymes and starter culture selection. Identifying the factors that influence the quality of the OBMS is essential and has a considerable economic impact (Silventoinen-Vejalainen*et al*., 2023). The α-amylase treatment and controlled fermentation techniques used in the production process will increase yields, decrease energy inputs, and ensure product consistency, thereby lowering overall production costs while maintaining high product quality (Xu *et al*., 2024; Silventoinen-Vejalainen*et al*., 2023).

The local production of oats can help boost local economies by lowering the transportation and import prices of this crop in areas where oats grow in large numbers. Oats grow best in cool and moist environments, which helps to cultivate oats in regions (e.g. Eastern Europe, Scandinavia, North America) where the cultivation of soya or nuts is not applicable (Kütt*et al*., 2023). The versatility of using oat yogurt in various global regions makes the process economically viable, thereby advancing food security and supporting local economies.

In general, the economic benefits of the oat yogurts are reduced raw material and production costs, utilization of by-products to produce value-added products that generate additional income, longer shelf life to minimize losses, enhanced value of the product in the burgeoning functional foods market, and contribution to local agricultural economies. All these factors make oat yogurt a cost-effective, scalable, and profitable dairy yogurt replacement, holding great potential for sustainable business growth (Le *et al.*, 2025; Silventoinen-Vejalainen*et al*., 2023; Gupta and Bisla, 2019; Kütt*et al*., 2023; Yu*et al*., 2023).

2.4 Production of Oat-Based Yogurt

2.4.1 Raw Material Preparation

Oat flakes were used to make oat milk and its formulations was adjusted to be similar to bovine milk properties. To prepare oat milk, 100 g of oat flakes was used to make 1 litre of oat milk. The flakes of oats were soaked in water for 12 hours overnight, covered with a clean cloth. The ingredients were thoroughly whizzed in a mixer grinder (about 2 minutes) to prepare oat milk. Milk was poured through a very fine sieve or muslin cloth into a bowl (Gupta and Bisla, 2019).

2.4.2 Heat Treatment

Heating the oat milk to 90°C for 3 minutes and then holding it at that temperature for 30 minutes was done to pasteurize the milk. After this, it was cooled to the temperature of inoculation (45°C) in a closed vessel (Gupta and Bisla, 2019).

2.4.3 Homogenization

The oat flour was initially blended at 10% dry matter content with water and homogenised with an Ultra-Turrax. The homogenised sample was subjected to treatment with an α-amylase (Silventoinen-Vejalainen*et al*., 2023).

2.4.4 Fermentation Technology

The starter culture strain was inoculated into the oat milk at the level of 2.0 g/100ml. The inoculated milk was allowed to incubate at 45°C until the pH reached 4.4 in about 5 hours (Gupta and Bisla, 2019). The study aimed to analyse the fermentation of oat milk with the microorganisms *Lactobacillus reuteri*and*Streptococcus thermophilus* to create a new probiotic product. No matter which starter culture was used, the desired pH below 4.2 was obtained after 12 h. The dominant species in all the samples was *S. thermophilus*, accounting for 38 to 99% of the microbial consortia. Lactic acid was produced between 1.6 and 2.8 g/L (Kütt*et al.*, 2023).

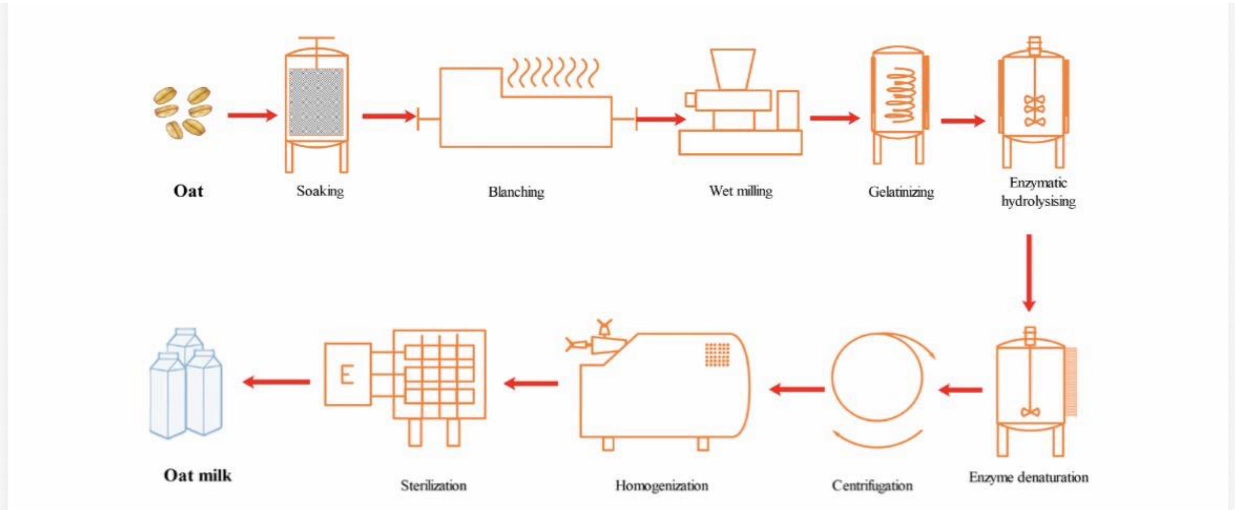


Figure 2. The manufacturing process of oat milk

Source: Foods, 2023.

2.4.5 Storage Technology

During refrigerated storage (4°C), the fermented product was analyzed for pH, acidity, β-glucan and oligosaccharides levels, colour, and rheological behaviour. All the formulations used were sufficient enough to make fermented foods and a minimum dose of each factor was used as the optimum dose. The formulation chosen yielded a starter's survival above 107cfu/ml, which was considered a functional food, and the starter's β-glucans were retained in the final product and had a positive effect on viscosity (Bernat*et al.*, 2015).

2.5 The impact of Extended Fermentation on nutritional properties.

2.5.1 Protein Digestibility

Oat yogurt is a great example of how fermentation, especially prolonged fermentation, can result in a more digestible and bioavailable protein, which can prove to be nutritionally beneficial and enhance absorption in the human gastrointestinal tract.

The use of fermentation has proven to increase the protein content of cereals by enhancing the amino acid content (lysine, methionine, and tryptophan) of grains like wheat, rice, oats, and corn (Das and Maria, 2017). This enhancement of the amino acid balance is a direct improvement in protein digestibility. Enzymes cause the proteins to break down into smaller pieces during fermentation and these smaller proteins are easier for the body to absorb than the intact protein of oats.

Lactic acid bacteria (LAB) are important for their proteolytic activity during the long fermentation period in oat-based systems. Fermentation enriches food with biological protein and essential amino acids and other nutrients (Kütt*et al.* 2023; Angelov*et al.* 2018). These enzymes have increased activity due to starter cultures and probiotics (*Lactobacillus plantarum, L. acidophilus, and S. thermophilus*) and will break down oat globulins and other storage proteins for a longer period of time as a result of the longer fermentation time.

This is because the protein content of oats is greater than that of wheat, the limiting amino acid in cereals (Paudel*et al*., 2021). This is further enhanced by fermentation. The fermentation with microbial cultures results in proteolysis, which breaks down proteins into peptides and amino acids, thereby improving the digestibility and nutritional quality of the oat yogurt product (Das and Maria, 2017; Kütt*et al*., 2023).

Several studies have confirmed that longer fermentation times are associated with higher amounts of bioactive peptides from fermented oat products with better digestibility. Fermentation processes are also involved in the production of peptides with better absorption and potential beneficial health effects in synbiotic oat yogurts, like ACE-inhibitory activity, (from various sources: Zhao *et al*., 2023; Chen *et al*., 2020).

When the fermentation lasts for a longer period of time (such as reducing pH below 4.2 in 12 hours or more, and continuing for longer), the pH drop and enzymatic reaction create an ideal environment for protein hydrolysis. With a lower pH, the counts of proteolytic strains such as *L. acidophilus, L. plantarum, and L. paracasei* also rise, thereby further contributing to the breakdown of proteins (Kütt*et al*., 2023). This yields better in vitro protein digestibility values for longer fermented oat yogurt than for shorter fermentations and unfermented oat bases.

Oat yogurts have advantages over dairy yogurts due to fermentation. Dairy proteins are easily digested, but the fermented oat proteins are more competitive because the anti-nutritional factors are less, and the availability of free amino acids is higher compared to the other proteins (Das and Maria, 2017; Gupta and Bisla, 2019). This is especially useful when making long-fermenting oats for a specific group of people who have certain nutritional requirements such as vegetarians, vegans, or those who are looking for plant-based high-quality protein sources.

Overall, the extended fermentation seems to have a positive effect on oat yogurts' protein digestibility due to the proteolytic action of LAB, the release of free amino acids, the degradation of complex storage proteins, and synergistic effects with β-glucan and other oat components. These modifications increase the nutritional worth of oat yogurt, making it a better functional food item (Das and Maria, 2017; Kütt*et al*., 2023; Paudel*et al*., 2021; Angelov*et al*., 2018).

2.5.2 Reduction of Anti-Nutritional Factors

The key nutrient change in oat yogurts by using extended fermentation is the marked decrease in anti-nutrients, especially phytic acid (phytate), which increases mineral bioavailability and nutrient absorption.

Cereals contain phytate in complexes with polyvalent cations, mainly iron, zinc, calcium, magnesium, and proteins, and optimum pH during fermentation will result in optimum enzymatic degradation of phytate. During enzymatic degradation, the amount of phytate may decrease several-fold and the iron, zinc, and calcium present in the food may increase several-fold (Das and Maria, 2017).

Phytate is one of the primary anti-nutrient compounds in oats and other cereals that can form insoluble complexes with essential minerals, which then cannot be absorbed in the human gut. The gradual decrease of pH (which can drop below pH 4.2 in 12 hours and get even lower during longer fermentation) puts the system in an acidic state, which triggers endogenous and microbial phytases. Enzymes break down phytate, releasing more bioavailable minerals (Das and Maria, 2017; Kütt*et al*., 2023).

Streptococcus thermophilus, Lactobacillus acidophilus, L. plantarum, and others are lactic acid bacteria (LAB) that play a direct role in the fermentation process of oat yogurt. At lower pHs, the number of these proteolytic and phytase-producing strains remains high, thus promoting degradation of anti-nutritional compounds (Kütt*et al*., 2023). Long fermentation (such as 24-48 hours) provides more complete enzymatic activity than the traditional fermentation period, leading to greater reduction in phytate and better nutritional profile.

This decrease in the content of anti-nutritional factors has a positive impact on the oat yogurt's overall nutritional quality. Extended fermentation releases minerals from phytate complexes, thereby enhancing the bioavailability of important micronutrients such as iron, zinc, calcium, and magnesium for the immune system, bone health, and metabolism (Das and Maria, 2017). Further, the fermentation resulted in an increase in protein digestibility while phytate also lessened its interference with protein digestion (Das and Maria, 2017; Paudel*et al.*, 2021).

2.5.3 Vitamin Enhancement

Oat yogurts are enhanced by lactic acid bacteria (LAB) during extended fermentation, especially with respect to the B-group vitamins. This can lead to a greater production and bioavailability of the essential vitamins in the oat-based matrices and consequently to the production of more nutritious functional foods.

Fermentation can enhance the B vitamins in cereal by using lactic acid bacteria (LAB). Lactic acid bacteria enhance the production of vitamin B2, B9, B11, and B12 (Das and Maria, 2017). Extended fermentation (usually 12–48 hours or longer) is a good time for vitamin biosynthesis because the starter cultures and probiotics (*Streptococcus thermophilus, Lactobacillus acidophilus, L. plantarum*, and other microorganism starter strains) are active for a longer period. As the pH drops and microorganisms grow, these bacteria naturally make and accumulate B vitamins in unfermented oats (Das and Maria, 2017; Kütt*et al.*, 2023).

Vitamins B2 (riboflavin), B9 (folate), B11, and B12 are especially boosted. Vitamins are critical in energy metabolism, red blood cell formation, DNA synthesis, and neurological function. The enhanced content of these in the fermentation process overcomes the widespread nutritional deficiencies of people, particularly vegetarians, and extended fermented oat yogurt could be exploited as a carrier of the vitamins (Das and Maria, 2017).

De novo synthesis by LAB and release of bound vitamins from the oat matrix. The longer fermentation goes on, the longer vitamins are released by microbial enzymes, which in turn enable additional bacterial production. The low PH levels (typically below 4.2 after 12 hours) also help to keep these water-soluble vitamins in place and continue to build (Kütt*et al.*, 2023; Das and Maria, 2017).

This effect is enhanced by using special starter cultures and probiotics in oat yogurt production. *Lactobacillus reuteri and S. thermophilus* are examples of strains used in fermented oat drinks, which are known to be very viable during long fermentation times and storage periods (Bernat*et al*., 2015; Kütt*et al*., 2023). The final product has a better nutritional value than short-fermented or unfermented oat beverages.

Other improvements in the nutrition of extended fermented oat yogurt include the increased digestibility of protein and increased bioavailability of minerals (because of the reduction in phytates). These modifications enable oat yogurt to be a highly functional food to combat various micronutrient deficiencies (Das and Maria, 2017; Paudel*et al*., 2021; Angelov*et al*., 2018).

2.5.4 Carbohydrate Changes

Extended fermentation results in several changes to the carbohydrates in oat yogurts, most notably the breakdown of complex starches and oligosaccharides by microorganisms into simpler sugars, lactic acid, and other by-products. Such changes increase digestibility, reduce gastrointestinal discomfort, and improve the nutritional composition of the product.

Microbial fermentation leads to a decrease in the level of carbohydrates as well as some non-digestible poly- and oligosaccharides, which reduce side effects such as abdominal distention and flatulence. Improvement in starch digestibility during fermentation is due to the breakdown of starch oligosaccharides. The enzymes bring about the cleavage of amylase and amylopectin to maltose and glucose (Das and Maria, 2017).

The major carbohydrates in an oat yogurt system are starch (the predominant carbohydrate, ~82% of the oat endosperm db) and β-glucan. Lactic acid bacteria (LAB) like *Streptococcus thermophilus, Lactobacillus acidophilus,* and *L. plantarum*are highly active in metabolizing available sugars during the extended fermentation (usually 12-48 hours or more). This leads to a gradual decrease in the total carbohydrate content and an increase in the lactic acid level (1.6–

2.8 g/L) and the pH below 4.2 in less than 12 hours, and further changes in the carbohydrate content occur during extended fermentation periods (Kütt*et al*., 2023; Das and Maria, 2017). Hydrolysis of starch to maltose and glucose increases the digestibility of starch and makes the carbohydrates more digestion-friendly. This is especially useful in oat matrices because of the potential of native starch to increase viscosity and negatively impact the sensory attributes. Longer fermentation time means more enzyme activity in the process, not only from the added amylases used in pre-treatment, but also from microbes, thus yielding a higher conversion rate of complex carbohydrates (Das and Maria, 2017; Silventoinen-Vejalainen*et al*., 2023; Xu *et al*., 2024).

Importantly, the main soluble fibre in oats, β-glucan, is fairly resistant to fermentation, and retains its prebiotic and cholesterol-lowering effects. Most studies have found that the amount of beta-glucan remains in the final fermented product as it contributes to the viscosity of the product and to its health benefits, while other carbohydrates are preferentially fermented (Bernat*et al*., 2015; Kütt*et al*., 2023; Angelov*et al*., 2018).

The changes in the carbohydrate content of these foods have several nutritional benefits. Increased reduction of non-digestible oligosaccharides leading to enhanced consumer tolerance in regard to potential flatulence and bloating. Lactic acid and other organic acids produced during the process not only lower the pH of the product, but also provide the optimum conditions for mineral bioavailability and protein digestibility. In addition, short-chain metabolites are beneficial for gut health as a source of energy for the colonic epithelial cells (Das and Maria, 2017; Djorgbenoo*et al*., 2023).

2.6 The Effects of Extended Fermentation on Microbial Properties.

2.6.1 Growth of Lactic Acid Bacteria (LAB)

Extended fermentation of oats is a process, that is central to the growth of Lactic Acid Bacteria (LAB). Longer fermentation periods enable dynamic changes in microbial populations and favour the predominance of certain strains, a higher product longevity, and better functional properties of the final product.

Metagenomic sequencing showed that *S. thermophilus* was the prevalent species ranging between 38% to 99% of the entire microbial consortia. In the fermented oat drinks, the population of *L. acidophilus, L. plantarum, and L. paracasei* further grew in the lower pH. (Kütt*et al*., 2023)

The final pH below 4.2 was reached after 12 h for oat drink fermentation using any of the starter cultures. This early acidification is caused by the high levels of growth of *S. thermophilus,* which can sometimes be the most abundant species in the consortium (Kütt*et al*., 2023). When fermentation is continued beyond the first 12 hours, there will be a selective pressure for acid-tolerant strains as the pH will continue to drop. The presence of *Lactobacillus acidophilus, L. plantarum,* and *L. paracies a*re seen to sustain or increase under these lower pH conditions and contributes to the overall microbial stability and functionality of the oat yogurt (Kütt*et al*., 2023).

Mixed starter cultures also help to achieve good growth of LAB during prolonged fermentation. Fermentation of oat milk with *Lactobacillus reuteri*and*Streptococcus thermophilus* was analyzed to develop a new probiotic product. The chosen formulation was not only able to support the survival of starters above 10^7 cfu/ml, but it also kept the series intact throughout the 28 days controlled (Bernat*et al*., 2015). This illustrates that using the right combination of strains, extended fermentation can keep high levels of beneficial LAB throughout the fermentation and subsequent storage periods under refrigeration conditions.

The growth of LAB is sensitive to both the level of inoculation and the incubation conditions. Starter culture was added to the oat milk in the amount of 2.0 g/100 ml. The inoculated milk was subjected to incubation at 45 °C until the milk had reached a pH of 4.4 after about 5 hours (Gupta and Bisla, 2019). If fermentation time is prolonged after this initial period then the LAB will continue to metabolise the remaining carbohydrates and produce more acid, thereby encouraging further changes in the population that will increase the probiotic potential and biopreservative effects of the oat yogurt.

The microbial consortium constantly changes over time during long fermentation. It is shown that the number of proteolytic and acid-resistant strains grows at lower pH levels, not only affecting the acidification but also the proteolysis, flavor formation, and inhibition of undesirable microorganisms (Kütt*et al*., 2023). The growth pattern of *S. thermophilus*followed by the increasing of *Lactobacillus* species is typical of the extended fermentation of oat matrices and leads to a stable microbial profile of the oat yogurt with good functionality.

Extended fermentation also results in high LAB viability, even in the storage process. There was no sign of poor survival during storage and in vitro digestive tract conditions when added to probiotic fermented oat products (Chen *et al*., 2020). This long shelf life is essential to provide probiotics to consumers, and is one of the advantages that set extended fermented oat yogurts apart from yogurts with shorter fermentation times.

2.6.2 Benefits

As defined by the proposed Food and Agriculture Organization (FAO) International Guidelines for the Appropriate Use of Probiotics in Animal Production, probiotics are ‘live microorganisms that, when fed at appropriate levels, provide a health benefit to the host’. The minimum number of viable probiotic bacteria should be 10^7–10^9 colony-forming units (cfu)/g or ml of a product at the time of consumption (Bernat*et al*., 2015).

2.7 Microbial Analysis Parameters

Microbial analysis parameters are crucial to assess the influence of longer fermentation on the quality, safety, and functionality of oat yogurts. Typical parameters monitored are viable cell counts (CFU/ml), pH, lactic acid, metagenomic sequencing for the composition of microbial consortium, survival during storage tests of probiotics, and survival during simulated gastrointestinal testing.

Lactobacilli that were viable were counted in the fermentation and storage at 4°C and after exposure to the simulated gastrointestinal tract conditions. It was observed that the survival rate was good both in storage and in the in vitro digestive tract (Chen *et al*., 2020).

In studies on fermented oat drinks, metagenomic sequencing is used to determine the microbial consortium dynamics. The metagenomic data indicated that *S. thermophilus* was the predominant species (38%-99%) in the total microbial consortia. At lower pH values, the population of *L. acidophilus, L. plantarum,* and *L. paracasei* continued to increase in fermented oat drinks (Kütt*et al*., 2023).

Another important microbial analysis parameter is the production of lactic acid. The lactic acid concentration obtained during fermentation was 1.6-2.8 g/L (Kütt*et al*., 2023). Acidification to the desired pH (less than 4.2) was achieved within 12 h, irrespective of the starter culture, which is another significant parameter for successful LAB activity in the course of long fermentation (Kütt*et al*., 2023).

Surveying of viable count is an essential tool to determine probiotic functionality. The chosen formulation was able to guarantee a survival rate > 107 cfu/ml after 28 days of a controlled period and was considered a functional food (Bernat*et al.*, 2015). This parameter shows that extended fermentation when optimized, supports long-term viability that is important for health benefits.

pH and acidity are typical parameters of microbial analysis used on a regular basis. Starter culture was added at the rate of 2.0 g/100 ml of oat milk. The inoculated milk was incubated at

45°C till the pH changed to 4.4 in about 5 hours (Gupta and Bisla, 2019). The pH continues to drop thereafter, indicating a continuation of metabolism within the LAB consortium.

Survival under simulated gastrointestinal conditions is an important quality criterion for probiotic-enriched oat yogurts. Good survival was noted both during storage and when exposed to the in vitro digestive tract conditions (Chen *et al*., 2020). This analysis makes sure that the microbial population that has been developed in an extended fermentation is able to produce probiotic effects once consumed in the fermented product.

The correlation between the rheological and physicochemical parameters and the microbial activity is often observed during long fermentation. Rheological measurements indicated that the fermented oat beverages formed weak gel-like structures, which could be an indirect indication of the EPS production of some LAB strains during the extended fermentation period (Kütt*et al*., 2023).

Volatile compound profiling (e.g., using GC-IMS) is sometimes provided as an additional analytical parameter for microbial analysis, since many of the aroma compounds are derived from LAB metabolism. During fermentation, the concentration of the most preferred volatiles, e.g., the diacetyl and acetoin, increased (Kütt*et al*., 2023).

2.8 The Effect of Extended Fermentation on Sensory Properties.

2.8.1 Sensory Changes at 12 Hours Fermentation

Oat yogurts experience significant sensory changes at 12 h of fermentation, mainly due to the rapid acidification and early production of some important volatile compounds by lactic acid bacteria (LAB). At this stage, the product assumes the characteristic sour flavor and starts to make strides in aroma and overall acceptability.

This pH level was obtained after 12 h of incubation, irrespective of the starter culture used (Kütt*et al*., 2023). This rapid acidification is followed by the appearance of a characteristic sour smell and taste, characteristic sensory properties of fermented oat drinks at this stage. The fermented oat drinks all had a sour smell and taste (Kütt*et al*., 2023).

At this stage, volatile compounds found were from ketone, alcohol, aldehyde, acid, and furan classes. Buttery and creamy notes were observed during fermentation with the concentration of the most preferred volatile components such as diacetyl and acetoin that began to balance the sourness (Kütt*et al*., 2023). These compounds are synthesized as a result of the metabolic activity of the dominant species *S. thermophilus*(between 38% and 99% of microbial consortia) and supporting *Lactobacillus*spp. (Kütt*et al*., 2023).

Functional ingredients such as oat β-glucan can further influence sensory changes up to 12 hours. The addition of 0.5% β-glucan greatly shortened the time required for lactic acid bacteria fermentation to obtain the desired pH of 4.6, from 3.5h to 3h (Zhao *et al*., 2023). This accelerated acidification affects the sourness pathway, promoting the production of desirable flavor compounds, which resulted in more pronounced fruity, sweet, and creamy flavor in the increase of acetic acid, acetone, 2,3 butanedione, 3-hydroxy-2-butanone, and ethyl acetate (Zhao *et al*., 2023).

Despite these positive changes, sensory evaluation at 12 hours may still show that the product is much more cereal than dairy, in terms of overall taste and smell (Kütt*et al*., 2023). The ‘cereal-like’ character is a frequent sensory characteristic for early-stage fermented oat yogurts, and is a natural property of the oat matrices as longer fermentation times are needed for the aroma to become richer and more complex.

2.8.2 The Sensory Changes at 24 Hours of Fermentation.

Oat yogurts show greater sensory differences at 24 hours of fermentation than at 12 hours, due to continued microbial activity which results in more acidification and formation of certain volatile compounds, as well as further development of texture. These changes result in a more sensory complex profile and maintain the cereal-like properties, typical of oat products.

The fermented oat drinks all had a sour smell and taste (Küttet *al*., 2023). After 24 hours, the sourness deepens and is more pronounced, as lactic acid and other organic acids continue to be produced. The levels of the most desirable volatiles (diacetyl and acetoin) rose during fermentation (Küttet *al*., 2023). These compounds add buttery, creamy, and yogurt-like notes to help round out the growing acidity and make the final flavor more nuanced.

The compounds detected were in the groups of ketone, alcohol, aldehyde, acids, and furan (Küttet *al*., 2023). Within 24 hours, the tendency to increase desirable ketones (such as diacetyl, acetoin) and acids starts to show up, resulting in a more aromatic profile. Despite this, sensory evaluation still revealed that none of the samples were dairy-related but rather cereals-related in terms of taste and odor (Küttet *al*., 2023). The long cereal association suggests a hint of depth in the flavors despite the fermentation, but the oat signatures are still present and it does not lead to a complete shift to a dairy-like flavor.

The use of functional ingredients like oat β-glucan may help to positively regulate sensory changes around this time. The supplementation of oat β-glucan significantly boosted the production of acetic acid, acetone, 2,3-butanedione, 3-hydroxy-2-butanone, and ethyl acetate, which contributed to the fruity, sweet, and creamy aroma and flavor (Zhao *et al*., 2023). While the main data of Zhao *et al*. (2023) are limited to earlier fermentation times, rapid acidification and the production of flavor compounds indicated that the improvement in earlier times would be even greater after 24 hours, which would help to reduce cereal flavor and increase the overall liking of the product.

Rheological changes at 24 hours also affect mouthfeel and sensory perceived qualities. The rheological analysis revealed the presence of weak gel-like structures in fermented oat beverages (Küttet *al*., 2023). At this point, the gel network is more mature than at 12 hours and the product has a more yogurt-like consistency but is still not as firm as dairy yogurt. This is also significantly enhanced by β-glucan, which further boosts plastic modulus (G') and water-holding capacity, resulting in better creaminess and less syneresis during sensory evaluation (Zhao *et al*., 2023).

For blended oat-cow milk systems, the values of 24-hour fermentation indicate an improved blending of the milk and optimized blending ratios (e.g., 60:40) produced an acceptable sourness and overall sensory scores (Gupta and Bisla, 2019). In pure oat yogurts, however, the 24-hour mark is frequently a compromise where sourness and volatile development are desirable, but cereal-like flavours may need extra measures (such as flavours or extended fermentation) to achieve consumer acceptance.

The overall result shows that at 24 hours of fermentation, oat yogurts exhibit a more pronounced sour aroma, greater levels of desirable volatiles (diacetyl, acetoin, and others), deeper weak gel structure, and higher creamy/fruity attributes, with the addition of β-glucan. These changes provide significant sensory improvements when compared to the shorter fermentation periods, but the products still have a very strong cereal attribute and are not dairy-like (Kütt*et al*., 2023; Zhao *et al*., 2023; Gupta and Bisla, 2019). Oat yogurts may require longer fermentation times (usually more than 24 hours) to achieve the best sensory characteristics by further developing the complexity of the aroma and texture.

2.8.3 The Sensory Changes at 48 Hours of Fermentation.

At 48 hours of extended fermentation, oat yogurts undergo further sensory maturation compared to earlier stages (12 and 24 hours). Continued microbial activity results in intensified acidification, more complex volatile profiles, and progressive texture development, although the products often retain a distinctly cereal-associated character rather than fully mimicking dairy yogurt.

Fermentation improved the flavor and texture of the product (Kütt*et al*., 2023). By 48 hours, the cumulative effects of prolonged LAB metabolism lead to a more balanced yet pronounced sourness, with increased concentrations of desirable aroma compounds. Sensory evaluation showed that all samples were associated with cereals and not dairy in terms of taste and odor, even after extended fermentation (Küttet *al*., 2023). This persistent cereal-like sensory identity is a consistent finding across studies, indicating that while 48-hour fermentation enhances certain attributes, it does not eliminate the inherent oat-derived notes.

Texture continues to evolve during extended fermentation. Rheological analysis showed the formation of weak gel-like structures in fermented oat drinks (Kütt*et al*., 2023). By 48 hours, the gel network is more consolidated than at earlier stages, resulting in improved mouthfeel, although it remains weaker than traditional dairy yogurt. The incorporation of 0.5% β-glucan supports this development by increasing plastic modulus (G') and water-holding capacity, leading to better creaminess and reduced syneresis during sensory assessment (Zhao *et al*., 2023).

In oat-cow milk blended yogurts, extended fermentation up to 48 hours allows better flavor integration in optimized ratios, but pure oat systems at this stage still tend to score moderately in overall acceptability due to the dominant cereal character (Gupta and Bisla, 2019). Sensory evaluation at this point typically notes increased sourness alongside improved aroma complexity, but the products are still perceived as more cereal-like than dairy-like (Kütt*et al*., 2023).

Overall, at 48 hours of fermentation, oat yogurts display intensified sour odor and taste, richer volatile profiles with elevated diacetyl, acetoin, and other desirable compounds, more developed (though still weak) gel texture, and enhanced creamy/fruity notes when fortified with β-glucan. While these changes represent substantial sensory improvement and better balance compared to shorter fermentation times, the products remain distinctly cereal-associated (Kütt*et al*., 2023; Zhao *et al*., 2023; Gupta and Bisla, 2019). This highlights the need for further optimization strategies, such as specific strain selection, flavor masking, or blending, when targeting extended fermentation for oat yogurts.

2.9 Texture Modification During Fermentation

One of the most obvious changes that takes place during longer fermentation of oat yogurts is the texture modification. This results in the formation of weak gel-like structures due to the interplay among acid production, exopolysaccharide (EPS) production by LAB, and the interaction between LAB and oat components (e.g. β-glucan and starch).

Weak gel-like structures were detected in fermented oat drinks by rheological analysis (Küttet *al*., 2023). The progressive acidification and microbial activity during prolonged fermentation are directly responsible for this weak gel formation. The viscosity of an oat base increases and the mouthfeel becomes more structured when the pH drops, usually below 4.2 in a period of 12 hours, and continues to fall. This is due to the coagulation of the oat proteins and starch, and a network is formed.

Adding oat β-glucan immensely improves the development of texture. The lactic acid bacteria-induced fermentation was significantly accelerated by the addition of 0.5% β-glucan, and it increased the plastic module (G') from 693 Pa to 764 Pa when fermented for 3 h; and improved the water-holding capacity from 77.29% to 82.15% (Zhao *et al*., 2023). The increase in G' (storage modulus which correlates with gel strength) and water-holding capacity lessens syneresis and helps to produce a creamier, more stable texture with longer fermentation times. For set-type oat yogurt production, enzymatic hydrolysis with long fermentation time is important for optimizing the texture. However, the controlled fermentation (Xu *et al*., 2024) optimized the enzymatic hydrolysis and fermentation processing to obtain set-type oat yogurt with desirable acidity and coagulated texture. Longer fermentation can be used for strains like *S. thermophilus*and *L. plantarum,* which can produce more complete EPS, leading to a more robust network of protein-starch and enhanced rheological properties.

The texture is fairly constant after longer fermentations during storage. Rheological properties of the fermented product were characterized during the storage time at 4 °C (Bernat*et al*., 2015). The stability is significant for commercial viability, since longer fermentations create a firmer gel, which will not break down during refrigerated storage.

Texture modification is also evident for blends of oats and cow milk. The ratio of 60:40 ratio of oat to cow milk had an acceptable texture for sensory evaluation after fermentation (Gupta and Bisla, 2019). In pure oat systems, however, placing the fortifying agents (e.g. β-glucan or stabilizers) in oats and giving them longer fermentation time is crucial to obtain a desirable mouthfeel (Silventoinen-Vejalainen*et al*., 2023; Kütt*et al*., 2023).

In summary, the texture modifications that occurred during a prolonged fermentation of oat yogurts were characterized by the formation of weak gel structures, rising G', increasing viscosity, better water holding capacity, and a decrease in syneresis. These changes are attributed to acidification, EPS production, and interaction with β-glucan, although with fewer changes in texture than traditional dairy yogurt, and sensorial properties are improved, as compared to shorter fermentation times (Kütt*et al*., 2023; Zhao *et al*., 2023; Xu *et al*., 2024; Bernat*et al*., 2015; Gupta and Bisla, 2019).

2.10 Challenges Associated with Extended Fermentation.

Plant-based drinks are not necessarily well balanced for the needs of dairy products and great amounts of fortification are necessary. For taste and odor, all the samples were related to cereals, not dairy, according to sensory evaluation. Issues regarding the viability of probiotics during production and storage including issues with pH, oxygen, temperature, and strain and ingredient interactions (de Souza *et al*., 2024).

2.11 Identified Areas

Research has extensively reported typical fermentations (usually 4-12 hours to reach desired pH) in dairy-based yogurt with prebiotics (Jaman*et al*. 2022; Heydari*et al*. 2011; Sarvari*et al*. 2014) and recent studies with oats on starter selection, enzyme optimisation, and short fermentations (Christenson, 2020; Xu *et al*. 2024; Benmeziane and Belleli, 2023; Küttet *al*. 2023). Yet, few studies provide empirical evidence on the impact of extended fermentation times (especially 12, 24, and 48 hours) on the nutritional (anti-nutrient reduction, bioactive improvement), microbial (viability, stability), and sensory characteristics of exclusively or predominantly oat-based yogurt. The majority of plant-based research focuses on texture issues through the use of additives (not time), and Nigerian or African studies focus on cereal-based fermentations without specific oat yogurt experimentation (Adesemoye*et al*., 2025). This justifies the current study to assess the effect of extended fermentation times on oat substrate degradation, probiotic viability, and consumer-related characteristics of a functional oat-based yogurt.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample and Materials Sourcing

The raw materials including oat (*Avena sativa L.*), were sourced from Ogbete main market Enugu, Nigeria. Oat milk was extracted using distilled water. Additional oat yogurt ingredientsincludingdates (dabino) and vanilla essence, were procured from the same local sources. Primary starters in yogurt were selected lactic acid bacteria (LAB): *Streptococcus thermophilus* and *Lactobacillusdelbrueckii*subsp. *bulgaricus* (Hoxha *et al*., 2023; Kutt*et al*., 2023). These were all transported in sterile Ziploc bags back to the Microbiology Laboratory at Godfrey Okoye University, Thinkers Corner Campus where the experimental laboratory work was carried out under aseptic conditions.

3.2 Experimental Design

In this study a Completely Randomized Design (CRD) with one experimental factor, fermentation time with four levels, T1 (0 hour, standard control), T2 (12 hour), T3 (24 hour), and T4 (48 hour) was used. The treatments were replicated to ensure reliability and statistical validity of the results. The experimental design was chosen because it is suitable for evaluating the effect of a single factor under laboratory conditions to study the fermentation of oat-based yogurt and it is consistent with the methodology used in other studies (Hoxha et al., 2023; Xu et al., 2024; Ziarno et al., 2025).

3.3 Sample Preparation

3.3.1 Oat Milk Production

Whole-grain oat flour at 80 grams per cup (1920 grams or 24cups) and Dates (dabino) were soaked in warm water to soften for 20 minutes then were slurred together with water (11,356ml) in a blender. Oat milkwasextracted with sterile muslin cloth. After the enzymes had converted some of the oat starch, the oat milk was heated to stop the enzymes from working any further. Then it was cooled and mixed under pressure to achieve an even distribution of all particles, resulting in a smooth and stable oat milk.Direct fermentation was done using the final oat milk base. This protocol provides adequate glucose levels and eliminates phase separation that is characteristic of oat systems (Xu *et al.*, 2024; Silventoinen-Vejalainen*et al.*, 2023; Zhou *et al.*, 2023). Anaerobic fermentation (lactic acid fermentation) was done with mixed LAB starter cultures The ready oat milk base was inoculated and fermented at 42°C in sterile plastic containers until the desired time elapsed (Hoxha *et al*., 2023; Kutt*et al*., 2023; Xu *et al*., 2024; Ziarno*et al*., 2025).

3.4 Microbial Analysis

3.4.1 Microbial Culture and Isolation from Oat-based Yogurt Samples

Sterile peptone water was used for the dilution of the yogurt samples to obtain serially diluted concentrations (up to 10^6) for plating the samples on sterile semi solid agar plates. For the first test tube, one mL of prepared yogurt was added into 9mL of peptone water and was homogenized properly before being transferred seriallyup to the 6th test tube and was discarded effectively. For the inoculation on the agar plates, 0.1mL of 3 dilution concentrations (10^2, 10^3 and 10^4) were transferred unto the surface of the agar plates.

3.4.2 Lactic Acid Bacteria Count

For the enumeration of lactic acid bacteria present in the fermented oat-based yogurt Samples, the MRS agar was used. The MRS agar was prepared according to manufacturer's instructions by sterilizing in an autoclave for 15 minutes at 121°C and was allowed to cool down to about 45°C. Utilizing the pour-plate method, 0.1mL of yogurt samples were transferred into sterile petri dishes, followed by about 20mL of the cooled sterilized MRS agar, and was allowed to gel completely before being inverted and transferred into an anaerobic incubator at 37°C for intervals of 12, 24 and 48 hours respectively. Colonies were enumerated as CFU/mL, and their equivalent log10 CFU/mL was calculated (Hoxha *et al*., 2023; Sarvari*et al.*, 2014).

3.4.3 Yeast and Mold Count

To further confirm the safety profile of the fermented oat-based yogurt sample, yeast and mold culture counts were conducted using the spread plate method on Potato Dextrose Agar and was incubated at 25°C from 3days to 5days. Counts were expressed as CFU/mL using the formula:

CFU/mL = (number of colonies × dilution factor) / sample weight. This method ensures the assessment of microbial safety and fermentation efficacy (AOAC International, 2019).

3.5 Nutritional Analysis of oat yogurt samples.

3.5.1 Proximate Composition

To determine the nutritional profile of the oat-based yogurt samples, proximate analysis for specific profile was conducted. The nutrient profile analyzed were moisture content, ash, crude protein, crude fat and carbohydrates as described below according to AOAC International, (2019); Xu *et al*., (2024); Ziarno*et al*., (2025)

3.5.1.1 Moisture Content:

About 2g of the yogurt sample (W_1) was weighed and transferred into pre-weighed crucible (W_0) and was dried in a hot air oven at 105°C for 4 hours until constant weight was achieved. This was allowed to cool completely at room temperature and was reweighed for the final weight (W_2).

The percentage% Moisture = $[(W_1 - W_2) / W_1] \times 100$.

3.5.1.2Ash Content:

Another measured 2g of the sample was transferred into pre-weighed crucible and was placed inside a muffle furnace at 550°C for 6 hours. After combustion reaction, this was allowed to cool completely, and the final weight was taken.

The percentage% Ash = (Weight of ash / Weight of sample) × 100.

3.5.1.3 Crude Protein:

Using the Kjeldahl method, 0.5g of sample was measured inside Kjeldahl flask with 10ml concentrated H_2SO_4 and a catalyst added into it. This was digested until clear solutions formed and was distilled with 40% NaOH. The distillate was collected in boric acid solution and was titrated with 0.1N HCl.

Crude Protein percentage is calculated by: % Nitrogen = [(Titer value × Normality of HCl × 14.007) / Weight of sample] × 100

% Crude Protein = % Nitrogen × 6.25.

3.5.1.4 Crude Fat:

Using the Soxhlet extraction method, 2g of sample was measured into a thimble and its content were extracted with petroleum ether for 6 hours, and the solvent was allowed to evaporate to dryness in the flask at 105°C. The solution was allowed to cool and was weighed again.

The percentage % Fat = [(Weight of fat / Weight of sample)] × 100.

3.5.1.5 Carbohydrate:

The percentage of carbohydrate is a measure of the difference of a 100 and the summed-up percentages of the other proximate scores

The Percentage% Carbohydrate = 100 - (% Moisture + % Ash + % Protein + %fibre + % Fat)

3.6 Sensory Evaluation

3.6.1 Panel Selection

A panel composed of individuals (n = 52) who were familiar with the attributes of yogurt were recruited and briefed for the sensory evaluation exercise with their ages ranging from 18-56 years.

3.6.2 Evaluation Method

A 9-point descriptive/hedonic scale was used to measure the texture, color, aroma, and taste of the oat yogurt in addition to the overall acceptability (Hoxha *et al.*, 2023; Zhou *et al.*, 2023).

3.7 Data Analysis

Data was analyzed in triplicates, and the findings were recorded in the form of mean ± standard deviation. Significant differences were established using one-way ANOVA. The findings were displayed in a table (Hoxha *et al.*, 2023; Parvarei*et al.*, 2021).

CHAPTER FOUR

RESULTS

4.1. Viable Bacteria Count (CFU/mL)

The results of viable bacteria count as observed in Table 4.1 shows the colony counts of nutrient agar having the consistent highest bacteria count within the first 12 hours at 1.02×10^7 CFU/mL, the 24-hour count at 1.17×10^7 CFU/mL and at 48 hours at 1.72×10^7 CFU/mL.In contrast to the deMann Rogosa and Sharpe Agar (MRS) had the second highest at all three-time intervals ranging from 8.7×10^6 CFU/mL, 9.5×10^6 CFU/mL and 1.09×10^7 CFU/mL respectively. There was no growth on the MacConkey agar plate.

Table 4.1. Bacteria Viable Count of Isolates

Agar Plates ID/Time Interval	Dilution Factor	Colonies Counted	Colony Forming Units (CFU/mL)	pH at Fermentation end
MRS 12 hours	10^2	68	6.8×10^4	4.78
	10^3	117	1.17×10^6	
	10^4	95	9.5×10^6	
MRS 24 hours	10^2	77	7.7×10^4	4.65
	10^3	103	1.03×10^6	
	10^4	87	8.7×10^6	
MRS 48 hours	10^2	TFTC	Nil	4.50
	10^3	36	3.6×10^5	
	10^4	109	1.09×10^7	
NA 12 hours	10^2	83	8.3×10^4	4.78
	10^3	116	1.16×10^6	
	10^4	102	1.02×10^7	
NA 24 hours	10^2	207	2.07×10^5	4.65
	10^3	193	1.93×10^6	
	10^4	117	1.17×10^7	
NA 48 hours	10^2	TNTC	Nil	4.50
	10^3	230	2.3×10^6	
	10^4	172	1.72×10^7	
MAC 12 hours	10^2	TFTC	Nil	Nil
	10^3	TFTC	Nil	
	10^4	TFTC	Nil	

MAC 24 hours	10^2	TFTC	Nil	Nil
	10^3	TFTC	Nil	
	10^4	TFTC	Nil	
MAC 48 hours	10^2	TFTC	Nil	Nil
	10^3	TFTC	Nil	
	10^4	TFTC	Nil	

Key: (MRS): deMann, Rogosa and Sharpe Agar; (NA): Nutrient Agar; (MAC): MacConkey Agar; (TFTC): Too Few To Count; (TNTC) Too Numerous To Count; (Nil): Non-viable count.

4.2. Viable Yeast and Fungi Count (CFU/mL)

The Yeast and fungi count were taken at intervals of 48 hours (2 days) each from the 3rd day counting after visible fungi cells were spotted on the potato dextrose agar plate (PDA) as seen in Table 4.2. The highest fungi count was recorded on the 3rd day with colony forming units of 1.77×10^7 CFU/mL, followed by the 5th day or 2nd count at 9.0×10^6 CFU/mL, and finally on the 7th day or 3rd count at 3.3×10^6 CFU/mL.

Table 4.2. Yeast and Fungi Viable Count

Agar plates ID and Time Interval	Dilution Factor	Colonies Counted	Colony Forming Units (CFU/mL)
PDA 3rd Day	10^2	255	2.55×10^5
	10^3	116	1.16×10^6
	10^4	177	1.77×10^7
PDA 5th Day	10^2	217	2.17×10^5
	10^3	183	1.83×10^6
	10^4	90	9.0×10^6
PDA 7th Day	10^2	200	2.8×10^5
	10^3	177	1.77×10^6
	10^4	33	3.3×10^6

Key: (PDA): Potato Dextrose Agar.

4.3. Proximate Analysis of Samples

The control sample had the most moisture and protein content, but recorded the least crude fat, fibre and protein content in comparison to the other samples as seen in Table 4.3. The 12-hour fermentation sample had the the most fat, protein, and fibre content in contrast to the other 2 fermented samples but recorded the least total carbohydrate percentage. The 24 and 48 hour fermented samples had declining values of nutrient components evaluated**.**

Table 4.3. Proximate Composition Analysis of Oat-based Yogurt Sample

Proximate Composition (%)	Control sample (%)	12-hour sample (%)	24-hour sample (%)	48-hour sample (%)
Moisture	41.5	46.75	40.52	39.96
Ash content	1.08	1.5	1.02	1
Crude Protein	4.9	11.05	9.89	10
Crude Fiber	2.35	3.9	1.55	0.85
Crude Fat	5.55	9.50	7.25	7.10
Carbohydrate	44.62	27.30	39.77	41.09

4.4. Sensory Evaluation Profile of Oat yogurt samples

Sensory evaluation of the samples indicated mixed results with declining acceptability of the oat yogurt samples from the control, 12th hour and to the 48th hour sample as observed in Table 4.4. Mean scores were calculated from individual scores of preferences according to a 1 to 9 hedonic scale with 1 being the least liked and 9 being the most liked.

Table 4.4. Sensory Evaluation Profile for Yogurt Samples

Sensory properties	Mean Age of 52 Panelists (18-47 years)	Control sample	12-hour sample	24-hour sample	48-hour sample
	26.83				
Taste		8.38 ± 0.88	6.71 ± 1.11	4.67 ± 1.81	2.57 ± 1.79
Aroma		7.22 ± 1.04	5.46 ± 1.35	3.76 ± 1.42	2.14 ± 1.55
Color		7.17 ± 1.21	5.89 ± 1.47	4.27 ± 1.59	2.96 ± 1.80
Texture		7.63 ± 1.16	5.52 ± 1.20	3.58 ± 1.53	1.77 ± 1.64
Overall acceptability		6.92	5.89	4.17	2.36

Key: Scores are mean averages across the 52 Panelists ± Standard Deviation across the mean averages for each sensory property as analyzed using single-factor ANOVA in Excel 2013**.**

Table 4.5: One-Way ANOVA for Sensory Evaluation

(a) Taste

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	965.428	3	321.809	155.104	<0.0001	2.649
Within Groups	423.260	204	2.075			
Total	1388.688	207				

(b) Aroma

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	743.101	3	247.700	137.553	<0.0001	2.649
Within Groups	367.356	204	1.801			
Total	1110.457	207				

(c) Colour

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	524.013	3	174.671	76.324	<0.0001	2.649
Within Groups	466.861	204	2.289			
Total	990.874	207				

(d) Texture

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	972.898	3	324.299	171.428	<0.0001	2.649
Within Groups	385.918	204	1.892			
Total	1358.816	207				

(e) Overall Acceptability

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	800.782	3	266.927	227.704	<0.0001	2.649
Within Groups	239.141	204	1.172			
Total	1039.923	207				

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1. Discussion

The study's rationale is centered on the fermentation of oat flour and date palm composite blend into yogurt using active starter culture as an alternative to dairy-based yogurt. The yogurt produced was fermented for a total period of 48 hours, and the pH and microbial load profile (viable bacteria and yeast count) were taken at different intervals (Table 4.1 and Table 4.2). The viable bacteria count recorded varied across the specific de Man,Rogosa, and Sharpe (MRS) agar and the nutrient agar at different dilution factors and at different fermentation time periods. Both agars had higher concentrations of bacteria at 6.8×10^4 to 1.09×10^7 CFU/mL for the MRS at 3 different concentrations and 8.3×10^4 to 1.72×10^7 CFU/mL for the Nutrient Agar, and this was different from the results of a similar study utilising tiger nut milk by Onyimba e*t al*. (2022), who recorded a lower microbial count profile. There was no growth on the MacConkey agar inoculated with the yogurt, as is expected, as the bacteria that naturally ferment milk and plant-basedalternative blends into yogurt are of Gram-positive origin, which are the lactic acid bacteria genera. Bacteria that grow on MacConkey agar are naturally Gram-negative in origin, and this serves as a differential medium in this regard. The inability of the bacteria in the oat-based yogurt to grow on this agar also served as a positive indicator and control to ensure the isolates are of the lactic acid bacteria genera, which were selectively grown on the MRS agar. On the other hand, the viable yeast count had moderate high concentration of isolates within the ranges of 2.17×10^5 to 1.77×10^7 CFU/mL as observed in Table 4.2, in the course of a 7-day incubation period. This was similar but a bit higher than the results of a similar study by Bristone*et al*. (2015), who recorded a total fungal count of 5.8 to 6.3×10^5 CFU/mL in tiger nut milk yogurt variant.

The results of the proximate composition of all oat-based yogurt samples showed a particular trend of increase and decrease of some of the components tested, from the control to the different fermented samples. The unfermented control sample had the most moisture, as its formulation remained unchanged during and after the test, and protein content, which may be due to the presence of pitted dates, which rather increased the existing protein of the oat flour. This also recorded the least fat content, as oat grains are known to have low dietary fats. The ash content of a food gives a visual understanding of the mineral elements contained in the food, and in this case the 12-hour sample had the most, followed by the unfermented, 24- and 48-hour samples respectively. This trend could indicate the loss and increment of mineral contents of the oat-based yogurt due to the benefits of an ideal fermentation process. The 12-hour sample having the highest ash content could indicate the presence of improved mineral content, as provided due to the metabolic and biochemical changes the oat flour samples have undergone. The crude fat content of the samples was recorded highest in the 12-hoursample and may be due to the pre-hydrolysis of the oat-based yogurt during fermentation by the lactic acid bacteria culture, which makes it easily digestible. This process likely increased the available fat content of the yogurt, making it more palatable and preferred as per the sensory evaluation analysis. The final carbohydrate content of the 12-hour sample, in contrast to the other 3 samples, was at 27.30%, and this was consistent with the result of a similar study by Baskar et al. (2022), who recorded an optimised carbohydrate content value of 27.00% for oat-based yogurt. This could indicate that the 12-hour fermentation time is most ideal to get the most nutritional content and benefits of oat-based yogurt.

The sensory evaluation of food products gives a better understanding of the organoleptic properties of that particular food, and in this study, this was done to ascertain precisely the palatability profile of the oat-based yogurt in terms of aroma, taste, texture, and color by the chosen panelists. Compared to cow's milk, oat milk tends to offer more vitamins and minerals, such as vitamin E, magnesium, and potassium. So, this points toward the fact that plant-based dairy alternatives might still deliver nutrients that some people don't get or simply wouldn't find in cow's milk in the same way (Collard and McCormick, 2021). The individual scores of the 52 Panelists who had a taste test of the different samples were statistically analyzed and noted as mean scores ± the standard deviations across the individual scores as detailed in the results (Table 4.3). The overall acceptability of the four attributes evaluated here varied from the control sample to the other three samples. The control sample, which was an unfermented blend of oat flour and date palm fruits, had the most acceptability at 6.92 in terms of taste, aroma, color, and texture as a result of the still present and noticeable hint of sweetness obtained from the date palm fruits. The noticeable decline in acceptability amongst all selected panelists occurred as a result of the different fermentation periods the yogurt underwent. At the 12-hour mark of fermentation, the mean score and acceptability of the yogurt samples were at a moderate level, with the taste having the best overall score at (6.71 ± 1.11) in comparison to the other attributes, which may be accounted for by the decrease in pH to 4.78 as a result of the beginning of the fermentation process. The overall acceptability of the 12-hour fermented sample at 5.89 was similar to the result of Grasso*et al.* (2020), who compared the sensory properties and acceptability of different plant-based yogurts to dairy yogurt and recorded the highest statistically significant acceptability at 5.95. The 24-hour mark of fermentation resulted in further decline of the oat-based yogurt samples' acceptability, with mean scores across all four attributes ranging between 3.58 and 4.67 ± standard deviations. This is as a result of a slight decrease in pH to 4.65 and the near depletion of all available sugar substrates in the oat yogurt. The final fermentation period of 48 hours recorded the lowest scores between 1.77 and 2.96. The yogurt samples were at their lowest pH of 4.50, an ideal pH for plant-based yogurt alternatives during fermentation. The decreased pH gave a highly acidic and unpalatable taste and texture to the yogurt, which most panelists rejected during the taste test.

CONCLUSION

In conclusion, the microbial, sensory, and nutritional properties of the oat-based yogurt indicate that the most ideal fermentation time for this plant-based yogurt alternative is within the 12th hour mark, as due to having the most probiotic and nutritional content as well as the best overall acceptability by the panelists in this study.

REFERENCES

AOAC International. (2019). *Official methods of analysis* (21st ed.). AOAC International.

Alemayehu, G. F., Forsido, S. F., Tola, Y. B., and Amare, E. (2023). Nutritional and phytochemical composition and associated health benefits of oat (*Avena sativa*) grains and oat-based fermented food products. *The Scientific World Journal*, 2023, 2770175.

Angelov, A., Yaneva-Marinova, T., and Gotcheva, V. (2018). Oats as a matrix of choice for developing fermented functional beverages. *Journal of Food Science and Technology*.

Benmeziane, F., and Belleili, I. (2023). An innovative approach: Formulation and evaluation of fermented oat milk using native yoghurt cultures. *Measurement: Food*, 12, 100113.

Bernat, N., Cháfer, M., González-Martínez, C., Rodríguez-García, J., and Chiralt, A. (2015). Optimisation of oat milk formulation to obtain fermented derivatives by using probiotic *Lactobacillus reuteri* microorganisms. *Food Science and Technology International*, 21(2), 145–157.

Bristone, C., Badau, M. H., Igwebuike, J. U., and Igwegbe, A. O. (2015). Production and evaluation of yoghurt from mixtures of cow milk, milk extract from soybean and tiger nut. *World Journal of Dairy and Food Sciences*, 10(2), 159–169.

Chen, L., Wu, D., Schlundt, J., and Conway, P. L. (2020). Development of a dairy-free fermented oat-based beverage with enhanced probiotic and bioactive properties. *Frontiers in Microbiology*, 11, 609734. https://doi.org/10.3389/fmicb.2020.609734

Christerson, M. (2020). Screening of starter cultures for oat-based non-dairy yoghurt. *Swedish University of Agricultural Sciences.*

Das, G., and Maria, J. M. (2017). Nutrient profile of fermented oats. *International Journal of Food Science and Nutrition*, 2(4), 69–71.

Decker, E. A., Rose, D. J., and Stewart, D. (2014). Processing of oats and the impact of processing operations on nutrition and health benefits. *British Journal of Nutrition*, 112(S2), S58–S64.

de Souza, M., Drunkler, D. A., and Colla, E. (2024). Probiotic functional yogurt: Challenges and opportunities. *Fermentation*, 10(1), 6. https://doi.org/10.3390/fermentation10010006

Djorgbenoo, R., Hu, J., Hu, C., and Sang, S. (2023). Fermented oats as a novel functional food. *Nutrients*, 15(16), Article 3521. https://doi.org/10.3390/nu15163521

Erem, E., and Kilic-Akyilmaz, M. (2024). The role of fermentation with lactic acid bacteria in quality and health effects of plant-based dairy analogues. *Comprehensive Reviews in Food Science and Food Safety*, 23(4), e13402.

Gupta, S., and Bisla, G. (2019). Nutritional and sensory characteristics of oat milk-based yoghurt. *International Journal of Applied Home Science*, 6(6-8), 261–265.

Heydari, S., Mortazavian, A. M., Ehsani, M. R., Mohammadifar, M. A., and Ezzatpanah, H. (2011). Biochemical, microbiological and sensory characteristics of probiotic yogurt containing various prebiotic compounds. *Italian Journal of Food Science*, 23(2), 153–163.

Hoxha, R., Evstatieva, Y., and Nikolova, D. (2023). Physicochemical, rheological, and sensory characteristics of yogurt fermented by lactic acid bacteria with probiotic potential and bioprotective properties. *Foods*, 12(12), 2552.

Jaman, S., Islam, M. Z., Sojib, M. S. I., Hasan, M. S., Khandakar, M. M. H., Bari, M. S., and Rashia, M. H. (2022). Physicochemical characteristics, sensory profile, probiotic, and starter culture viability of synbiotic yogurt. *Journal of Advanced Veterinary and Animal Research*, 9(4), 694–701.

Kütt, M.-L., Orgusaar, K., Stulova, I., Priidik, R., Pismennõi, D., Vaikma, H., Kallastu, A., Zhogoleva, A., Morell, I., and Kriščiunaite, T. (2023). Starter culture growth dynamics and sensory properties of fermented oat drink. *Heliyon*, 9(5), e15627. https://doi.org/10.1016/j.heliyon.2023.e15627

Le, M. S., Hermansen, C., and Vuong, Q. V. (2025). Oat milk by-product: A review of nutrition, processing and applications of oat pulp. *Food Reviews International*, 41(5), 1538–1575. https://doi.org/10.1080/87559129.2025.2450263

Łopusiewicz, Ł. (2024). Comparison of homemade and commercial plant-based drinks (almond, oat, soy) fermented with yogurt starter culture for fresh consumption. *Fermentation*, 10(1), 35.

Onyimba, I. A., Chomini, M. S., Job, M. O., Njoku, A. I., Onoja, J. A., Isaac, I. C., Isaac, D. C., and Ngene, A. C. (2022). Evaluation of the Suitability of Tigernut Milk and Tigernut-Cow Composite Milks for Yoghurt Production. *European Journal of Biology and Biotechnology*, 3(2), 38–44.

Parvarei, M. M., Khorshidian, N., Fazeli, M. R., Mortazavian, A. M., Nezhad, S. S., Mortazavi, S. A., and Golabchifar, A. A. (2021a). Comparative effects of probiotic and paraprobiotic addition on microbiological, biochemical and physical properties of yogurt. *Food Research International*, 140, 110030.

Parvarei, M. M., Khorshidian, N., Fazeli, M. R., Mortazavian, A. M., Nezhad, S. S., Mortazavi, S. A.,andGolabchifar, A. A. (2021b). Comparative effect of probiotic and paraprobiotic addition on physicochemical, chemometric and microstructural properties of yogurt. *LWT - Food Science and Technology*, 144, 111177.

Paudel, D., Dhungana, B., Caffe, M., andKrishnan, P. (2021). A review of health-beneficial properties of oats. *Foods*, 10(11), Article 2591. https://doi.org/10.3390/foods10112591

Pua, A., Tang, V. C. Y., Goh, R. M. V., Sun, J., Lassabliere, B., and Liu, S. Q. (2022). Ingredients, processing, and fermentation: Addressing the organoleptic boundaries of plant-based dairy analogues. *Foods*, 11(6), 875.

Sarvari, F., Mortazavian, A. M., and Fazeli, M. R. (2014). Biochemical characteristics and viability of probiotic and yogurt bacteria in yogurt during the fermentation and refrigerated storage. *Applied Food Biotechnology*, 1(1), 55–61.

Silventoinen-Vejalainen, P., Sneck, A.-M., Nordlund, E., and Rosa-Sibakov, N. (2023). Influence of oat flour characteristics on the physicochemical properties of oat-based milk substitutes. *Food Hydrocolloids*, 147, 109402.
https://doi.org/10.1016/j.foodhyd.2023.109402

Xu, W., Wu, X., Xia, C., Guo, Z., Zhai, Z., Cheng, Y., and Qiu, J. (2024). Optimization of enzymatic hydrolysis and fermentation processing for set-type oat yogurt with favorable acidity and coagulated texture. *Foods*, 13(13), 4180.

Yang, S.-Y., andYoon, K.-S. (2022). Effect of probiotic lactic acid bacteria (LAB) on the quality and safety of Greek yogurt. *Foods*, 11(23), 3799.

Yu, Q., Qian, J., Guo, Y., Qian, H., Yao, W., and Cheng, Y. (2023). Applicable strains, processing techniques and health benefits of fermented oat beverages: A review. *Foods*, 12(8), 1708. https://doi.org/10.3390/foods12081708

Zhao, P., Li, N., Chen, L., Guo, Y., Huang, Y., Tong, L., Wang, L., Fan, B., Wang, F., and Liu, L. (2023). Effects of oat β-glucan on the textural and sensory properties of low-fat set type pea protein yogurt. *Molecules*, 28(7), 3067. https://doi.org/10.3390/molecules28073067

Zhou, S., Jia, Q., Cui, L., Dai, Y., Li, R., Tang, J., and Lu, J. (2023). Physical–chemical and sensory quality of oat milk produced using different cultivars. *Foods*, 12(6), 1165.

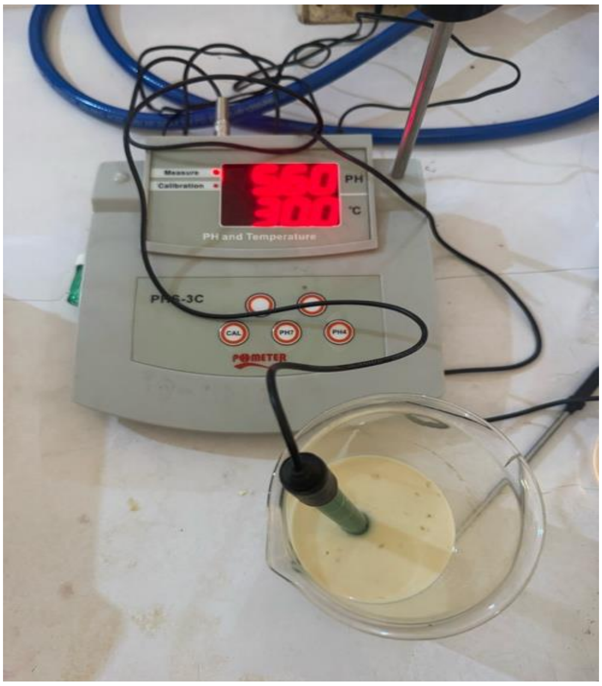
Ziarno, M., Zaręba, D., Kowalska, E., and Florowski, T. (2025a). A study into the effects of chosen lactic acid bacteria cultures on the quality characteristics of fermented dairy, dairy-oat, and oat beverages. *Applied Sciences*, 15(7), 3714.

Ziarno, M., Zaręba, D., Kowalska, E., and Florowski, T. (2025b). The effect of varying oat beverage ratios on the characteristics of fermented dairy-oat beverages. *Applied Sciences*, 15(7), 3219.

APPENDIX

Appendix 1: Picture showing laboratory process involving serial dilution

Appendix 2: Picture showing proximate ash content sample in the crucible



Appendix 3: Picture showing pH measurement of 12 hours sample

Appendix 4: Picture showing mold count after 7 days of storage